Review

Two Nonthermal Technologies for Food Safety and Quality—Ultrasound and High Pressure Homogenization: Effects on Microorganisms, Advances, and Possibilities: A Review

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ABSTRACT

Some nonthermal technologies have gained special interest as alternative approaches to thermal treatments. High pressure homogenization (HPH) and ultrasound (US) are two of the most promising approaches. They rely upon two different modes of action, although they share some mechanisms or ways of actions (mechanic burden against cells, cavitation and micronization, primary targets being the cell wall and the membrane, temperature and pressure playing important roles for their antimicrobial potential, and their effect on cells can be either positive or negative). HPH is generally used in milk and dairy products to break lipid micelles, whereas US is used for mixing and/or to obtain active compounds of food. HPH and US have been tested on pathogens and spoilers with different effects; thus, the main goal of this article is to describe how US and HPH act on biological systems, with a focus on antimicrobial activity, mode of action, positive effects, and equipment. The article is composed of three main parts: (i) an overview of US and HPH, with a focus on some results covered by other reviews (mode of action toward microorganisms and effect on enzymes) and some new data (positive effect and modulation of metabolism); (ii) a tentative approach for a comparative resistance of microorganisms; and (iii) future perspectives.

HIGHLIGHTS

- High pressure homogenization (HPH) and ultrasound (US) can be used for food preservation.
- HPH and US exert a mechanic burden against microorganisms.
- HPH and US can be used for liquid foods.
- HPH and US are promising strategies, although further efforts are required.

Key words: Cavitation; Equipment; Mechanic burden; Micronization; Mode of action

Food safety and quality are challenges and primary goals worldwide; the Centers for Disease Control and Prevention, in fact, estimates that, each year, 48 million people get sick from foodborne illnesses, 128,000 are hospitalized, and 3,000 die (32).

This global threat is generally counteracted by food preservation (FP). According to Lado and Yousef (78) and Van Impe et al. (137), FP aims to disturb homeostasis; if microorganisms cannot obtain homeostasis and respond to stress, they either are unable to grow or they die. The core of FP is to create a stress or an unfavorable condition and to reversibly or irreversibly modify homeostasis and to thereby achieve microbial inhibition or inactivation.

Generally, FP is achieved by pasteurization and sterilization; however, it can cause some negative changes in the nutritional and sensorial quality of foods (16). Thus, consumer demands for safety and minimally processed foods with high-quality attributes have encouraged the food industry and scientific researchers to design alternative nonthermal technologies (NTTs) to produce foods with a minimum of changes induced by the technologies themselves (16, 21, 71). Definitions for NTT can be found in the articles of Tiwari et al. (130) and Van Impe et al. (137): NTTs are approaches that are effective at sublethal or ambient temperatures and that lead to minimal or no impacts on key nutritional and quality food parameters.

The key findings on NTT, reported by Van Impe et al. (137), can be summarized as follows: (i) NTTs are effective on vegetative cells, whereas the inactivation of spores can be achieved by combining multiple hurdles; (ii) NTTs result in higher inactivation rates in model systems than they do in foods; and (iii) NTTs are effective in liquid foods.

Figure 1 shows the most important NTTs and the main approaches they are based on (physical and chemical). The focus of this article is on two methods: ultrasound (US) and high pressure homogenization (HPH). The most important phenomenon related to US is sonoporation or sonochemistry.
try, whereas in HPH, a fluid is forced through a small gap (a homogenizing valve). However, both methods share some mechanisms or ways of actions, like their mechanical burden against cells, cavitation and micronization, their primary targets being cell walls and membranes, and temperature and pressure having important roles in their antimicrobial potential. In addition, they can be both used for liquid foods (46, 105, 137), and their effects on microorganisms can be either positive (stimulation and modulation of some functional properties) or negative (inhibition and inactivation).

Therefore, the main goal of this article is to describe how US and HPH act on biological systems, with a special emphasis on the factors triggering the antimicrobial activity, the mode of action on microorganisms and proteins, the positive effects on some microbial properties (adhesion, surface properties, and enzyme release), the equipment, and the level of microbial inactivation, with some examples of application.

The article is composed of three main parts: an overview of US and HPH, a tentative approach for a comparative resistance of microorganisms, and future perspectives. The overview of US and HPH shows data reported in other reviews and some new traits (positive effects of homogenization and sonication on microorganisms); the novelty of this section is not on its contents but in its covering of the most important topics.

ULTRASOUND

Definition and use. US can be classified as a nonwater-based or reduced water-usage technology; US uses sounds with a frequency beyond the human ear (frequencies from 2 Hz to 20 MHz) (98). Sonic waves can be classified in three groups: (i) infrasonic (2 to 20 Hz); (ii) acoustic (20 Hz to 20 kHz), which includes the region for human hearing (16 to 18 Hz); and (iii) ultrasonic or US (20 kHz to 20 MHz).

US is generally defined as sonic waves with a frequency beyond human hearing; it comprise three ranges with different practical implications and applications: (i) power US (20 to 100 kHz) for processing applications, (ii) high frequency or extended range for sonochemistry (20 kHz to 2 MHz), and (iii) diagnostic ultrasound (5 to 10 MHz). From an application perspective, a second classification is based upon frequency and intensity; thus, US can be classified as high intensity and low frequency (intensity at 10 to 1,000 W/cm², frequency from 20 to 100 kHz) or low intensity and high frequency (intensity, <1 W/cm²; frequency, >100 kHz). High-intensity US can be used for FP because it generally employs power levels high enough to generate cavitation and to exert an antimicrobial effect. In contrast, high-frequency US is used for nondestructive analyses because it does not produce cavitation, and the sonic waves produce zero or no changes in the materials through which they pass.

In the food industry, US was used in the past for a variety of foods, such as milk and dairy products, vegetables and olives, fish and shellfish, meat, and beverages; the applications, because of the cavitation and related effects induced by US (see following sections), included the inactivation of microorganisms and enzymes, the production of emulsions and nanoemulsions, the extraction of bioactive compounds and oils from vegetable cells, and the
TABLE 1. Overview of the use of US in the food industry

<table>
<thead>
<tr>
<th>Application</th>
<th>Description</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microbial inactivation</td>
<td>Bacteria (lactobacilli and other gram-positive bacteria, pseudomonas, enterobacteria, and staphylococci), fungi (Candida, Pichia, Rhodotorula, Saccharomyces, Zygossaccharomyces, Aspergillus, Fusarium, Penicillium, and Mucor species), and phytoviruses</td>
<td>7, 16, 20, 89</td>
</tr>
<tr>
<td>Enzyme inactivation or enhancement</td>
<td>Inactivation of pectic enzymes and enhancement of the activity of α-amylase and amyloglucosidase</td>
<td>16, 89, 122, 142</td>
</tr>
<tr>
<td>Extraction</td>
<td>Extraction of essential oils and bioactive molecules from plants, proteins, polysaccharides, pigments, cellulose nanofibers, and xylolucans</td>
<td>6, 34, 89</td>
</tr>
<tr>
<td>Freezing and crystallization</td>
<td>Increase of the number of nucleation sites and nucleation rate</td>
<td>6, 34, 89</td>
</tr>
<tr>
<td>Emulsification</td>
<td>Production of micro- and nanoemulsions</td>
<td>6, 89</td>
</tr>
<tr>
<td>Modulation of bacteria metabolism</td>
<td>Delay of acidification in rice beverages and laboratory media</td>
<td>11, 114</td>
</tr>
<tr>
<td>Effect on biofilm</td>
<td>Removal of biofilm on equipment and enhancement of biofilm formation by probiotic bacteria</td>
<td>17, 53</td>
</tr>
<tr>
<td>Other applications</td>
<td>Defoaming, deaeration, filtration, pickling, drying, defrosting, fractionation, wine aging, and extrusion</td>
<td>6, 34, 89</td>
</tr>
</tbody>
</table>

removal of microorganisms from a surface (cleaning effect). Apart from the destructive action, US can modulate or enhance biofilm formation and bacterial metabolism or cause desirable changes in the physical and chemical structure of matrices (defoaming, deaeration, filtration, pickling, drying, defrosting, fractionation, accelerated wine aging, and extrusion). Tables 1 and 2 offer some details about the various applications; in the following pages, there is a description of US for microbial inactivation and for the modulation of metabolism or biofilm formation.

US in biological systems. According to Sango et al. (116), the possibility of using US for FP is linked to two main phenomena, generally referred as “acoustic cavitation” and “acoustic streaming.” In a liquid medium, the acoustic waves create areas of compression and expansion because of bubble formation. Bubbles, in fact, grow until they become instable and implode, thereby increasing temperature (up to 5,000°C) and pressure (1,000 atm) (95, 103, 116). The acoustic streaming is a consequence of acoustic cavitation and relies upon the dissipation of the energy of acoustic vibration through shear energy and turbulence.

A secondary mode of action is linked to the formation of reactive species and free radicals, which can act on DNA (116, 137). Wu et al. (144) described the mode of action of US on yeasts by focusing on the release of proteins and polysaccharides and suggested a two-step mechanism as follows: US acts upon the cell wall without damaging cell membrane; however, prolonged US treatments disrupt the cell membrane and contribute to the release of a burst of intracellular proteins. There are at least four effects, which include morphological changes of cells, the thinning or disruption of cell membranes, the weakening of spore coats via cavitation, and a possible effect on DNA, as a result of free radicals (58, 59, 116, 137).

This two-step mechanism may also be suggested for bacteria; however, for these microorganisms, cellular membranes and capsules could have a significant role, as postulated by the models of Gao et al. (58, 59) and Krasovitski et al. (76). Krasovitski et al. (76) suggested that the membrane could be intrinsically able to adsorb mechanical energy from US and to transform it into expansions and contractions in the intramembrane space.

Gao et al. (58, 59) pointed out the role of the capsule, which is soft and can dampen the mechanical effects of

TABLE 2. Applications of US for some foods

<table>
<thead>
<tr>
<th>Food</th>
<th>Application (references)</th>
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<tbody>
<tr>
<td>Milk and dairy beverages, rehydrated powder milk</td>
<td>Inactivation of pathogens (milk, rehydrated powdered milk, infant formula, skim milk, and red-grape) (5, 100, 116)</td>
</tr>
<tr>
<td></td>
<td>Homogenization of dairy beverages (probiotic milk, chocolate milk, whey-grape juice drink) (137)</td>
</tr>
<tr>
<td>Juices and other beverages</td>
<td>Inactivation of spoiling microorganisms and pathogens (orange, apple, pineapple, mango, red-fruits, and mulberry) (5)</td>
</tr>
<tr>
<td></td>
<td>Inactivation of enzymes (100)</td>
</tr>
<tr>
<td>Vegetables</td>
<td>Inactivation of pathogens in fresh lettuce, cherry tomatoes, iceberg lettuce, celery, and fresh pepper (5)</td>
</tr>
<tr>
<td>Meat</td>
<td>Improvement of meat tenderness (decrease of Warner-Bratzler force, increase of proteolysis, and improved disruption of myofibrillar structures) (21, 35, 150)</td>
</tr>
<tr>
<td>Fish and shellfish</td>
<td>Assisted extraction and fast determination of arsenic, selenium, vanadium, and nickel in fish and shellfish (91)</td>
</tr>
<tr>
<td>Table olives</td>
<td>Enhancement of debittering in NaOH-free table olives (62)</td>
</tr>
</tbody>
</table>

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cavitation. For this latter model, the ability of the capsule to dampen the lethal effects of US depends on some key factors, such as shear forces, US power, and the level of the target microorganism.

Recently, Ojha et al. (98) described the lethal effects of US and postulated the existence of a unifying mechanism, called sonoporation (the formation of transient cavities or pores on cell membranes from cavitation). Sonoporation can be the result of at least six different mechanisms, and the lethal effects could be caused by one or more of them. The mechanisms can be described as follows:

1. **Cavitation**: Bubbles increase in size and implode near the cell membrane.
2. **Push**: Increasing bubbles touch and push the cell membrane.
3. **Pull**: Bubbles pull the membrane during the compression phase, thus disrupting it.
4. **Jetting**: Bubbles experience an asymmetric collapse and create a funnel-shaped jet.
5. **Streaming**: The stream of fluid around the oscillating bubbles create a shearing effect on the membrane, leading to an injury or rupture.
6. **Translation**: High-intensity US radiation forces diffuse across the membrane, and during this phase, bubbles lose their shells while passing through the cell membrane.

**US for FP: factors, foods, and microorganisms.** The use of US as a suitable FP approach has been proposed and reviewed in the past by many authors. Although the treatments found in the literature are not equivalent, it is possible to point out some variables that have a major role in the effectiveness of the treatment:

- **Energy**: Several studies highlighted the significance of power and the length of time for US treatment, but they also stressed the importance of the interaction between those variables, which means that the leading variable for the antimicrobial effect was the total energy dissipated into the system (18–20, 34, 58, 59, 72, 136). A leading variable in this field is heat; the collapse of bubbles, in fact, generates local heating (up to 5,000°C), which significantly contributes to microbial inactivation (137). The roles of energy and heat suggest the need for further experiments to design treatments that combine power and duration, without the adverse effect of an uncontrolled increase in temperature, which in turn, could contribute to negative effects on sensory and chemical traits. Such a perspective could be the design for new equipment with “on-line” control of temperature.

- **Temperature**: Temperature affects the antimicrobial action of US, which is the basis for designing combined treatments, also known as thermosonication (2, 4). The enhancement is the result of two different actions: temperature exerting an antimicrobial effect per se and enhanced cavitation and sonoporation of cells.

- **Kinds of microorganisms and size of cells**: Feng et al. (54) and Kentish and Feng (74) reviewed the D-values of different microorganisms for US treatment and proposed a resistance hit as follows: spores > fungi (molds) > yeasts > gram-positive bacteria > gram-negative bacteria, which makes the bacterial spores the most resistant targets and the gram-negative bacteria the most susceptible. That resistance hit relies on different factors, such as the size and shape of the cell and the morphology of its external layers. The size is probably the leading factor for the different trends between fungi and bacteria because larger cells are generally more sensible because the surface exposed to US is greater (34). Chemat et al. (34) also reported a significant effect of shape, showing that cocci are more resistant than bacilli because of the relationship between cell surface and volume.

On the other hand, there is a debate on the different susceptibility of gram-positive and gram-negative bacteria. Some authors reported that gram-positive microorganisms are more resistant than gram-negative ones are because of their thicker cell wall, which provides a protection against US effects (34, 51). However, other studies reported no significant differences between these groups of bacteria (69, 116). There are also significant differences depending on the strain and the species; Bevilacqua et al. (20) reported that *Pseudomonas putida* and *Salmonella* sp. were the most resistant gram-negative microorganisms, whereas *Escherichia coli* was the most sensitive. *Staphylococcus aureus* was the most resistant target among gram-positive bacteria, whereas lactic acid bacteria and bifidobacteria showed an intermediate trend. Bevilacqua et al. (19) also found a pronounced strain or species dependence on US effect for yeasts. They studied the antiyeast effects (for *Saccharomyces cerevisiae, Candida norvegica, Pichia membranifaciens, Wickerhamomyces anomalus, Zygosaccharomyces bailii*, and *Zygosaccharomyces rouxi*) of this treatment in different kinds of juices (orange, apple, pineapple, strawberry, and red-fruit). *W. anomalus* was the most resistant target, followed by *S. cerevisiae* and *Z. rouxi*, and finally, by *P. membranifaciens* and *Z. bailii*; however, the different resistance abilities were not related to cell shape because similar yeasts showed different behaviors. The difference in the external layers between gram-positive and gram-negative bacteria probably affects the way US acts on cells. Iorio et al. (69) studied the inactivation of *E. coli* O157:H7 and *Listeria monocytogenes* in almond milk and found that the treatment exerted different effects on the growth kinetics of the pathogens, i.e., the prolongation of the lag phase in *L. monocytogenes* and a decrease of the growth rate in *E. coli*. The prolongation of the lag phase was a sign of a stronger, sublethal injury on *L. monocytogenes*, with an impairment of growth, and the microorganism required time to adapt to the environment and, probably, to restore its metabolism. Once the metabolism was restored, the microorganisms fully restored the enzymatic pool; thus, the difference in the growth rate was not significant. In contrast, with *E. coli* the effect was less because the microorganism did not experience a lag phase; however, the targets of US were not completely restored because the growth rate was significantly less. Few data are available on viruses and
protozoa, although some authors have reported the potentialities of US (1, 34, 117).

- **Initial cell number:** Few data are available on this topic, although some results are available in Gao et al. (59). They found that the resistance of Enterobacter aerogenes and Aureobasidium pullulans increased when the initial cell number increased because of an enhanced aggregation ratio at higher concentrations.

- **Pulse:** Pulse is processing parameter that is able to affect the antimicrobial effect of US. In fact, sonic waves can be applied either continuously or discontinuously through pulses (64); however, the role of this factor is a matter of debate. Hashemi (64) studied both a continuous and a pulsed treatment to prolong the shelf life of Mirabelle plum and found that the kind of treatment did not affect the extent of the antimicrobial effect; however, pulsed treatments resulted in better preservation of some chemicophysical traits (solubility of solids, amount of ascorbate, color, and texture). Hashemi et al. (65) studied the effects of sonication, alone or combined with other treatments, to inactivate L. monocytogenes, Shigella sonnei, Byssoschlamys fulva, and Saccharomyces cerevisiae. They found a possible role for pulses, although the weight of that parameter was less than that found for other variables. Similar results were found by Bevilacqua et al. (18), who studied the effects of US on the spores of Fusarium oxysporum and developed a mathematical model describing the quantitative effects of power, duration of treatment, and pulse. Thus, they found that pulse was not significant as a single term but was when in interaction with other variables. Those results, along with other data (72) and evidence reported above suggest that pulse could indirectly affect the antimicrobial effects of US because it is connected to the total energy.

- **Agitation:** Some indirect evidences suggest that agitation could have a significant role. Agitation could be used to improve the efficiency of sonication as an extraction procedure (41); in addition, it could exert a role in the inactivation of pathogens (3, 88), probably because of the mixing effect of sonication, the better dissolution of antimicrobials, and the increased surface exposed to cavitation. It is not known the quantitative link between agitation and effect and whether agitation is a secondary or primary effect.

**Modulation of metabolism and biofilm formation.** US can exert a dual effect on microorganisms: a lethal effect or the stimulation of growth, depending on the intensity and the frequency. That dual effect was described and reviewed for biofilm by Erriu et al. (53). As reported above, high-intensity US impairs the membranes and causes a strong loss of viability (112), whereas low-intensity US stimulates bacterial metabolism and increases the transport of oxygen and nutrients to deeper layers of the biofilm (53, 109). The positive effect of US on biofilm formation was confirmed by Bevilacqua et al. (17); they studied the effect of US on biofilm formation by Acidipropionibacterium jensenii and Propionibacterium freudenreichii and found that the treatment improved the stability of the biofilm and counteracted the detachment of cells from surfaces. An increase in biofilm formation and stability was also found for Lactobacillus reuteri, which showed a 10-fold greater adhesion to Caco-2 cells after US treatment (114).

The positive effect on biofilm formation and adhesion could be linked to the increase of hydrophobicity (17, 114) and membrane permeability (17). US increased the hydrophobicity of L. reuteri from 3 to 25% and from 15 to 18% to 30 to 35% for propionibacteria. That effect could be related to the increased adhesion because hydrophobicity is linked to the first stage of probiotic adhesion in the gut (adhesion to mucus) (45), and promotes the second stage of adhesion to specific cell-wall components of mucosa (63). A secondary mechanism related to adhesion was reported by Tabatabaei and Mortazavi (128); they described a clumping-like effect (an increase in autoaggregation) in Lactobacillus acidophilus, Lactobacillus casei, and Lactococcus lactis subsp. cremoris after US exposure.

The effect of permeability was suggested by Bevilacqua et al. (17), who found an increased release of nucleic acids and proteins after US exposure.

Another interesting application of US is attenuation, which is a method to delay acidification and counteract postacidification in active drinks containing probiotic microorganisms (11, 17, 114). This treatment does not affect the viability or functional characteristics of the probiotic microorganisms and could be performed using different methods (such as homogenization, US, heat-treatment, or freezing). US at a power of 50 to 80 W was successfully applied to delay acidification of L. reuteri, L. plantarum, L. casei, bifidobacteria, and propionibacteria, both in an organic rice beverage and in laboratory media (11, 17, 114).

**Equipment and energy requirements.** US laboratory devices include horn and bath types (68). In a US horn, a titanium cylinder contains the transducer, which results in a 100-µm vibration amplitude and high intensity immediately below the probe, but the intensity drastically decreases away from the device. This device can be used for small volumes and to treat microbial suspensions (11, 17, 20, 114). In contrast, a US bath uses a diffuse acoustic energy, and there are several transducers distributed around the base; this system, however, provides less intensity (74).

Horn-type devices can be also used for industrial applications (74); however, additional or modified tools, such as radial, ring, and/or radial arrays of transducers (94, 147, 149) improve the efficiency of the treatment. Some industrial devices can now process up to 1,000-L volumes (100).

The energy inputs for US are a function of different variables, such as its power, the design of the equipment, and the duration of the treatment and its pulse. Generally, US is generated by electric energy supplied to a piezoelectric material (transducer), which converts the input energy into mechanical vibrations. The amount of energy dissipated can be estimated through the following formula:
Energy dissipated = $k \times f^2 \times A^2$

where $k$ is a constant, $A$ is the amplitude, and $f$ is the frequency of the wave.

Unfortunately, a fraction of energy is lost as heat, thus reducing the efficiency of the treatment.

There is no consensus on the energy requirements for US because the data available in the literature are produced through devices with different designs and energy inputs. The energy requirements for US were reviewed by Sango et al. (116), who collected data from different articles. For a treatment of milk at 90 μm for 9 min at 60°C (an assisted US treatment), the energy required was 0.19 J/mL (67); in contrast, conventional pasteurization of juice requires 35 J/mL and a pulsed-electric field treatment requires 100 to 400 J/mL (8, 61, 75, 119).

The results from Sulaiman et al. (123) do not confirm the idea of US as a lower-input treatment because they found that a US treatment (24 kHz, 1.3 W/g, 33°C) required an energy input of 1,233 kJ/kg, whereas a thermal treatment at 65°C for 15 min required an input of 240 kJ/kg. Therefore, further effort is required to find an effective balance between the energy needs of US and those of equivalent thermal treatments or other approaches.

**HIGH PRESSURE HOMOGENIZATION**

**Definition and effects on microorganisms.** Traditionally, the word homogenization refers to the ability to produce particles with a uniform-size distribution by forcing the liquid through a disruption valve (49, 105, 148). The inventor of this process was Gaulin in 1900; since then, homogenization has been introduced in food industry for different purposes (such as preparation and stabilization of emulsions, creation of physical changes, and cell disruption) (46).

Currently, the terms high pressure homogenization or high dynamic pressure are used to designate a dynamic process working with fluids for short processing times and at pressure less than high hydrostatic pressure (49, 131, 134). Pressure levels are from 20 to 200 MPa (for conventional treatments) and up to 400 MPa for ultrahigh pressure homogenization.

The geometry of a homogenizer is very simple, mainly consisting of a pump and a homogenizing valve (42, 85, 97). The pump forces the fluid into a small, narrow gap between the valve seat and the piston or pump (42). The input pressure of the fluid is very high and is controlled by adjusting the opening of the homogenizing valve; it leaves the gap in the form of a radial jet that stagnates on the ring. The shift from high (input) to very low (exit) pressures is responsible for the physical and antimicrobial effects of HPH as well as some of its physical phenomena (such as cavitation, turbulence, shear forces, and micronization) (46, 49, 103).

Diels and Michiels (46) described the basic phenomena responsible for cell disruption and microbial inactivation and highlighted at least four possible mechanisms:

1. Rapid pressure drops near the entrance and the rate of pressure drop (23), which could be responsible for a phenomenon similar to the explosion of microbial cells.
2. Turbulence and effect on valves and rings, which are responsible for the mechanical injuries of cells (46).
3. Velocity fluctuations; Doulah et al. (50) suggested that when the kinetic energy of oscillatory movements exceeds the strength of the cell wall, the result is the disruption of microorganisms.
4. Cavitation and shock waves (118), which produce a cavity collapse similar to that described for US.

The extent of the antimicrobial action of HPH depends on several factors, such as its microbial and physiological parameters (such as the kinds of microorganisms, the cell concentrations, the physiological states, and whether cells or spores are being affected), its process parameters (such as the pressure, the number of passes, and the temperature increase), and the characteristics of the fluids (such as its viscosity, any additives, and the composition of the matrix) (46).

The first factor that has a major role in resistance to HPH is the surface: the larger the surface, the greater the microorganism’s sensitivity. Therefore, yeasts are more susceptible than bacteria (14, 15, 46). For bacteria, it is debated whether there is a difference between gram-positive and gram-negative microorganisms. Kelemen and Sharpe (73), Vachon et al. (135), and Wuytack et al. (146) found greater resistance from gram-positive microorganisms and suggested a protective role for peptidoglycan. Bevilacqua et al. (15) confirmed the lower resistance of gram-negative bacteria, with pseudomonads and enterobacteria being highly susceptible.

Among the gram-positive microorganisms, lactic acid bacteria possess intrinsic resistance and require multiple-pass treatments to be inactivated (14, 15, 79, 80, 104, 106). The same trend was found for bifidobacteria (15). The effect of HPH on bacteria and yeasts is mainly linked to its mechanical burden from spatial pressure, impingement, and cavitation (46).

**Antibacterial and antifungal effects.** Generally, HPH is used to inactivate Yersinia enterocolitica, E. coli, Salmonella sp., L. monocytogenes, S. aureus, Clostridium perfringens, Alicyclobacillus acidoterrestris, Geobacillus stearothermophilus, Pseudomonas fluorescens and P. putida, Lactobacillus spp., and Bifidobacterium spp. (28, 36, 46, 57, 87, 105, 113, 121).

HPH was also tested on molds (Aspergillus, Fusarium, Penicillium, and Mucor species) and showed promising bioactivity trends in both model and real systems (10, 26, 39). Fungal spores are highly resistant to the treatment and require multiple-pass treatments at 120 or 150 MPa (26, 39). Corbo et al. (39) also found a discoloration of the mycelium of Penicillium sp. because of sublethal injury on the surviving spores. The color of mycelium is generally the result of the production of conidia over the border of the colony because the wall of the conidia of different molds introduces a characteristic pigmentation (green in Penicil-
Therefore, the discoloration of mold could be the sign of the stress on the target because the microorganism seems to lose the ability to produce conidia or, simply, pigmented conidia.

Figure 2 proposes a simplified model of the antimicrobial effect of HPH. Each microorganism has a unique trend. For resistance hits, spores (fungal and bacterial) are the most resistant targets and have greater resistance than vegetative forms have. In addition, bacteria are more resistant than yeasts. Figure 2 also reports some key findings for spores (the effect on bacterial and fungal spores), as well as the effect on bacteria and the possibility of resistance hits (gram-positive bacteria are more resistant, and among them, lactic acid bacteria possess intrinsic resistance). Finally, Figure 2 proposes an overview of the factors exerting antimicrobial action, as detailed above.

**Effect on bacterial spores.** Bacterial spores are generally resistant to homogenization (12, 87, 121), and complete inactivation can be achieved only at pressures ranging between 300 and 350 MPa (60).

Lopes et al. (87) described a three-step model for the inactivation of bacterial spores by HPH. Homogenization induces the release of dipicolinic acid (DPA) and ions, as suggested by Chaves-López et al. (33). Because of that release, the core is hydrated, and the spores germinate and are inactivated. The mechanisms for spore germination can be different, depending on the pressure. At low pressures (100 to 200 MPa), nutrient receptors appear activated and thus contribute to spore hydration (120). When higher pressure levels are applied (up to 500 MPa), there is a nonphysiological opening of the Ca-DPA channels, with the release of DPA triggering germination and a subsequent loss of pressure and heat resistance (87, 99).

Sevenich and Mathys (121) proposed a model for germination induced by pressure in *Bacillus subtilis* and *Clostridium perfringens*. In *B. subtilis*, pressure induces the activation of some key receptors (i.e., GerAA-AB-AC, GerBA-BB-BC, and GerKA-KB-KC), which are, in turn, responsible for the activation of GerD and ions, followed by Ca-DPA release. After DPA release, there is a cascade of events (i.e., core hydration, cortex deformation, and hydrolysis), activation of other receptors (i.e., CwIJ and SleB), and spore germination. In *C. perfringens*, the receptors GerKA-KC, Ger AA, and GerKB are activated, which could directly induce germination (or induce it via core hydration), followed by cortex deformation and hydrolysis.

**HPH for positive modulation of probiotic and functional traits.** HPH can also cause positive changes and modulate the metabolic activity of microorganisms (11, 105). Lanciotti et al. (79, 80) reported that sublethal HPH treatment controlled the fermentation kinetics of bacterial strains used as starters and modified their metabolic profiles, leading to products with enhanced sensorial properties. In addition, HPH improved the viability of *L. acidophilus* and *Lactobacillus paracasei* in Caciotta cheese. Tabanelli et al. (127) confirmed the positive effect of HPH on the sensory profile and found modulation of volatile compounds produced by two probiotic strains.

In addition, HPH at low pressures can positively affect the probiotic properties, such as hydrophobicity, of some microorganisms, increase the resistance to acid and bile conditions throughout the transit to the duodenum, and
## TABLE 3. Comparative resistance of pathogens (4-D reduction in the viable count) and examples of US and HPH applications in food

<table>
<thead>
<tr>
<th>US Reference</th>
<th>HPH Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Salmonella</strong></td>
<td></td>
</tr>
<tr>
<td>Typhimurium</td>
<td></td>
</tr>
<tr>
<td>• Horn type-285W/20-25 kHz for 6 min</td>
<td></td>
</tr>
<tr>
<td>• Carrot juice</td>
<td></td>
</tr>
<tr>
<td>84</td>
<td>Senftberg</td>
</tr>
<tr>
<td>Enteritidis</td>
<td></td>
</tr>
<tr>
<td>• Horn type-104W/20 kHz for 30 min</td>
<td></td>
</tr>
<tr>
<td>• Liquid whole egg</td>
<td></td>
</tr>
<tr>
<td>• The reduction was 2.3 log CFU/mL</td>
<td></td>
</tr>
<tr>
<td>129</td>
<td>Enteritidis</td>
</tr>
<tr>
<td>S. enterica</td>
<td></td>
</tr>
<tr>
<td>• Horn type-78-104W/20 kHz for 6 min</td>
<td></td>
</tr>
<tr>
<td>• Rice beverage</td>
<td></td>
</tr>
<tr>
<td>27</td>
<td>S. enterica</td>
</tr>
<tr>
<td><strong>Escherichia coli</strong></td>
<td></td>
</tr>
<tr>
<td>K-12</td>
<td></td>
</tr>
<tr>
<td>• Probe (20 kHz, 70 W) for 16–29 min</td>
<td></td>
</tr>
<tr>
<td>• Laboratory media</td>
<td></td>
</tr>
<tr>
<td>• Combination of ultrasonic probe (20 kHz, 70 W) and a water bath (33 kHz, 96 W) for 19–26 min</td>
<td></td>
</tr>
<tr>
<td>• Laboratory media</td>
<td></td>
</tr>
<tr>
<td>68</td>
<td>K-12</td>
</tr>
<tr>
<td>O157:H7</td>
<td></td>
</tr>
<tr>
<td>• Bench-top equipment (40 kHz, 400 W/L); time &gt; 5 min</td>
<td></td>
</tr>
<tr>
<td>• Sprouts</td>
<td></td>
</tr>
<tr>
<td>• Treatment was combined with fumaric acid and electrolyzed water</td>
<td></td>
</tr>
<tr>
<td>97</td>
<td>E. coli</td>
</tr>
<tr>
<td><strong>Listeria spp.</strong></td>
<td></td>
</tr>
<tr>
<td>L. monocytogenes</td>
<td></td>
</tr>
<tr>
<td>• Bench-top equipment (40 kHz, 400 W/L); time &gt; 5 min</td>
<td></td>
</tr>
<tr>
<td>• Sprouts</td>
<td></td>
</tr>
<tr>
<td>• Treatment was combined with fumaric acid and electrolyzed water</td>
<td></td>
</tr>
<tr>
<td>97</td>
<td>L. monocytogenes</td>
</tr>
<tr>
<td>L. innocua</td>
<td></td>
</tr>
<tr>
<td>• Combination of ultrasonic probe (20 kHz, 70 W) and a water bath (33 kHz, 96 W) for 39 min</td>
<td></td>
</tr>
<tr>
<td>• Laboratory media</td>
<td></td>
</tr>
<tr>
<td>• Ultrasonic batch, 600 W for 10 min</td>
<td></td>
</tr>
<tr>
<td>• Laboratory media</td>
<td></td>
</tr>
<tr>
<td>68</td>
<td>L. innocua</td>
</tr>
<tr>
<td>• Probe, 20 KHz, 500 W for 8–20 min</td>
<td></td>
</tr>
<tr>
<td>• Water</td>
<td></td>
</tr>
<tr>
<td>• The treatment was combined with ZnO (40 mM)</td>
<td></td>
</tr>
<tr>
<td>47</td>
<td>L. innocua</td>
</tr>
<tr>
<td><strong>Staphylococcus aureus</strong></td>
<td></td>
</tr>
<tr>
<td>• Probe, 20 kHz, 600 W</td>
<td></td>
</tr>
<tr>
<td>• Saline solution</td>
<td></td>
</tr>
<tr>
<td>• Treatment was combined with electrolyzed water</td>
<td></td>
</tr>
<tr>
<td>82</td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td><strong>Ultra-high pressure homogenizer, 300 MPa</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Continuous HPH with a ball valve, 4 cycles at 100 MPa</strong></td>
<td></td>
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<tr>
<td><strong>Liquid whole egg</strong></td>
<td></td>
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<tr>
<td><strong>Egg white</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Ultra-high pressure homogenizer, 4 cycles at 150 MPa</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Egg white</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Stansted type valve, 200/210 MPa</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Whole milk (pathogen reduced by 2 log CFU/mL) and laboratory medium (reduction of 5 log CFU/mL)</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Ultrahigh pressure homogenizer, 300 MPa</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Orange and grape juice</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Bench-top high-pressure homogenizer, 250 MPa</strong></td>
<td></td>
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<tr>
<td><strong>Apple and carrot juices</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Bench-top HPH, 250 MPa</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Apple and carrot juices</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Stansted type valve, 200/210 MPa</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Whole milk (pathogen reduced by 2 log CFU/mL) and laboratory medium (reduction of 5 log CFU/mL)</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Ultrahigh pressure homogenizer; treatments: 1 cycle/230 MPa; 4 cycles/150 MPa</strong></td>
<td></td>
</tr>
</tbody>
</table>
increase the immunomodulation as well as the enzymatic patterns (24, 96, 124–126). These effects could be attributed to modifications in the outermost cellular structure (127), such as the reduction of the level of cyclic fatty acids and the increase in the degree of unsaturation (125).

Bevilacqua et al. (11) proposed HPH (multiple passes at 100 MPa) as a tool for attenuation to counteract the acidification of promising probiotic strains in an organic rice drink and to avoid the postacidification of the product throughout storage. The treatment, in fact, delayed acidification by 4 days and reduced the maximum extent of the acidification.

Effects on proteins. HPH significantly affects the structure and the bioactivity of enzymes (25, 49), and there are at least two variables, responsible of this change:

1. Pressure: Molecular structure and chemical bonds are strongly influenced by high pressure; the changes are reversible at 100 MPa and irreversible at 200 MPa. The changes are mainly related to significant alterations in the tertiary structure (49). The most important change caused by HPH is the unfolding of proteins: native monomers, dimers, and protein aggregates are shifted to unfolded molecules. Additional changes reported in the literature are the creation of monomers from dimers and, to a lesser extent, the formation of aggregates.

2. Cavitation: Cavitation and related phenomena (turbulence and shear forces) create strong variations in pressure and temperature, as well as intense turbulent eddies or fluid flows over a surface. Above a speed break point, intense, small vortices are formed that cause a disordered movement of particles, thus reducing their size (46, 49).

The effect of HPH on enzymes depends on the source of the enzymes and the processing parameters of the homogenization (pressure, temperature, and number of passes) (49). Proteases and lysozymes were positively affected by the treatment because some authors reported an increase of their activity. Vannini et al. (138) and Iucci et al. (70) studied the bioactivity of lysozyme from chicken eggs combined with HPH up to 130 MPa, against E. coli, Pseudomonas fragi, Salmonella Enteritidis, and L. monocytogenes. Thus, they found an increase in the bioactivity of lysozyme and suggested that the enhancement was the result of a partial unfolding of the enzyme and the formation of a hydrophobic pocket.

Tribst et al. (133) found a controversial effect on a commercial protease. They tested the bioactivity of an enzyme after a treatment at 20 and at 60°C and found a positive effect at 20°C (increased by 20%) and a reduction (60%) of enzyme activity at 60°C. According to the authors, HPH could affect the exposure of the active sites of enzymes, thus improving the interaction enzyme and substrate at nonoptimal temperatures. Other enzymes positively affected by HPH were amyloglucosidase (increased by 5 to 80% at 0 to 200 MPa, with a pH ranging from 2.9 to 6.5) and glucose oxidase (activity increased by 25 to 400%) (132).
Other authors reported a partial or complete inactivation for other enzymes, such as proteases from *P. fluorescens* (110), pectin methyl-esterase (29, 77, 139), papain (86), trypsin (85), alkaline phosphatase (42), lactoperoxidase (42, 66, 81), lipase (42), and polyphenoloxidase (108). Few enzymes were found resistant or not affected by HPH, such as trypsin (82, 83) and a protease from *Rhizomucor miehei* (81).

The effects on proteins and food structure, combined with the cavitation and shear forces, are the requisites for use of HPH in the production of emulsions (148) and other applications. For example, a frontier method used HPH at 30 to 80°C to produce starch nanocapsules by mixing a starch slurry and an aqueous solution (143).

**Equipment, devices, and energy requirements.** The design of the homogenization valve is the core of HPH equipment. Basically, HPH valves are designed in such a way that the flow passes through a narrow gap between the valve rod and the seat, which accelerates the fluid velocity (85). For microbiological purposes, Engler (52) described at least six valve configurations (standard, cell rupture, grooved, knife edge, conical, and ball cell disruption). Currently, there are hundreds of patents for homogenization valves (93); however, those valves can be grouped into three main classes:

- **Counter-jet microchannel:** The stream is divided into two channels, where the rate of the fluid experiences a 10-fold increase. Finally, the two streams are forced to impinge in the interaction chamber. The maximum pressure and flow rate are, respectively, 200 MPa and 1,000 L/h. The main benefit of this equipment is that it does not contain movable parts.

- **Radial diffuser annular:** This valve is composed of an axial face and a mobile seat. The pressure is controlled by adjusting the slit width (from 15 to 75 μm) and the upstream rate. The maximum pressure and flow rate are, respectively, 200 MPa and 5,000 L/h; a possible drawback is the temperature increase (20°C for 100 MPa for water).

- **Axial flow through an orifice valve:** The fluid enters the valve axially and the diameter of the inlet pipe experiences a threefold reduction and an increase in fluid rate. Because of the rate increase, the fluid collides with the needle and the valve seat. The maximum pressure and flow rate are, respectively, 200 MPa and 90 L/h; the drawback is the temperature increase.

Few data are available on the energy requirements of HPH. A possible drawback for HPH is the loss of a significant portion of energy as heat. Cortés-Muñoz et al. (40) reported that in a ultrahigh pressure homogenization 41 to 63% of the energy was lost as heat.

That drawback could be counteracted with a new equipment design, as proposed by Donsi et al. (48), combining the classical design (pump and valve) with a heat exchanger to reduce the temperature of the fluid after treatment.

**COMPARATIVE RESISTANCE OF SPOILERS AND PATHOGENS TO US AND HPH APPLICATIONS IN FOODS**

The International Commission on Microbiological Specifications for Foods proposed a scheme for managing microbial risks and introduced the concept of a food safety objective (37). For FP, a food safety objective could be the extent of the microbial reduction required to ensure an appropriate level of protection. Although the equipment and the tools proposed in the literature for US and HPH are different and use different levels of energy, the best criterion to assess the performances of these methods in foods requires a brief overview of the conditions needed to achieve a target level of spoiler and pathogen inactivation.

There is no universal criterion, useful for all microorganisms and foods; however, some targets or desired reduction levels can be chosen based on literature reports. For many foods, a 4-log reduction was proposed as a criterion for NTTs in the past (31).

As a basic criterion, the focus was on some target microorganisms (four pathogens: *L. monocytogenes*, *S. aureus*, *E. coli*, and *Salmonella* spp.; and spoilers: *Pseudomonas* spp. and yeasts) and on the results published in the past 5 to 6 years. Tables 3 and 4 provide a synopsis of important data found in the literature. The choice of the articles to include in the tables relied upon the following key factors: (i) application in food matrices and/or in conditions simulating an effective FP; and (ii) a focus on results with comparable tools and equipment.

The analysis of the available literature suggests some key points:

1. There are some foods in which both technologies were successfully applied, i.e., juices and milk, and assured an appropriate level of protection with a reduction of at least 4-D in pathogens. HPH was also used in liquid egg products.

2. Generally, the most used equipment for US was a probe-based device working at 20 to 25 kHz (for some applications, at 40 kHz); the input power varied (from 20 to 30 W to 600 W or more). Concerning HPH, two tools were used worldwide: equipment with a ball valve and a Stansted-type device (Stansted Fluid Power Products, Harlow, UK). Another important variable is the highest level of pressure (P_max) because, for some applications, the classical homogenizers were used (P_max 200 MPa), whereas for some foods, a new generation of homogenizers was tested (ultrahigh pressure homogenization or ultrahigh pressure homogenizers).

3. US and HPH did not exert negative effects on the sensory scores, and many times contributed to a better bioavailability of some ingredients.

4. The effect on bacterial spores was less studied, as well as the effect on fungal spores, because of their intrinsic resistance, requiring the use of stronger operating conditions or the design of combined approaches.
TABLE 4. Comparative resistance of *Pseudomonas* spp. and yeasts (4-D reduction in the viable count) and examples of US and HPP applications in food

<table>
<thead>
<tr>
<th>US</th>
<th>Reference(s)</th>
<th>HPP</th>
<th>Reference</th>
</tr>
</thead>
</table>
| *P. fluorescens* | • Bench-top ultrasonic equipment, 400 W/L for 5 min
  • Fresh-cut kale
  • Treatment combined with thermal processing; the microorganisms was reduced by 3 log CFU/mL | 90
  • *P. fluorescens* with a ball valve 250 MPa
  • Skim milk | 111 |
| *P. putida* | • Probe, 20 kHz, 100 W
  • The pathogen was reduced by 2 log CFU/mL in water, whereas in milk, the treatment exerted a bacteriostatic effect | 20
  • Protease by *P. fluorescens* | 44 |
| *Saccharomyces cerevisiae* | • Probe, 20 kHz, 600 W for 10–30 min
  • Apple juice
  • US alone reduced the yeast by 2 log CFU/mL, whereas the combination of US with pulsed light exerted a stronger effect (4-D reduction) | 55, 56
  • *S. cerevisiae* | 92 |
| *Saccharomyces bayanus* | • Probe, 2000 W, 20 kHz, 5–15 min
  • Citrate buffer
  • The medium was first heated to 57°C | 38
  • *S. bayanus* | 13 |
| *Pichia membranifaciens,*
  *Wickerhamomyces anomalus,*
  *Zygosaccharomyces bailii,* Z. rouxii,* and Candida norvegica* | • Probe, 20–80 W, 20 kHz, 2–6 min, pulse at 2–6 s
  • Strawberry, orange, apple, pineapple, and red-fruits | 19
  • *Z. bailii* | 107 |

For each microorganism, the first point reports the kind of equipment; the second, the matrix; and the third, some notes (if available).

5. Many authors studied the reduction in the viable count of pathogens and spoilers immediately after the treatment; however, some researchers stressed the importance of the induction of a viable, but not cultivable, state that could significantly affect both the safety and the shelf life (84). There are few data on the effect of the treatments during the shelf life of foods, and both US and HPP could ensure a shelf life comparable to pasteurized foods (days or weeks), but the records available on the most important databases (PubMed [National Center for Biotechnology Information, Bethesda, MD], Scopus [Elsevier, Amsterdam, the Netherlands], Web of Science [Clarivate Analytics, Philadelphia, PA]) do not include robust data on the possibility of using US or HPP as alternative strategies to sterilization. Data on laboratory media, buffers, and water are available,
but a challenge test in foods for long periods (6 months to 1 year) has not, to our knowledge, been created.

6. A problem for the use of HPH and US is that the experiments were performed on different species or strains; however, it is well known that strain dependence can greatly affect the outcomes of these treatments. In addition, the level of inoculum and the physiological state of cells are other factors able to significantly influence the results. Therefore, a robust approach of comparative resistance is not possible.

Future efforts are required to design standardized methods to determine (i) an indicator for strains and microorganisms for each kind of food, (ii) the resistance or susceptibility hit, and (iii) the conditions required to perform equivalent experiments worldwide.

The development of standardized guidelines is the primary requisite for an effective spread of these technologies at industrial level.

A FINAL OVERVIEW ON THE BENEFITS, LIMITATIONS, AND FUTURE EFFORTS

HPH and US are promising strategies and can be used for FP or other purposes (such as modulation of metabolism, modification of enzyme activity, cleaning, or modification of the rheology of a product). They can be used for liquid foods, although in the case of US, some recent publications and patents have proposed an upgrade for some solid foods (vegetable).

In conclusion, we provide some of the benefits of these two technologies and some of their possible limitations, which require further effort toward improving HPH and US in FP.

Benefits.
1. Safe processes in which pressure and temperature act synergistically on microbial inactivation.
2. Nutritional quality unaffected or enhanced. This topic was not addressed in this article; however, many authors report that both US and HPH do not affect some compounds, such as vitamins and phenols in a negative way (5, 71, 98). This topic could be a promising path toward designing friendly technology able to produce fresh-like foods.
3. The possibility of using these technologies for multiple purposes.

Limitations.
1. HPH and US do not guarantee sterilization (spore inactivation) or a shelf life as long as that of thermally treated products; therefore, it is necessary to combine a series of devices, plants, or approaches in a “system” that allows the sterilization.
2. They can be successfully applied only on liquid foods.
3. Energy use needs to be defined clearly.
4. High variability currently exists in the operating conditions used to assess the potential of US and HPH.
5. The shelf life of US and HPH treated foods is not comparable to that of sterilized foods.

These limitations are not drawbacks; they are issues to be addressed and to suggest possible paths toward future research efforts to:
1. Design effective treatments that are able to ensure longer shelf life (at least some months);
2. Evaluate the cost of treatments and the energy required to design and optimize environmentally friendly approaches;
3. Improve the approaches for using US on solid foods;
4. Define possible guidelines and international methods (e.g., indicator microorganisms, levels of inoculum, among others) needed to produce an effective possibility of comparing data produced in different countries; and
5. Spread information about one of the most important benefits of US and HPH: they are ambivalent technologies and so could act as bactericidal or bacteriostatic tools, but at the same time, they offer the possibility of improving and/or modifying the technological and the functional traits of probiotic microorganisms and starter cultures.

REFERENCES
33. Dolan, H. L., L. J. Bastarrachea, and R. V. Tikekar. 2018. Inactivation of *Listeria innocua* by combined treatment of low-


