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Effect of sonication on microwave inactivation of *Escherichia coli* in an orange juice beverage

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Abstract

The survival of *Escherichia coli* ATCC 25922 in orange juice treated with microwave and/or ultrasound was evaluated; Weibull model was fitted to survival curves to describe inactivation kinetics; and the effect of combined microwave-ultrasound treatments was assessed. Ultrasonic treatment (42 kHz) has no significant effect on the survival rate reduction (1.3 log for 60 min of sonication) of *E. coli*. However, sonication can increase the efficiency of microwave inactivation. The use of ultrasound as pre-treatment was more effective than when used in post-treatment, that is, a reduction of 8.0 log was achieved by a combination of ultrasound (20 min) followed by a microwave (900 W/30 s), while 4.0 log was obtained when these both processes were reversed. The scale parameter α estimated from microwave-inactivation kinetics combined with an ultrasound pre-treatment was found to be lower than those obtained with a post-treatment, which, in turn, are lower than those estimated for microwave or ultrasound alone. These results demonstrate the effectiveness of ultrasound as a pre-treatment to improve the inactivation of *E. coli* by microwave processing in orange juice beverages.

Practical applications

High-temperature short-time (HTST) type processes are preferred by the food industry to reduce the adverse thermal degradation in food quality while ensuring food safety for liquid food. The microwave heating is fast and can significantly reduce the come-up time to the desired process temperature. The hydrodynamic action of ultrasound on microorganisms is a great potential to improve microbial decontamination efficiency by microwave processing. This study contributes to the design and control of an effective combination treatment of ultrasound and microwave to improve pasteurization processes for orange juice beverages.

1 | INTRODUCTION

Fruit juices contain a wide variety and an important complex nutrients mixture for health. These nutrients provide good conditions for the growth not only of spoilage microorganisms, but also of foodborne pathogens. Depending on the type of juice, *Escherichia coli*, *Listeria monocytogenes*, *Cryptosporidium* and *Salmonella* are considered relevant pathogens for processes designed to control the effectiveness of disinfection treatments (Lima Tribst, de Souza Sant'Ana, & de Massaguer, 2009). Fruit juice treatments rely on the thermal pasteurization and their inherent acidity. However, concerns about their microbiological safety have arisen due to a number of pathogen-related outbreaks, including *E. coli* O157: H7 (Eblen et al., 2004).

Most fruit juices like orange juice have pH values that are in the acidic range (<4.5). Classically, pathogen growth would not be observed under such acidic pH values. Foster (2001) reported that *E. coli* strains displayed much greater resistance to pH acid and can develop several acid stress survival mechanisms. In the processing of orange juice, *E. coli* strains are of concern because they are resistant to acidic environments and high-temperatures, therefore, alternatives to traditional pasteurization are a challenge (Chung, Bang, & Drake, 2006). Heat treatment is commonly used to inactivate microorganisms in liquid foods in order to improve food safety and extend shelf life (Uemura, Kobayashi, & Inoue, 2010). New technologies are emerging to prevent the negative impact of high temperatures on the quality characteristics of pasteurized juices. In this context, nonthermal processes, such as ultraviolet radiation, have been proposed (Rodríguez, Oteiza, Giannuzzi, & Zaritzky, 2017), pulsed electric field treatment (Gurtler, Rivera, Zhang, & Geveke, 2010), ozone treatment (Patil, Bourke, Frias, Tiwari, & Cullen, 2009), dynamic high pressure (Tahiri, Makhlouf, Paquin, & Fliss, 2006), pectin methyl esterase (Torres, González-M, Klotz, & Rodrigo, 2016), clarification (Anvarian, Smith, & Overton, 2016), cold atmospheric plasma (Dasan & Boyaci, 2018) or combination of these processes with mild heat treatments (Rifna, Singh, Chakraborty, & Dwivedi, 2019). The use of appropriate alternatives to conventional thermal preservation techniques is recommended in order to ensure the quality of fruit juices that consumers require (Jiménez-Sánchez, Lozano-Sánchez, Segura-Carretero, & Fernández-Gutiérrez, 2017).

Ultrasound (US) is also one of the innovative non-thermal technologies that can ensure microbial inactivation while preserving fruit juice quality. It is considered to be energy efficient and environmentally friendly with reduced chemical and physical hazards (Mohideen et al., 2015). The mode of action of ultrasound on microorganisms in liquids involves hydrodynamic effects (intracellular cavitation and microstreaming phenomenon) and formation of radicals that disrupt cell structure (Tiwari, Muthukumarappan, O'Donnell, & Cullen, 2008). However, as a preservation method, the application of ultrasound alone is not effective enough to kill all microorganisms. In addition, a high level of ultrasound power can adversely affect the nutritional and sensory properties of foods (Ferrario, Alzamora, & Guerrero, 2015).

The microwave (MW) heating instead of conventional heat exchangers offers certain advantages for heat treatment, such as rapid volumetric heating, lower equipment surface temperature, uniform heating, energy efficiency and possible preservation of food quality (Chandrasekaran, Ramanathan, & Basak, 2013). Many studies have demonstrated the great potential of microwave radiation in the microbial inactivation in various foods, particularly in liquid foods (Benlloch-Tinoco, Martínez-Navarrete, & Rodrigo, 2014). Indeed, High-temperature short-time (HTST) type processes are preferred by the food processing industry to reduce the adverse thermal degradation in food quality while ensuring food safety for liquid food (Varghese, Pandey, Radhakrishna, & Bawa, 2014). In this sense, the advantage of microwave heating is that the process is fast and can significantly reduce the come-up time (CUT) to the desired process temperature (Di Rosa, Bressan, Leone, Falqui, & Chiofalo, 2019). Woo, Rhee, and Park (2000) have investigated the mechanism of microbial cell inactivation by microwave heating, they revealed that in addition to inactivation, the microwave radiation caused severe damage on the surface of *Escherichia coli* cells, and there was no significant change in *Bacillus subtilis* cells.

Therefore, the combination of ultrasound with microwave can induce synergistic effects in terms of quality retention and efficiency in microbial inactivation, as well as in energy saving (Rostami et al., 2018). Furthermore, the application of this combined technique in fruit juice processing has a great potential, since milder temperatures can be used to achieve microbial decontamination, while preserving the original characteristics of fruit juices (Bhat, Kamaruddin, Min-Tze, & Karim, 2011).

The aims of this work were (a) to experimentally evaluate the survival of *Escherichia coli* ATCC 25922 cells in orange juice treated by microwave and/or ultrasound, (b) fit the Weibull model to describe and compare the inactivation kinetics, and (c) assessing the combined effects of microwave-ultrasound treatment.

2 | MATERIALS AND METHODS

2.1 | Bacterial strain and culture conditions

Experiments were conducted on *Escherichia coli* ATCC 25922 obtained from Pasteur Institute Collection (Alger, Algeria). The strain was maintained on tryptone soy agar (TSA; Conda, pronadisa, spain) at 4°C until used. Young cultures were prepared by suspending colonies in Tryptone Soy Broth (TSB; Conda, pronadisa, spain) and incubated at 37°C for 18 hours. The bacterial cells were then recovered by centrifugation (4,000 g for 15 min at 4°C) (Cabassi, Falanga, & Romani, 2017).

2.2 | Orange juice inoculation

The experiments were performed using commercial orange juice beverage with a pH of 3.27 and soluble solids of 12°Brix. Orange juice was filtered to remove the pulp and inoculated with the concentrated suspension of *E. coli* ATCC 29522 previously prepared. Inoculated juice was then adjusted to a final concentration of approximately 10^8 UFC/ml (Cabassi et al., 2017).

2.3 | Microwave and ultrasound treatment procedure

The ultrasound treatment was performed by sonication of 40 ml of inoculated orange juice in an ultrasonic bath (42 kHz) at different times between 5 and 60 min. The sample temperature was maintained at 20 ± 2°C by gradually adding ice to the ultrasonic bath. Irradiation was carried out in a household microwave (Samsung GE614ST, Malaysia) operating at 2450 MHz. Inoculated orange juice samples of

40 ml were placed in the microwave cavity and irradiated at 300, 600, and 900 W for different exposure time between 5 and 35 s. After completion of treatment, the sample was quickly removed from the cavity and immediately cooled in an ice bath.

The ultrasound as a pre- or post-treatment effect on the microwave inactivation kinetics of *E. coli* ATCC 25922 was investigated. The combined treatments applied between microwave and ultrasound were: 10, 20, and 30 min for pre- or post-ultrasound treatment and 300, 600, or 900 W for microwave inactivation kinetics. The experimental procedure was the same as described previously. All experiments were carried out in triplicate.

2.4 | Enumeration of survival cells

Aliquots (1 ml) of treated and untreated samples were serially diluted in sterile physiological water and spread-plated in triplicate onto Eosin methylene blue medium EMB. Surviving bacterial count was determined at 37°C after 24 hr of incubation.

2.5 | Modeling inactivation kinetics and statistical methods

The survival rate at time *t*, denoted *S*, was calculated as the change in the ratio of viable *E. coli* ATCC 25922 at any time $N^{(t)}$ (CFU/ml) to the initial number of microorganisms $N^{(0)}$ (CFU/ml) as control:

$$S = \frac{N^{(t)}}{N^{(0)}} \tag{1}$$

The decrease in the log-survival rate data of *E. coli* ATCC 25922 obtained in thermo-sonication and microwave treatments was described by the modified Weibull model (Mafart, Couvert, Gaillard, & Leguérinel, 2002):

$$\log(S) = -\left(\frac{t}{\alpha}\right)^{\beta} \tag{2}$$

where α is the time of the first decimal reduction, can be called scale parameter, and β is the so-called shape parameter. In order to compare the time of the first decimal reduction of the obtained inactivation kinetics: Equation (1) was fitted for a second time by setting β equal to the mean of the previously estimated β.

Modified Weibull model parameters were estimated using nonlinear regression with a curve fitting toolbox (MATLAB 6.5, The Math-Works Inc., Natick, MA). The root mean squared error (RMSE) between all experimental $Y^{(t)}$and predicted data, adjusted coefficients of determination (R^2_{adjusted}) and confidence intervals (calculated with 95% of probability) were used as goodness-of-fit indicators for the estimated parameters.

Statistical significance ($p < .05$) of the effect of the combination treatments was assessed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. The survival rates were also statistically compared to control (initial number of microorganisms) using ANOVA followed by Dunnett's post hoc test.

3 | RESULTS AND DISCUSSION

3.1 | Modeling and kinetic parameter estimation

The first-order kinetic model described by the *D* and *z* parameters is widely used to describe the microorganism inactivation kinetics (Benlloch-Tinoco, Martínez-Navarrete et al., 2014). However, the survival curves of microorganism inactivation kinetics are not often log-linear. Peleg and Cole (1998) have described the survival curves based on a Weibull distribution of microorganism resistance. This model was then modified by Mafart et al. (2002) to give practical significance for its parameters. In the present study, the modified Weibull model was well fitted to the experimental survival data of *E. coli* ATCC 25922, as illustrated in Figures 1, 2, and 3. Furthermore, the R^2_{adjusted} (0.942–0.998) and the RMSE (0.255 in average for all treatments) values reported in Table 1 indicate that the modified Weibull model predictions describe the experimental data satisfactorily.

3.2 | Ultrasound effect

Figure 1 shows the inactivation kinetic of *Escherichia coli* ATCC 25922 in an ultrasonic bath at a frequency of 42 kHz. The shape parameter β was found as 0.5 ± 0.02 (Table 1), which is clearly seen as an upwardly concavity curve with a more pronounced drag. This curve shape can be explained either by the presence of aggregates or "flocs" in the suspension, or the distribution of resistances within the population,

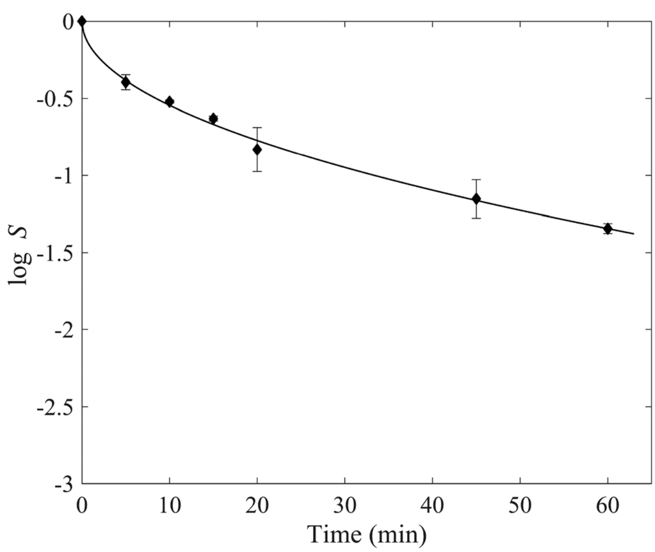

FIGURE 1 Inactivation kinetic of *E. coli* ATCC 25922 by ultrasonic (42 KHz) treatment. Error bars represent the *SD* of the mean ($n = 3$)

that is, the most sensitive members of the population are inactivated first and the most resistant remain, or due to the physical protection provided by agglutination of the inactivated cells (Peleg & Penchina, 2000).The same shape of the curve (β < 1) was obtained by Ugarte-Romero, Feng, Martin, Cadwallader, and Robinson (2006) for *E. coli* K12 treated by ultrasounds (20 kHz) at 5 different temperatures in apple cider.

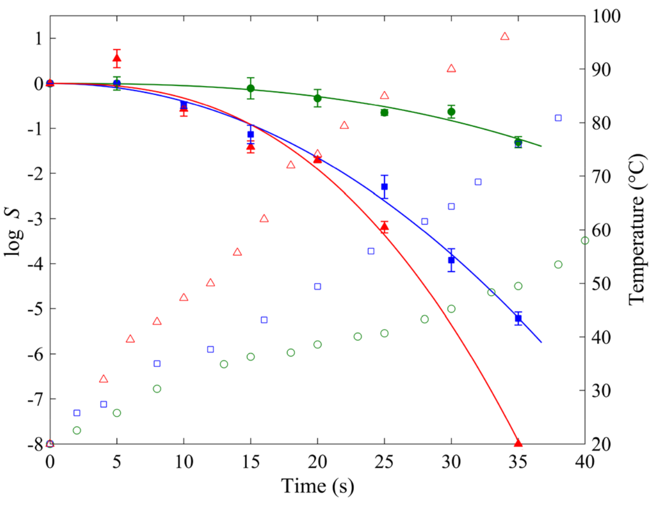

FIGURE 2 Fitting of the modified Weibull model (solid lines) to the experimental survival rate $S^{(t)}$ (symbols) of *E. coli* ATCC 25922 subjected to microwave treatments (● [in green] 300, ■ [in blue] 600, and ▲ [in red] 900 W). Time-temperature profiles are represented by symbols (◦ [in green] 300, □ [in blue] 600, and Δ [in red] 900 W). Error bars represent the *SD* of the mean (*n* = 3)

Increasing the duration of the ultrasound treatment results in a reduction in the number of *E. coli* ATCC 25922 in orange juice (Figure 1). However, it can be seen that there is no substantial reduction in the survival rate. Ultrasound treatments applied at a frequency of 42 kHz have a low impact on the inactivation of *E. coli* ATCC 25922. Indeed, the maximum reduction achieved is 1.3 log of the population after 60 min of ultrasound treatment. Char, Mitilinaki, Guerrero, and Alzamora (2010) reported that ultrasound treatment (20 kHz, 95 μm-wave amplitude) in orange juice caused a reduction in *E. coli* ATCC 35218 of up to 2.2 log after 20 min.

Patil, Bourke, Kelly, Frías, and Cullen (2009) and Joyce, Phull, Lorimer, and Mason (2003) concluded that the efficacy of ultrasound treatment was found to be a function not only of environmental conditions and treatment durations, but also a function of frequency. Most studies reported the efficacy of high frequency ultrasound on *E. coli* survival in fruit juices (Yildiz, Pokhrel, Unluturk, & Barbosa-Cánovas, 2019).

In the present study, the choice of using a low-power ultrasonic bath may reduce the viscosity and pectin content of the juice, thus improving the clarity of the juice. In addition, it has been demonstrated that, sonication increases the amount of bioactive compounds in orange juice (Guerrouj, Sánchez-Rubio, Taboada-Rodríguez, Cava-Roda, & Marín-Iniesta, 2016). In contrast, high-power ultrasonic can

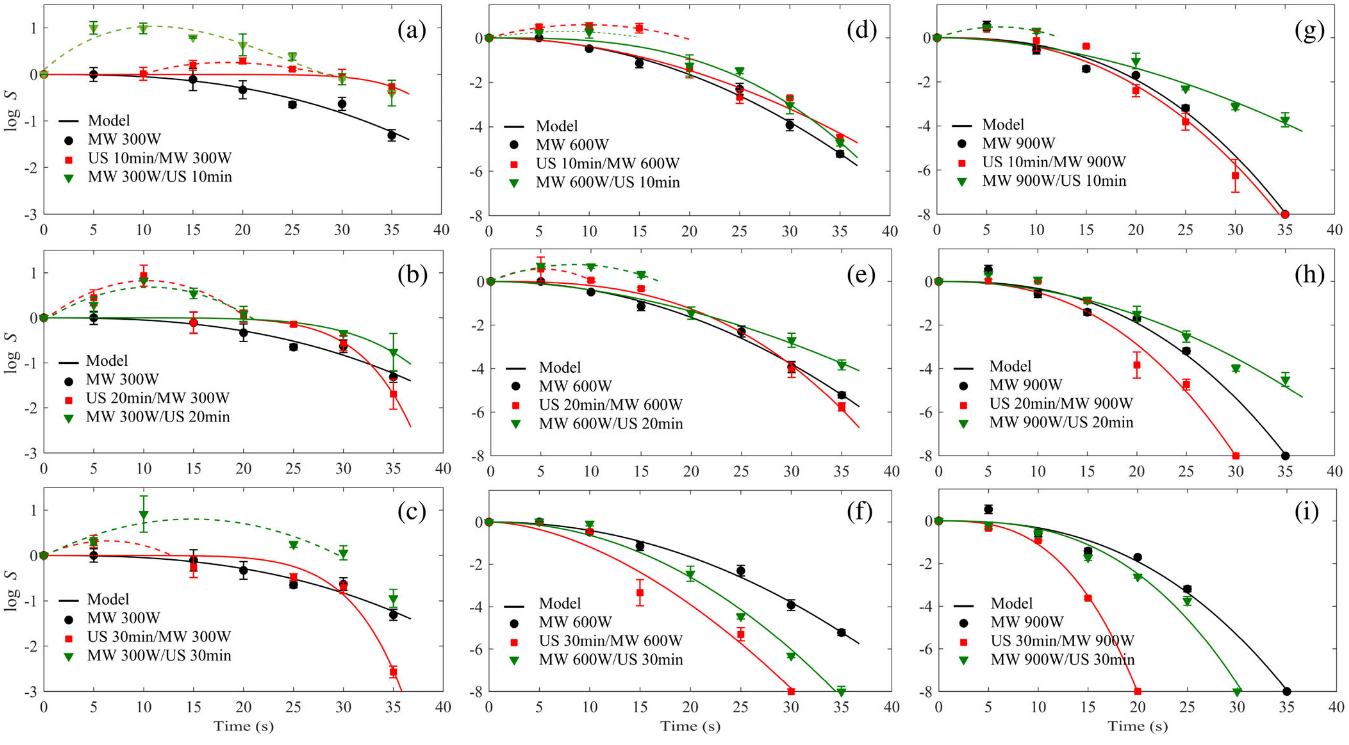

FIGURE 3 Fitting of the modified Weibull model (solid lines) to the experimental survival rate $S^{(t)}$ (symbols) of *E. coli* ATCC 25922 subjected to combined microwave (300, 600, and 900 W) and ultrasonic (42 kHz) treatments. The dashed lines are curves added as a visual guide to highlight an increase in survival rate. Error bars represent the *SD* of the mean (*n* = 3)

TABLE 1 Estimated scale (α) and shape (β) parameters for the fit of survival rate $S^{(t)}$ at different treatments of *Escherichia coli* ATCC 25922

Treatments		α	β	$R^2_{adjusted}$	RMSE	α^a (β = 2.1)
US treatment only		33.34 ± 2.67	0.5 ± 0.05	0.995	0.034	nd
MO treatment only						
300 W		32.3 ± 2.8	2.6 ± 1.4	0.942	0.103	32.5 ± 2.6
600 W		15.7 ± 2.3	2.1 ± 0.4	0.991	0.189	16.1 ± 0.5
900 W		15.5 ± 2.8	2.5 ± 0.6	0.982	0.384	13.6 ± 0.8
US pre-treatment						
300 W	US 10 min	40.1 ± 3.2	10.0 ± 5.4	0.993	0.010	nd
	US 20 min	32.6 ± 0.6	7.3 ± 1.8	0.991	0.061	nd
	US 30 min	30.6 ± 2.6	7.0 ± 4.6	0.955	0.218	nd
600 W	US 10 min	16.4 ± 7.1	1.9 ± 1.3	0.946	0.384	17.5 ± 1.3
	US 20 min	18.4 ± 6.2	2.7 ± 1.5	0.992	0.243	15.6 ± 1.2
	US 30 min	9.1 ± 5.0	1.7 ± 0.9	0.969	0.595	13.0 ± 0.4
900 W	US 10 min	14.5 ± 2.8	2.4 ± 0.6	0.985	0.388	13.2 ± 0.6
	US 20 min	13.1 ± 3.5	2.5 ± 0.9	0.974	0.469	11.6 ± 0.8
	US 30 min	9.7 ± 1.0	2.9 ± 0.4	0.998	0.127	7.8 ± 0.6
US post-treatment						
300 W	US 10 min	nd	nd	nd	nd	nd
	US 20 min	36.4 ± 1.0	6.6 ± 2.1	0.979	0.186	nd
	US 30 min	nd	nd	nd	nd	nd
600 W	US 10 min	20.5 ± 3.4	2.9 ± 1.0	0.981	0.250	17.7 ± 1.5
	US 20 min	16.8 ± 4.6	1.8 ± 0.7	0.995	0.129	16.0 ± 1.2
	US 30 min	12.6 ± 2.6	2.1 ± 0.5	0.988	0.351	13.0 ± 0.4
900 W	US 10 min	16.8 ± 5.5	1.8 ± 1.0	0.968	0.269	18.3 ± 1.5
	US 20 min	16.2 ± 3.4	2.0 ± 0.7	0.976	0.267	16.7 ± 0.9
	US 30 min	14.3 ± 3.4	2.8 ± 1.0	0.974	0.435	12.0 ± 0.9

Note: Parameter value ± confidence interval (p = .05). RMSE, Root Mean Squared Error between experimental and estimated data (log $S^{(t)}$). nd, not determined because the fit did not converge.
[a]Fitted for a second time by setting β equal to 2.1 (the mean of previously estimated β).

cause undesirable changes in the rheology of the juice (Chemat & Khan, 2011). The use of ultrasound in food products has proved to be advantageous in many processes. Although low-power ultrasound has been reported to be poorly effective in bacterial inactivation when used alone, there are increasing reports of changes in physicochemical parameters or component structures and the degradation of certain compounds. Studies show that orange juice quality parameters such as color, ascorbic acid reduction, b-carotene content and hydroxyl radical formation can be altered by ultrasonic treatment (Pingret, Fabiano-Tixier, & Chemat, 2013).

3.3 | Microwave effect

Figure 2 shows the fitting of the modified Weibull model to the experimental survival rate kinetics of *E. coli* ATCC 25922 subjected to microwave treatments at 300, 600 and 900 W, and the corresponding time–temperature profiles. Downward concavity (shoulder) of the curves can be interpreted as evidence that accumulated damage caused by a constant exposure to irradiation, and the destruction rate increases with exposure time. The observed reductions of survival rates become statistically significant (p < .05) only from 20, 10, and 10 s of treatment at 300, 600, and 900 W, respectively. The β values shown in Table 1 do not seem to show a clear trend with changing microwave power. This observation is consistent with the findings of some previous studies (Béchet, Laviale, Arsapin, Bonnefond, & Bernard, 2017; Fernández, López, Bernardo, Condón, & Raso, 2007; Hassani, Mañas, Pagán, & Condón, 2007; van Boekel, 2002; Virto, Sanz, Álvarez, Condón, & Raso, 2006; Zhang et al., 2018); they reported that the shape parameter β was independent of heat-inactivation temperature. Given the influence of inactivation conditions on the shape of survival curves and in order to quantify the microwave effect on *E. coli* ATCC 25922, the scaling parameter α of each survival curve was re-estimated at a fixed β value of 2.1 for each inactivation condition. An increase in microwave power resulted in increased inactivation rate: at 900 W the estimated scaling parameter α (13.6 ± 0.8 s) was the lowest, and at 600 W (16.1 ± 0.5 s) it was about twice as low as that obtained for 300 W (32.5 ± 2.6 s). Total mortality of *E. coli* ATCC 25922 was observed at 900 W after 35 s and achieved a reduction of 5.2 log at 600 W and 1.3 log at 300 W. These reductions were obtained with a linear trend of the time–temperature profiles, reaching temperatures of about 96, 76, and 50°C for 900, 600, and 300 W respectively.

Picouet, Landl, Abadias, Castellari, and Viñas (2009) reported that a 1 log reduction of *E. coli* O157: H7 was obtained in apple puree using irradiation at 652 W for 35 s. A 5 log reduction of *Listeria monocytogenes* was obtained during microwave heating of kiwi fruit puree at a time of 75 and 82 s at 900 and 1,000 W, respectively (Benlloch-Tinoco, Pina-Pérez, Martínez-Navarrete, & Rodrigo, 2014). Canumir, Celis, de Bruijn, and Vidal (2002) indicated that microwave pasteurization of inoculated apple juice with *Escherichia coli* at 720 and 900 W for 60 and 90 s resulted in a reduction of 2 and 4 log, respectively. Comparing the estimated decimal reduction times, authors have found no significant difference between conventional pasteurization and microwave processing. However, Siguemoto, Gut, Martinez, and Rodrigo (2018) demonstrated that microwave heating is more effective than conventional thermal treatment for *Escherichia coli* O157: H7 and *Listeria monocytogenes* inactivation in apple juice. Furthermore, it has been reported that the exposure effect to microwave irradiation of microorganisms suggests the existence of specific effect (non-thermal) (Shamis, Croft, Taube, Crawford, & Ivanova, 2012). Nevertheless, the topic of non-thermal effects of microwave on microbial inactivation remains controversial in the literature (Kubo et al., 2020).

3.4 | Combination effect

The ultrasound pre-treatment can increase the efficiency of the microwave inactivation of *E. coli* ATCC 25922 in orange juice. A microwave treatment at 600 W for 30 s combined with an ultrasound pre-treatment for 20 and 30 min provided respectively a reduction of 4.1 and 8.0 log, and only 2.7 and 6.3 log when the sonication is carried out in post-treatment. The same is true for 900 W, where it can be seen that the microwave treatment during 30 s followed by ultrasound at 20 min results in a destruction of 4.0 log, while a population reduction of 8.0 log is achieved when the ultrasound used in pre-treatment. These results demonstrate that ultrasound pre-treatment can reduce the microwave treatment time.

Figure 3 shows the fitting of the modified Weibull model to the experimental survival rates *S* of *E. coli* ATCC 25922 under combined microwave (300, 600, and 900 W) and ultrasound (42 kHz) treatments. During the first seconds of inactivation, it can be seen that in Figure 3a,c,d,e,g there is a small increase (<1 log) in the survival rate (highlighted by curves with dashed lines), indicating declumping effect that occurred as a result of the sonication treatment. This effect is more pronounced ($p < .05$) for the microwave inactivation kinetics at a power of 300 W with sonication as post-treatment (Figure 3a,b,c). Moreover, it is associated with longer shoulder periods of the survival curves obtained by combination treatments. For example, the observed reduction rates become statistically significant ($p < .05$) from 15 s at 900 W combined with a short ultrasound treatment (10 min), while it is 10s for 900 W without combination. However, this time is reduced to 0 s when the ultrasound treatment time is extended to 30 min. This declumping phenomenon was observed by Joyce et al. (2003) for *Bacillus subtilis* which was sonicated at low frequency by a probe (20 kHz) and an ultrasonic bath (38 kHz) without temperature control. It was also reported that sonication at low powers (40 kHz) resulted in a declumping effect on cyanobacteria over the first 2 min (Joyce, Wu, & Mason, 2010; Wu, Joyce, & Mason, 2012). This effect is reflected by the fact that ultrasound can disaggregate bacterial clumps producing more individual cells. The colony forming unit (CFU) enumeration reflects the viability of cells after treatment, although it is important to note that CFU can be a single cell or a group of cells. Then, if ultrasound triggers the cells, more CFUs will be formed (Mason, Duckhouse, Joyce, & Lorimer, 2003).

ANOVA results revealed that there was no significant difference ($p > .05$) between the different treatments shown in Figure 3d,e. It also revealed that there was no significant effect of short ultrasound pre-treatment (10 min) combined with 900 W treatment (Figure 3g). As shown in Figure 3g,h, the microwave treatment alone at 900 W was more effective when combined with short ultrasound treatments (10 and 20 min) because the latter produces a declumping effect, thus increasing the microbial load. However, the inactivation effect of ultrasound during post-treatment is more pronounced with a treatment time of 30 min (Figure 3f,i). An overlap of the kinetic inactivation curves is observed in Figure 3a,b,c. This suggests that the major effect of low power microwave and the ultrasound combination on the inactivation rate (*S*) is attributed to the dispersion of bacterial clumps with little inactivation effect. In other words, the declumping effect induced by sonication masks the actual inactivation rate. Indeed, the expected effect of microwave and ultrasound combination did not occur at 300 W.

Table 1 shows that values of the shape parameter β are greater than 1 and do not show any particular trend for microwave and ultrasound combination treatments. Nevertheless, high values were observed for combinations at 300 W. By setting β to 2.1, it can be seen that values of α decrease with increase in the ultrasound treatment time at the same irradiation power. The estimated α for combinations using ultrasound as pre-treatment are lower than those estimated for post-treatments, which, in turn, are lower than those estimated for microwave or ultrasound alone. This provides further evidence of the effectiveness of ultrasound as a pre-treatment to improve the inactivation of *E. coli* ATCC 25922 by microwave processing.

Wang, Zhao, Liao, and Hu (2010) found that treatment of three different strains of *Alicyclobacillus* by ultrasonic waves followed by microwave (400 and 900 W) was significantly better than the treatment of microwave followed by ultrasonic waves, and this combination had a markedly better effect than separate microwave processing. The effectiveness of ultrasound as a pre-treatment was also observed for thermal inactivation at high temperatures (70, 80, 85, 90, and 95°C) of *Alicyclobacillus acidoterrestris* spores in apple juice (Tremarin, Canbaz, Brandão, & Silva, 2019). Char et al. (2010) also reported the greater efficacy of simultaneous ultrasonic and UV-C treatment of orange and apple juices against *E. coli* ATCC 35218 compared to individual treatments. They found that a combination of high intensity ultrasound followed by UV-C was more effective than combination UV-C followed by high intensity ultrasound treatment.

Finally, Microwave coupled ultrasound treatment applied to orange juice can be an alternative to conventional thermal pasteurization, with potential for industrial application.

4 | CONCLUSION

This present study evaluated the effectiveness of microwaves and ultrasounds and their combination to inactivate the cells of *Escherichia coli* ATCC 25922 in the orange juice beverage. Variations in the time-course of survival rate were well represented by a modified Weibull model. Ultrasonic bath treatment (42 kHz) has no substantial effect on the survival rate reduction of *E. coli*, as only 1.3 log reduction was obtained for 60 min of sonication. However, sonication can increase the efficiency of microwave inactivation. The combination treatment of an ultrasound bath followed by a microwave gives better results than using ultrasonic bath as a post-treatment: a reduction of 8.0 log was obtained by a combination of ultrasound treatment (20 min) followed by a microwave (900 W/30 s). In food processing, it is recommended to develop HTST (High-Temperature Short-Time) processes to eliminate pathogenic bacteria in liquid foods while preserving quality. The combination of microwave and ultrasound processes allows to obtain a significant reduction in microbial load with short times. This work is useful to predict and optimize pasteurization processes for fruit juice beverages particularly orange juice beverages, where the presence of acid and high-temperature resistant strains such as *E. coli* ATCC 25922 is of concern.

AUTHOR CONTRIBUTIONS

Ourdia-Nouara Kernou: Conceptualization; data curation; funding acquisition; investigation; writing-original draft; writing-review and editing. **Amine BELBAHI:** Conceptualization; formal analysis; methodology; validation; writing-review and editing. **Akila Amir:** Investigation; writing-review and editing. **Kenza Bedjaoui:** Investigation; writing-review and editing. **Kamelia Kerdouche:** Writing-review and editing. **Sofiane Dairi:** Investigation; writing-review and editing. **Omar Aoun:** Methodology; writing-review and editing. **Madani Khodir:** Project administration; supervision.

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