International Journal of
Analytical and Applied Chemistry
ISSN: 2582-5933
Volume 8, Issue 2, 2022
DOI (Journal): 10.37628/IJAAC:

http://chemical.journalspub.info/index.php?journal=JAAC&page=index

Review IJAAC

Ultrasound-Microwave Technologies as Alternative Methods for Inactivation Bacterias in Fruit Juice

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Abstract

In the food industry, it is common practice to resort to methods that require maintaining high temperatures for just a limited amount of time in order to increase the product's quality while simultaneously lowering its risk of contamination. They provide a contribution toward the reduction of the potentially catastrophic impacts of heat deterioration. In fact, the undulating technologies are being employed for this purpose in increasing amounts; by using microwave heating, which is both speedy and efficient, the amount of time that is required to attain the necessary processing temperature may be greatly reduced. In addition to this, the impact that ultrasound has on microorganisms may also play a role in the achievement of a successful outcome during an operation. A significant advantage of using this treatment approach is the sanitization of fruit juice via microwave and ultrasonic therapy. In fact, when the microbiological activity of a number of different fruit juices is tracked over a considerable amount of time, an increased level of stability and a decreased rate of degradation are seen. In recent years, there has been a growth in the overall number of articles that are published in this sector. In this short study, sonication, microwaves, and a combination of the two are compared for their ability to kill the germs that cause juices and drinks to go bad.

Keywords: Ultrasound, microwave, juice, bacteria, inactivation

INTRODUCTION

Microorganisms, which include bacterias, yeasts, mold, algae, and protozoa, were the first life forms to emerge on Earth. These organisms are all-pervasive and inhabit every habitats. Despite the fact that they are necessary for both human and the environment, there are undesirable pathogenic types that must be treated and eradicated.

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Received Date: November 19, 2022
Accepted Date: November 25, 2022
Published Date: November 30, 2022

Citation: Ourdia-Nouara Kernou, Amine Belbahi, Ghania Kaanin-Boudraa, Khadidja Adel, Pr. Khodir Madani. A Review: Ultrasound-Microwave Technologies as Alternative Methods for Inactivation Bacterias in Fruit Juice. International Journal of Analytical and Applied Chemistry. 2022; 8(2): 31–40p.

Among novel microorganism destruction techniques are wave methods such as microwaves and ultrasounds. The latter are physical methods aimed at replacing chemical treatments, providing a better image of natural preservation. Many published studies have demonstrated the efficacy of these techniques in destroying microorganisms [1]. Indeed, these studies show that microwaves and ultrasound can cause a significant reduction in bacteria, even to the point of elimination. These techniques could be used to treat fruit juices, which offer favorable growth conditions not only for spoilage microorganisms, but also for foodborne pathogens. One of the innovative technologies to ensure the microbial inactivation in fruit juices while maintaining their quality and disinfecting the wastewater is ultrasound (US). It is believed to be more physically and chemically safe, environmentally friendly, and energy-efficient [2]. In addition to hydrodynamic effects (such as the microstreaming phenomena and intracellular cavitation), the method of action of ultrasound on microorganisms in liquids also includes the generation of radicals that destroy cellular structure [3]. However, as a method of preservation, ultrasonic technology alone cannot completely eradicate all bacteria. Additionally, excessive ultrasonic power may be detrimental to food's sensory and nutritional qualities [4]. Moreover, it has been reported that ultrasound treatments (at room temperature) alone may be ineffective in inactivating some bacterial species. To achieve a significant microbial reduction, a high power and a lengthy treatment time are required, resulting in high energy costs. Microwave (MW) heating for heat treatment has some advantages over conventional heat exchangers, including rapid volumetric heating, lower equipment surface temperatures, uniform heating, energy efficiency, and the potential to maintain food quality [5]. Numerous studies have shown how effective microwave radiation is at killing microorganisms in a variety of foods, especially liquid ones [6]. Indeed, the food industry favors HTST (High Temperature Short Time) process in order to reduce thermal degradation harmful to the quality of food while assuring the food safety of liquid food. Microwave technology is advantageous in this situation since it speeds up the heating process and significantly cuts down on the amount of time needed to attain the optimum processing temperature [7]. Woo, *et al.* [8] investigated how microwave radiation inactivated microbial cells and found that, in addition to inactivation, *Escherichia coli* cells were severely damaged on the surface while *Bacillus subtilis* cells were unaffected. Combination of various novel techniques demonstrated the ability to regulate enzymatic activities, the microbial load and improve the food safety of juices [9].

FRUIT JUICE

Typically, the thermal pasteurization procedure is used to extend the shelf life of food goods. Additionally, it is advantageous to stop the development of dangerous germs in certain beverages, such as fruit juices. High temperatures, however, may result in different nutritional and biochemical changes in the final product. The majority of consumers prefer to have juices extracted that have a fresh taste and minimize loss of nutrients. They also want to retain their quality through the addition of nutrients and antioxidants. This is beneficial for both the producers and the consumers as it increases the health marketing value of the product. Due to their potential to process fluids at much lower temperatures and apparent minimal impact on the sensory and nutritional qualities of fresh juices, the use of ultrasonics and microwaves as processing technologies has drawn a lot of attention as a viable alternative to using the traditional thermal processing. According to the current abundance of publications in this area, many researchers are very interested in their ability to maintain juice quality while increasing bioactive ingredients and shelf life by reducing spoilage microorganisms.

ULTRASOUND

Ultrasounds, in their basic definition, are pressure waves whose frequency is equal to or greater than 20 kHz, it devices typically operate at frequencies between 20 kHz and 10 MHz. Higher power ultrasound, sometimes known as “power ultrasound,” operates between 20 and 100 kHz and has the capacity to cause cavitation, which is used to render bacteria inactive [10]. However, the cavitation that results from pressure fluctuations brought about by the ultrasonic waves during the ultrasonic process is what kills the bacteria.

Mechanism of Microbial Inactivation Ultrasound

During an ultrasound procedure, which involves the creation and growth of micro gas bubbles, a phenomenon known as cavitation occurs. It can cause highshear forces and turbulence. Collapse of the gas bubbles also results in quick temperature shifts, greater than 5000 K, and pressure > 199 MPa on a microscale. Cavitation can also result in the segregation of water vapors from the surrounding air, which may lead to the creation of free radicals. These free radicals can then recombine to form hydrogen peroxide [11]. The effects of this phenomenon on various organisms can also cause damage to their cell structures. It can also inactivate certain enzymes [12]. The inactivity of enzymes is caused by the degradation of their biological polymers, this process can result in loss of enzyme activity. Dolas, Saravanan and Kaur [12] have characterized the main pathways of microbial inactivation by ultrasound (Figure 1), They explained the various mechanisms by which microbes inactivate by ultrasound. The impact waves generated by the collapsing bubbles and the breaking of the membrane's structures cause the perturbation of the cell's constituents. Free radicals are then formed, which damage the DNA of the microbe. During the process, the hydrogen peroxide produced by the reaction is also bacteriostatic and bactericidal. The application of heat and ultrasonics to inhibit the fungi was proposed. These methods can be performed at the same time and are capable of producing different results: (i) The release of various compounds (calcium-dipicolinic acid (DPA), fatty acids, acyl glycerol and glycolipids) after the treatment of fungi with ultrasound damage the exosporium, which then results in a reduction in resistance, and (ii) a subsequent thermal inactivation [13].

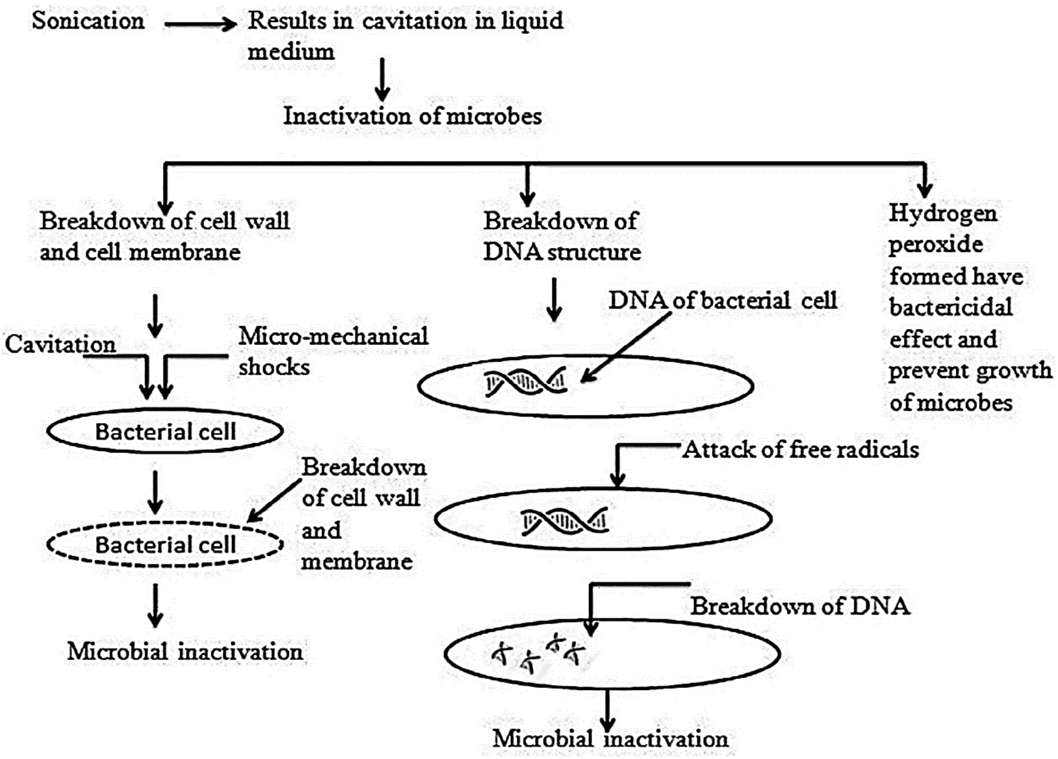

Figure 1. Ultrasound microbial inactivation mechanism [12].

Ultrasound is capable of inactivating bacteria and decayed bacterial clusters by a number of different physical, chemical and mechanical effects, it can eliminate surface contamination and biofilms [14]. The implosive production, development, and collapse of micro-and nano-bubbles in a fluid are caused by acoustic cavitations that are created when fluids are exposed to increased mechanical pressure waves. Due to the bubbles' large specific surface area, which boosts gas diffusion, strong local heating (about 5000°C), and high pressure are produced (1000 ATM) [15]. The lethality of ultrasound has also been reported for the reduction of several microorganisms.

Table 1 summarizes some of the impacts of ultrasound on the microbial cell.

Table 1. Some ultrasound effects on the micro-organism cell [10].

Ultrasound	Effects on bacterials cells
Cavitation	• -Thinning of bacterias • -Pores formation and disruption of cell membranes. • -Repture of cell wall structura release of cytoplsmic content
The destruction by free radicals	• -DNA repture and fragmentation

Several factors are involved in the ultrasound's effectiveness in destroying microorganisms, namely: *(a) Factors intrinsic to microorganisms:* It is commonly believed that sensitivity of some microorganisms increases with cell size. For instance, bacilli are more sensitive to different types of bacteria than they are to cocci. On the other hand, aerobic bacteria are more likely to be resistant to certain types of Gram-positive and Gram-negative bacteria [16]. (b) *Composition of the medium:* Pagan, *et al.* [17] investigated the effects of ultrasound (20 kHz, 200 kPa, 117 µm, 40°C) on the bacteria known as Listeria. They found that milk has a protection against harmful microorganisms. They also discovered that an acidic pH can increase the inactivation of these organisms. The inactivation rate of Salmonella when treated with ultrasound in a model medium is 4 log, while it is less than 1 log in egg and 2 log in milk. (c) *Duration of treatment:* The number of survivors decreases dramatically following an ultrasound treatment. This is similar to how other physical therapies work [18], (d) *Amplitude:* The lethality of ultrasound treatments increases exponentially with the amplitude of the wave [17]. (e) *Temperature and pressures* [19]. Although eliminating germs during food production cannot yet be accomplished using ultrasound, it may be successful when combined with pressure and heat. The benefits of this technology are its capacity to lower enzyme and microbial activity levels and its energy effectiveness. Future applications for ultrasonic technology might include thermosonication and mano-thermosonication.

MICROWAVE

Many natural and artificial sources emit energy in form of electro-magnetic waves characterized by an electrical field and magnetic field. Depending on their frequency and energy, these waves are classified as “ionising radiations” (X-rays and gamma rays) or “non-ionizing radiations” (visible, ultraviolet, infrared, radiofrequencies and microwaves, low frequency). *Microwaves (UHF:* Ulra High Frequency) are in the range of frequencies between 300 and 3000 MHz. The microwave technology was born with the conception of the radar around 1930, then widely developed in many fields (industrial, scientific and medical) [20].

Mechanisme of Microbial Inactivation Microwave

For many years, microwaves have been extensively researched as a potential alternative energy source for sterilization or decontamination. In addition, the widespread use of the microwave oven, which operates at a frequency of 2.45 GHz, has contributed to the development of studies on the interactions between microwaves and microorganisms at this frequency [21]. The existence of these effects has led to much controversy. For example, in the area of bacterial decontamination, studies have shown that microwaves, applied under the same temperature conditions as traditional heating, have a greater bactericidal effect than the latter. Some authors believe that there is a non-thermal bactericidal effect of microwaves, while others refute its existence. It seems that at present the existence of these non-thermal effects is accepted, but their nature and importance remain to be determined.

There are two proposed processes for the microwave inactivation of microorganisms:

- According to the main process, microorganisms will be entirely rendered inert by microwaves by heating via mechanisms similar to those seen in other thermal processes, such as denaturing of enzymes, nucleic acids, proteins, or other important components, as well as disruption of cell membranes [22].
- Non-thermal effects are involved in the second procedure. The likelihood of a non-thermal influence from microwaves is still an issue of debate [23]. Researchers observe that microorganisms irradiated with microwaves are destroyed at temperatures below their Thermal Death Point and show greater damage than cells treated with conventional heating at similar temperatures [24]. The authors sproposed that the athermal impact of microwaves is associated to changing protein structures by altering the direction of biochemical reactions [25].

The34nvestingations on the impacts of non-thermal microwaves on microorganisms in food preparation are shown in Table 2.

Table 2. Microwaves' non-thermal microorganism impacts on food processing.

System	Temperature control	Target	Methode	Non-thermal effects
2.45 GHz domestic oven	Post exposure measurement	*Staphylococcus aureus* in the culture medium	Conventional heating vs. similar temperature	Yes
2.45 GHz domestic oven	Measurement after exposure and mixing	*Escherichia coli* in culture medium	Conventional heating vs. similar temperature	No
Household stove 2.45 GHz	Optical fiber sensor	*Clostridium sporogenes* (spores) in the culture medium	1. Conventional heating vs. similar temperature, 2. Simultaneous cooling to maintain the fluid at sub-lethal temperatures	No
2.45 GHz domestic oven	Temperature measured before and after exposure	*P. acidovorans, Pseudomonas aeruginosa, S. epidermidis Staphylococcus aureus,* in the culture medium	Conventional heating vs. similar temperature	Yes
2.45 GHz microwave dryer and heat exchanger	Temperature sensor at inlet and outlet	Yeast, *Escherichia coli*, Pediococcus spp., *Enterobacter aerogenes Listeria innocua* and yeast in the various media	Maintaining the fluid at below-lethal temperatures by simultaneous cooling	No
Multi-mode cavity for 18 GHz microwave	Optical fiber sensor	*Escherichia coli* and *Staphylococcus aureus* in raw meat	Alternating exposure to keep the sample's temperature below the point of death	Yes
Focused 2.45 GHz microwave system	Optical fiber sensor	*Escherichia coli* in culture medium	Total destruction. Comparing with the conventional treatment	No
2.45 GHz household oven	Optical fiber sensor	*Listeria monocytogenes and Escherichia coli* in the apple juice	Comparison of modified kinetic models for conventional and microwave heating	Yes

Although electric fields have been known to affect the activity of microorganisms, their precise effects on the development and maintenance of microorganisms are not well known. Several studies have been presented that provide conflicting results due to lack of detail and improper control over the processing parameters. Non-thermal effects can also be observed in the development and maintenance of microorganisms. These effects can be distinguished from thermal effects by their various parameters, such as their frequency, power, and temperature distribution. Even though, the presence of non-thermal effect may be observed in the development and maintenance of micro-organisms, their precise effects are not well known. This is because the methods used to measure and analyze the activity of these organisms may not provide reliable results. Therefore, the use of a well-designed and integrated system is required in order to obtain reliable results. Electric field heating is generally considered to be safe for food safety, it is necessary to explore the effects of its temperature distribution on the product's internal temperature [26].

PASTEURISATION AND STERILIZATION

The food business uses conventional sterilization and pasteurization methods to render certain bacteria inactive and lengthen product shelf lives. However, they can also cause spoilage due to the presence of resistant organisms. This is due to the potential deleterious effects of the large quantities of energy consumed in these procedures on the sensory qualities and nutritional value of the finished product [27]. Along with having a detrimental influence on the goods, pasteurization and sterilization methods entail substantial energy expenditures that may have a big impact on how food is produced.

This is because the use of high temperature processes can lead to the contamination of food with harmful microorganisms Table 3.

Table 3. Consequences of sterilizing using ultrasonic settings on micro-organisms.

Sample	Experimental Parameters	Effect of ultrasound
Escherichia coli in the cactus pear juice	Amplitude 60%, 70%, 80% and 90%), 20 kHz, 1500W, at 1, 3 and 5 min	Complete inactivation was seen in both fruit juices after 5 minutes of ultrasound treatment at most of the amplitude levels, measured over 5 days
Spores inactivation of A*licyclobacillus acidoterrestris* in the orange juice	24 kHz, 460Wcm-^{2}, 33W and 105Wcm-^{2}, 162W	Thermosonication needed temperatures at lease 8°C lower than those of the heat treatments to obtain the same inactivation of the spores. (D75°C value of 49 min for 20.2 W/mL vs. 217 min for 0.33 W/mL). (D85°C has decreased in time from 69 to 29 min)
Escherichia coli and *Saccharomyces cerevisiae* in pomegranate juice	20 kHz amplitudes (50, 75, and 100%) and times (0, 6, 12, 18, 24, and 30 min)	More than a 5 log inactivation of *E. coli* and a 1.36 log inactivation of S. cerevisiae
Staphylococcus aureus in orange juice	Amplitude 80%, 20 kHz and discontinued pulses (10 s, 5 s)	The sonication at 20, 30 and 40 ◦C resulted in a decrease of 4.02 ± 0.52, 3.80 ± 0.49 and 4.30 ± 0.74 log of *S. aureus* after treatments of 90, 60 and 60 min, respectively.
Shigella dysenteriae, Salmonella Typhi, Shigella flexneri and *Listeria monocytogenes* in lemon juice	UP100H; 100 W, 30 kHz 0–8 min. A probe (10 mm in the diameter)	Starting populations of *S. Typhi, S. dysenteriae, L. monocytogenes*, and *S. flexneri* (7.2–7.6 log CFU/ml) in lemon juice were dramatically reduced to 6.2, 5.7, 5.1, and 5 log CFU/ml, respectively, when submitted to the US alone for 8 minutes.
The total number of microorganisms aerobic lactic acid bacteria, coliforms bacteria in tomato juice	20 kHz, 28 and 40 W cm^{-2}, 2.5, and 10 min	The tomato juice sonication process has contributed to the decrease in the number of microorganisms analyzed
Vegetative cells and spores of *Alicyclobacillus acidoterrestris* in the orange juice	20, 40, and 60 kHz, 3 L water capacity, 1000 W	20/40 kHz, 24 min, and 64°C produced the optimal best results. Vegetative cells and AAT spores were inhibited by 2 and 4 logs

Most commonly, microwave pasteurization is used to treat various fluid food products, such as milk and fresh juices.

Salazar-González, *et al.* [28] explained how microwave pasteurization can be performed safely and effectively for various types of fluid food products. The most desirable scenario is that the dielectric properties of the products are determined to determine the conditions under which microwave energy can be applied. Due to limited reports on the various applications of microwave pasteurization, further research is necessary to develop techniques that are effective for several food products, including fruits and vegetables, salt and sugar. In recent years, the commercial use of microwave heating has gained wide acceptance. For instance, the FDA allowed using microwave in the production of sweet potatoes. Tajchakavit, *et al.* [29] investigated the kinetic parameters of the destruction of *Lactobacillus plantarum* and *Saccharomyces cerevisiae* in the apple juice by continuous-flow microwave (700 W, 2,450 MHz) conventional heating and thermal heating of water baths. The juice was then heated to selected exit temperatures of 52.5°C to 65°C. The researchers subjected the samples to various heat treatments, such as an agitated water bath, over a temperature rage of 50 to 80°C. Results show that the degradation rate increased with temperature. The adjusted D values from microwave heaters were significantly less than those from conventional heaters. The rate of destruction of the studied microorganisms during microwave heating was around 6 times higher than conventional thermal heating. The objective of the study by Canumir, *et al.* [30] was to evaluate the influence of microwave pasteurization on the microbilogenic qualities of apple juice. The samples were inoculated with *E. coli* and treated at different power levels (900, 720, 450 and 270 W) from 40s to 90s. The results have been compared to those of conventional pasteurization (83°C for 30 s). Results indicate that pasteurization at 900 and 720 W for 60 to 90 seconds resulted in a 2 to 4 log loss. Although there was no significant variation between the microwave pasteurization and the conventional pasteurization. D values were smallest at 900 and 720 W corresponded to the maximal temperatures of 70.3 and 76.2°C, respectively; these technologies were greater than the temperature that thermally inactivates *E. coli*. Mendes-Oliveira, *et al.* [31] study the inactivation kinetics of *Salmonella Typhimurium and Escherichia coli* O157:H7 in fresh apple juice during microwave pasteurisation at temperatures ranging from 80 to 90°C, i.e. at the conditions used in conventional pasteurisation. The juices inoculated have been treated at different power levels (600 W, 720 W) and times of treatment (5s, 10s, 15s, 20s, 25s). A 5-log reduction of *E. coli* O157:H7 and *S. Typhimurium* in fresh apple juice was obtained when treatment times were greater than 20s and 25s for the 720 and 600 W power levels, with final temperatures reaching 90 and 80°C respectively.

COMBINATION EFFECT OF MICROWAVE ULTRASOUND

Few studies have been carried out on the inactivation of bacteria by microwave and ultrasound coupling. The results of the study of Hosseinzadeh Samani, *et al.* [32] revealed that the combined system's ability to remove *E.coli* from sour cherries (SJC) juice was remarkably effective when compared to the control method . The researchers noted that the system's ability to achieve high temperatures and expose the bacteria to ultrasonic exposure time were also effective. The combined effect of temperature and ultrasound was more significant than the individual effect. The optimal processing condition was achieved with a power microwave of 352.21W, a temperature of 49.94°C, and a 5-minute exposure time. Kernou, *et al.* [33] examined the effects of combined ultrasound and microwave treatment on the survival of *E.coli* ATCC 25922 in orange juice. They also used a Weibull model to describe the inactivation kinetics. Although the treatment with ultrasound (42 kHz) resulted in a reduction in the survival rate (1.3 log for 60 min of sonication), it did not affect the inactivation kinetics. The results indicated that sonication could increase the efficiency of the treatment. The researchers noted that the use of ultrasound as a pre-treatment significantly reduced the number of log (8log) produced by the combined treatment with microwave (900 W/30 s) and ultrasound (20 min). When these two processes were reversed, the number of log units produced by the combined treatment was 4.0log. The scale parameter that is estimated from the inactivation kinetics of the two methods is lower than that of the post-treatment. The results of the study revealed that the use of ultrasound as a pre-treatment significantly reduced the number of *E. coli* log units produced by the combined treatment with microwave and ultrasound. In order to study the effects of various combination treatments on the inactivation of multiple strains of Alicyclobacillus (*Alicyclobacillus acidiphilus* DSM 14558T, *Alicyclobacillus acidoterrestris* DSM 3922T and *Alicyclobacillus cycloheptanicus* DSM 4006T), Wang, *et al.* [34] conducted a study. They used a combination of ultrasonic (10min or 30min) and microwave waves to inactivate the vegetative cells of different strains. The results indicated that the DSM 3922T strain was the most susceptible to the combination of ultrasound and microwave. When the power and exposure time of the two methods were increased, the effectiveness of the combined treatment improved. On the other hand, the treatment of ultrasound followed by microwave was superior to that of the microwave. The combined ultrasound and microwave treatment resulted in a better effect than the single-wave processing alone. However, it did not have the same effect on the DSM 4006T strain. The inactivation rate was increased when the 30-minute treatment with the combined UW (400 W for 20 min) + MW (900 W for 10 min) was performed. Zia, *et al.* [35, 36] studied the combination effect of microwave (MW) treatment (2450 MHz for 120 s), ultrasound (US) (24 KHz, 20°C for 20 min) and the combined treatment (MW-US) on the stability and quality of sugarcane juice (SCJ) for 21 days of storage at 4°C. At time zero, all treatments demonstrated a significant reduction in overall microbial cell counts compared to the control. The results for microbial counts indicated that better microbial safety and longer shelf life were achieved by MW-US than either microwave treatment alone or ultrasonic treatment alone. Similarly, Pérez-Grijalva, *et al.* [37–44] suggested that the combination of MW and US can provide an effective alternative to the traditional processing of berries. It can also improve the quality of the juice [45–50].

CONCLUSION

The microorganism is stronger in certain bacteria. Microwaves or ultrasound (US) by themselves are unable to render these microorganisms inactive. In a short amount of time, combining microwave and ultrasonic procedures may significantly reduce the microbial burden. A better outcome is obtained when ultrasound is used in conjunction with microwaves after the first therapy. Since it has been able to sterilize juice without compromising its nutritional value and achieve the 5-log reductions necessary by FDA rules, ultrasonic-microwave coupling looks to be a workable method for pasteurizing fruit juice. It is expected that the food industry will adopt more of this technology in the near future to provide better food to consumers.

Acknowledgments

The authors would like to express their acknowledgment 295 to Algeria's Ministry of Higher Education and Scientific Research.

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Ourdia-Nouara Kernou: writing-review and editing.
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Khadidja Adel: Writing-review and editing,
Madani Khodir: Project administration; supervision.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

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