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ORIGINAL RESEARCH ARTICLE

Effect of microwave treatment for wine microbial stabilisation: Potential use of a Weibullian mathematical model

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ABSTRACT

The aim of this research was to investigate the efficiency of microwaves (MW) for wine microbiological stabilization and thereby assess the potential application of a Weibullian mathematical model in wine industry. The study focused on (i) the influence of treatment time (come-up time and temperature maintenance) and temperature on a simple wine-like matrix, as well as (ii) the specific resistance of yeast and bacteria selected according to their representativeness in red wines (*A. aceti* ATCC_15973, *S. cerevisiae* FX10, *B. bruxellensis* CRBO_L0619 and AWRI_1499), and finally (iii) the potential usefulness of a new mathematical model adapted to heat treatment to evaluate strain sensitivity. This research aimed to show that microwave treatment decreases the main wine-associated microorganism populations and that the reduction in viable counts mainly depends on the MW treatment temperature. Complete microbiological stabilisation was obtained under a temperature of 50 °C a very short treatment time, except in the case of *A. aceti* ATCC_15973. Furthermore, the Weibull parameters analyses showed that heat inactivation a wine microorganism studied did not follow first-order kinetics, indicating that we should change our way of studying thermal death curves. The findings from this study suggest that MW could be useful for the wine industry as a complement or an alternative to sulphite utilisation.

KEYWORDS: microwaves, thermal death curves, wine, Weibull model

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INTRODUCTION

In oenology, adding SO_2 to wine is the most common way of stabilising it in terms of growth microorganisms and of protecting it from oxidation (Ribéreau-Gayon *et al*., 2006). Wine consumers are nowadays showing an increasing preference for the limitation of sulfite (SO_2) utilisation (Costanigro *et al*., 2014). Indeed, an excessive intake of SO_2 can lead to a number of physical reactions by sensitive people, such as headaches, asthma, skin rashes and bradycardia (Silva *et al*., 2019; Vally *et al*., 2009). Microbial stabilisation via physical technologies (e.g., filtration, pulsed light and ultraviolet radiation) is one of the latest topics of research in the field (Lisanti *et al*., 2019; Pilard *et al*., 2021; Trevisan *et al*., 2020). Thus, in recent years, microwaves (MW) represent an interesting alternative for the food industry.

Microwaves are unionising electromagnetic waves belonging to the portion of the electromagnetic spectrum with wavelengths of 1 mm to 1 m and corresponding frequencies between 300 MHz and 300 GHz (Letellier & Budzinski, 1999). Two frequencies are often used for MW heating: 0.915 and 2.45 GHz. MW technology is based on its direct impact on polar materials/solvents. Two phenomena occur simultaneously during the treatment: dipole rotation results from the changing MW electromagnetic field and ionic conduction is caused by the changing electric field (Jiao *et al*., 2014). The microwaves are produced by a magnetron, a high-powered vacuum tube that generates microwaves. The magnetron converts electric energy into an oscillating electromagnetic field and conductors of waves, which reflect the electric field internally and thus transfer it to the heating chamber. In fact, microwave consists of two perpendicular oscillating fields: an electric field and a magnetic field. The electric field is responsible for the generation of heat, interacting with molecules via the two aforementioned modes of action, dipolar rotation and ionic conduction. In dipolar rotation, a molecule rotates back and forth constantly, attempting to align its dipole with the ever-oscillating electric field; the friction between each rotating molecule generates heat. In ionic conduction, a free ion or ionic species moves translationally through space, attempting to align with the changing electric field; as in dipolar rotation, the friction between these moving species generates heat.

MW mainly depend on the resistance of food molecules to this flow of ions, as well as the collisions between the molecules. Therefore, the higher the concentration of ions in food, the more frequent the collisions and the sharper the rise in temperature. In the case of high frequency, this realignment occurs a million times per second and the temperature rises very quickly. MW operating conditions (i.e., frequency and temperature) and the properties of the food (i.e., composition and structure) will affect microbial viability (Chipley, 1980). Finally, the influence of matrices is essential, because water absorbs MW energy. This process results in a reduction in come-up time and improves the ability to preserve the organoleptic characteristics of different types of juices, in comparison to classical thermal treatment (Arjmandi *et al*., 2016; Igual *et al*., 2010; Jiao *et al*., 2014; Martins *et al*., 2021).

Over the past few years, several studies have explored the possibility of using MW in the wine sector. Research has mainly focused on the possibility of increasing polyphenols extraction phenomena for red wine (Carew e*t al*., 2014) or making use of extracted high-value compounds (i.e., the waste and by-products) (Meija *et al*., 2019; Romero-Díez *et al*., 2019). Carew *et al*. (2014) have suggested that MW treatment carried out before alcoholic fermentation produces wine with higher concentrations of total phenolics, anthocyanins, tannins and pigmented tannins. They have also suggested that with specific operating parameters (peak temperature at 70 to 71 °C during 10 min) this process could induce microbiological stabilisation and reduce the opportunity for the colonisation of musts by aerobic spoilage microorganisms. However, the treatment time is long and any impact on organoleptic properties and aromas would need to be investigated.

At present, no studies on the microbiological aspects of finished wine have focused on the optimisation of MW operating parameters (e.g., time and energy). Given the alcohol content and low pH of wine, the thermo-tolerance of typical spoilage microorganisms can be achieved with mild heating (temperature around 60 °C for 30 s up to a few minutes). These parameters are sufficient to achieve sterility, but the high disparity for all microorganisms needs to be further studied (Ribéreau-Gayon *et al*., 2006). For example, Reveron *et al*. (2003) studied the thermal resistance of *S. cerevisiae* (CMOJ896) isolated from Pilsen beer (5 % v/v of alcohol) produced in an industrial process. They showed that the decimal reduction times of *S. cerevisiae* (CMOJ896) are $D_{47°C}$ = 3.16 min, $D_{48°C}$ = 2.65 min, $D_{49°C}$ = 1.74 min, $D_{50°C}$ = 0.68 min and $D_{60°C}$ = 0.01 min when initial cell concentration was 10^4 cells/mL.

These thermal treatments can also have an impact on the organoleptic characteristics of wine. The main concern expressed by winemakers is the negative effects of heat on wine sensory properties and longevity (Ribéreau-Gayon *et al*., 2006). Yuan *et al*. (2020) argue that microwave irradiation impacts some important physicochemical properties of young red wine (Cabernet-Sauvignon); the results of the principal component analysis showed that there was a big difference between the untreated red wine and the treated red wine under different microwave conditions. Their results thus suggest that microwave technology can change some physicochemical properties of red wine (changes in total phenolic compounds, total monomeric anthocyanins, titratable acidity and DPPH-free radical scavenging activity), and promote aging. However, the optimisation of operating conditions to ensure microbial stabilisation has not been investigated. Microwave technology offers a number of advantages, such as selective material heating, temperature homogeneity, rapid and non-contact heating, quick start-up and stopping, and the ability to treat in situ.

Microwave technology offers similar benefits to pasteurisation, but with reduced exposure time.

The aforementioned thermal effects of MW radiation are potentially linked to specific athermal effects. Research on their presence or absence (Herrero *et al.*, 2008) tends to prove that non-thermal effects result from a direct stabilising interaction between the electric field and specific (polar) molecules. The authors tested four synthetic transformations without raising the temperature, but they were not able to prove the occurrence of non-thermal effects. Dreyfuss and Chipley (1980) were the first researchers to prove this phenomenon in a study on the effects of MW on *Staphylococcus aureus* at sublethal temperatures; they proved the existence of a phenomenon different to thermal heating that resulted in the altered activity of various metabolic enzymes.

The application of heating treatments, like MW treatment, in food protection is usually based on the assumption that the survival curves of microbial cells follow first-order kinetics; the time of decimal reduction (D-value) can thus be calculated at a given temperature from the linear relationship between the decimal logarithm of the number of surviving microorganisms and the treatment duration (Stumbo, 1973). However, in most cases the survival curve of heated microorganisms is not linear, but has a downward concavity (presence of a shoulder) or an upward concavity (presence of a tail). First-order kinetics assumes that a microbial population is homogeneous in terms of heat resistance and that all the cells will react in the same way to the applied stress. Meanwhile, some researchers have developed the opposing assumption that a microbial population is polydispersed and a heating process at a given temperature during a variable length of time will cause death or resistance of the cells, depending on their physiological state (Peleg, 1999; Peleg & Cole, 2000; Peleg & Cole, 1998; Smith, 1991). They have thus developed a model based on the Weibull distribution that has the main advantage of describing downward concave survival curves ($\beta > 1$) and upward concave curves ($\beta < 1$), as well as the traditional linear case ($\beta = 1$).

In this study, we investigated the influence of treatment duration and temperature on microbial stabilisation in a simple wine-like matrix. Different species (*Saccharomyces cerevisiae*, *Œnococcus œni*, *Acetobacter aceti* and *Brettanomyces bruxellensis*) were studied due to their oenological importance and thermal death curves were determined for each case. Moreover, the usefulness of a new mathematical predictive model (Weibull model) that is more adapted to thermal treatment was assessed.

MATERIALS AND METHODS

1. Strains and growth conditions

Four types of microorganisms were chosen based on their representativeness in red wines. These microorganisms were a lactic acid bacteria *O. œni* CRBO_14243, one strain of acetic bacteria *A. aceti* ATCC_15973, *S. cerevisiae* FX10 and two strains of yeast *B. bruxellensis* CRBO_L0619 and AWRI_1499. All the strains were obtained from the "Centre of biological oenological resources" (CRBO) of the University of Bordeaux. Both the bacterial strains were grown in a liquid grape juice medium containing 250 mL/L commercial juice, 5 g/L yeast extract, 1 mL/L Tween 80 adjusted to pH 4. Yeast strains were grown in a liquid YPG medium for yeast containing 10 g/L yeast extract, 10 g/L bactopeptone, 20 g/L glucose adjusted to pH 5. The bacterial cultures were incubated for approx. 7 days at 25 °C and the yeast for between 2 and 5 days at 30 °C until the exponential phase. Trials were performed by inoculating 150 mL of modified grape must (containing 250 mL/L grape juice, 5 g/L yeast extract, 1 mL/L Tween 80 adjusted to pH 4 with 12 % ethanol (v/v)) with around 10^6 CFU/mL strains from a freshly prepared culture.

The populations in the cultures were monitored by fluorescence microscopy.

2. Microwave Experimental Setup for Microbial Stabilisation

6 mL of previously prepared modified grape juice (see Section 1.) was poured into glass test tubes, which were inserted into a waveguide that had been drilled to the

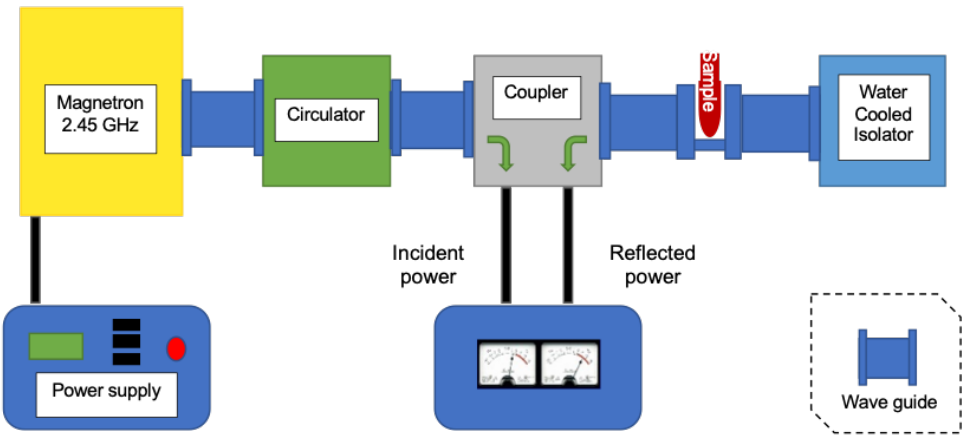


FIGURE 1. Microwaves experimental set-up for microbial stabilisation.

appropriate diameter. Microwaves treatment assays were carried out with the laboratory setup shown in Figure 1. A magnetron of 2.45 GHz was used and the electromagnetic energy was sent through the waveguide structure and coupler and into the sample cavity. Each temperature (40 °C, 45 °C, 47 °C, 50 °C, 60 °C and 70 °C) was tested for all microorganisms in triplicate. The control samples were not treated.

Preliminary tests were carried out to determine the microwave power and the exposure time to quickly obtain the desired temperatures and be able to take samples for analysis. Seven temperatures were tested and the microwave power was fixed to 100 W for 40 °C, 45 °C, 47 °C and 50 °C and 150 W for 60 °C and 70 °C. The temperature in the liquid was read in real time by a Luxtron optic fiber (PFA/teflon®) thermocouple connected to the data logger thermometer (fluroptic Luxtron model 790, Luxtron corp., Santa Clara, CA).

The microwave power was chosen based on the temperature, which rose quickly inside the chamber due to the low quantity of liquid. This temperature kinetics allow sampling for the microbial survival analyses. When the desired temperature of the liquid had been reached, the microwave emission was stopped in order to prevent the temperature from rising and to thus maintain a constant temperature within the chamber of the experimental set-up. Samples (200 µL) were taken every 10 s during the heat-up stage and while the temperature was constant, and placed into an Eppendorf. Then, the microbial survival was immediately investigated.

3. Microbiological Analysis

Microorganism survival was monitored over time during come-up, and during and after microwave exposure. Serial dilutions were carried out on all the samples, which were plated in duplicate on different media. A solid grape juice medium was used for the bacteria (*O. œni* and *A. aceti*), which contained 250 mL/L commercial grape juice, 5 g/L yeast extract, 20 g/L agar, 1 mL/L Tween 80 adjusted to pH 4.8. A YPG medium was used for yeast, which comprised 10 g/L yeast extract, 10 g/L bactopeptone, 20 g/L glucose, 20 g/L agar, and was adjusted to pH 5). The *B. bruxellensis* colonies were counted after 7 days of incubation at 30 °C, *S. cerevisiae* after 2 days at 30 °C, and *O. œni* and *A. aceti* after 7 days at 25 °C.

4. Mathematical Modelling of Data

The mean data corresponding to the inactivation of each microorganism resulting from each microwave treatment were fitted using first-order kinetics, and D-values were determined in each case. The calculated decimal reduction times, the D-value or the time to destroy 90 % of the population was computed for each temperature as the negative slopes of the linear portion of the log survival population (CFU/mL) versus time.

In a comparison of this log-linear model, the data were fitted to the Weibullian model (Boekel, 2002; Peleg & Cole, 1998). Over the years, the Weibullian model has been extensively applied to describe the inactivation of microorganisms in food for non-linear kinetics.

For the survival curve, the cumulative function is:

(1) $S(t) = exp\left(-\left(\frac{t}{\alpha}\right)^{\beta}\right)$

And

(2) $LogS(t) = \frac{-1}{2.303}\left(\frac{t}{\alpha}\right)^{\beta}$

with the two parameters of distribution α (the scale parameter - a characteristic of time) and β (the shape parameter)

where (3) $S(t) = \frac{LogN(t)}{LogN0}$

N is the number of microorganisms (CFU/mL) surviving the treatment at time t and N_0 is the initial number of microorganisms (CFU/mL).

The α and β parameters were estimated using the Excel equation solver and GRG (Generalised Reduced Gradient) by minimising the root mean square error (RMSE) between the model and experimental data.

The goodness of fit between the experimental and predicted data was assessed by using the regression coefficient R^2 and RMSE. The higher the R^2 value and the lower the RMSE value, the better the fit was considered to be.

5. Cell observations by TEM and SEM

5.1. Wine samples

A red wine from Bordeaux area naturally contaminated with *B. bruxellensis*, *S. cerevisiae* and lactic acid bacteria was treated by means of microwaves (50 °C, 60 °C and 70 °C) at 150 W during 20 s in the experimental setup previously described in Section 2. The control sample did not undergo microwave treatment. The triplicate samples were then pooled per condition before microscopy preparation.

5.2. Transmission Electronic Microscopy

Cell pellets from the samples were fixed for 3 h in 0.1 mol/L sodium cacodylate buffer (pH 7.2) supplemented with 2 % glutaraldehyde at room temperature. They were rinsed twice in cacodylate buffer and recovered by centrifugation (8,000 × g, 3 min). Cell inclusions were done in 1 % agarose and postfixed with (i) 1 % osmium tetroxide containing 1.5 % potassium cyanoferrate for 1 h at room temperature in the dark, and (ii) with 3 % uranyl acetate for 45 min at 4 °C in the dark. They were washed out with water 3 times for 5 min and gradually dehydrated in ethanol (50 % to 100 %) and embedded in EponTM. Thin sections (60 nm) were collected on 150-mesh copper grids, before examination with a HITACHI H7650 TEM (Bordeaux Imaging Center, CNRS-INSERM, Bordeaux University) at 80 kV.

5.3. Scanning Electronic Microscopy

Cells in suspension were deposited on lamellae (12 mm) coated with polylysine. Cell fixation was performed in 0.2 mol/L sodium cacodylate buffer (pH 7.2) supplemented

TABLE 1. D-values obtained for *S. cerevisiae* FX10, *O. œni* 14243, *A. aceti* ATCC15973 and *B. bruxellensis* (1499 and L0619) after MW treatments.

Temperature (°C)	D-values (min)				
	S. cerevisiae FX10	*O. œni* CRBO_14243	*B. bruxellensis* AWRI_1499	*B. bruxellensis* CRBO_L0619	*A. aceti* ATCC_15973
40	-	-	-	-	-
45	12.39	10.74	3.27	6.59	3.51
47	6.38	3.34	1.68	2.73	2.92
50	0.060	0.050	0.053	0.060	0.259
60	0.059	0.050	0.050	0.056	0.082
70	0.060	0.050	0.028	0.055	0.078

with 2.5 % glutaraldehyde at 4 °C overnight. The lamellas were rinsed twice in cacodylate buffer and then twice with water. They were gradually dehydrated in ethanol (30 % to 100 %) and desiccated by critical point drying (CPD) with CO_2. The observations were realised using Zeiss GeminiSEM300 (Bordeaux Imaging Center, CNRS-INSERM, Bordeaux University) with a coating system Q150T (Quorum technologies).

RESULTS AND DISCUSSION

The thermal treatment of foods is widespread, and has proved to be effective by the resulting inactivation of spoilage and pathogenic microorganisms. In our field of wine, we chose to study different types of microorganisms based on their oenological importance. Thus, the spoilage microorganisms *Acetobacter aceti* and *Brettanomyces bruxellensis* were chosen, as well as the technological microorganisms *Saccharomyces cerevisiae* and *Œnococcus œni*. Thermal Death Curves (TDC) were generated in order to investigate the effect of temperature on the viability of these four species.

Several temperatures set points were established (40, 45, 47, 50, 60 and 70 °C) and the mean viability of each microorganism (CFU/mL) as a function of time was evaluated.

In Figure 2a showing TDC at 70 °C, most of the viability loss can be seen to occur during MW heating (within the first 30 s). The *B. bruxellensis* AWRI_1499 strain seems to be more sensitive to temperature, showing a drop in number of the survival population in the first 20 s. Regarding the D-values (Table 1), we can see that they are very similar between 0.05 to 0.078 min, except for those of *B. bruxellensis* AWRI_1499, which had the lowest D-values at 70 °C (0.028 min). No correction to the come-up time (CUT) was done for the D-values because the CUT was too high in comparison to the microorganism's sensitivity to temperature. Indeed, the temperature set point at 70 °C (150 W) occurred after 40 s of MW heating, when no viable cells remained.

In Figure 2b, TDC at 60 °C shows the same trend as TDC at 70 °C, with a total viability loss before the temperature set point was reached. As in the aforementioned case, the *B. bruxellensis* AWRI_1499 strain was more sensitive to temperature than *B. bruxellensis* L0619 and the other species studied here. Nevertheless, the D-value of *B. bruxellensis* AWRI_1499 was the same as that of *O. œni* CRBO_14243 (0.05 min), indicating that they have the same sensitivity at this temperature.

It can be seen in Figure 2c that when the temperature set point was reduced to 50 °C in the MW treatment at 100 W, none of the species survived after 10 s, except for *A. aceti* ATCC_15973. Indeed, the surviving population of *A. aceti* ATCC_15973 was stable at around 10^2 CFU/mL by the end of the experiment. Moreover, we can observe that the surviving population of *B. bruxellensis* CRBO_ L0619 was around 4 log after 40 s, while the populations of the other species were under the detection threshold. The D-value is 0.26 min for *A. aceti* ATCC_15973 and 0.06 for *B. bruxellensis* CRBO_ L0619, compared to 0.05 min for the other strains. Fabrizio *et al*. (2015) studied the thermal inactivation of four strains of *B. bruxellensis* in hot water treatments at four different temperatures (55 °C, 57.5 °C, 60 °C and 62.5 °C). The D-values calculated were between 1.3 and 1.05 min at 55 °C, and 0.325 and 0.345 min at 60 °C. The authors noticed that thermal sensitivity is a strain-specific characteristic. In our case, the D-values of the *B. bruxellensis* strains were lower than those observed by Fabrizio *et al*. (2015) which could be explained by the aforementioned strain-specific sensitivity.

At a lower temperature (47 °C), the MW treatment did not totally decrease the microorganism's population (Figure 2d); the D-values at 47 °C were higher than at the other temperatures. *S. cerevisiae* FX10 is the strain that will hypothetically require the most time to destroy 90 % of the population (6 min at 47 °C) while *B. bruxellensis* AWRI_1499 is the most sensitive strain (1.68 min at 47 °C).

In Figure 2e, we can see that after 100 s at 45 °C, all the tested species underwent less than 1 log of population reduction. As before, the D-values are higher than at 47 °C, and *B. bruxellensis* AWRI_1499 has the lowest D-value of all the species tested. Couto *et al*. (2005) have also studied thermal inactivation of yeast *Dekkera/Brettanomyces* in tartrate buffer and in wine. They observed higher D-values at 45 and 50 °C, with, 17.4 min and 3.8 min respectively for *D. bruxellensis* PYCC4801 in tartrate buffer.

Finally, no effect of temperature was observed at 40 °C (Figure 2f), except for *B. bruxellensis* AWRI_1499,

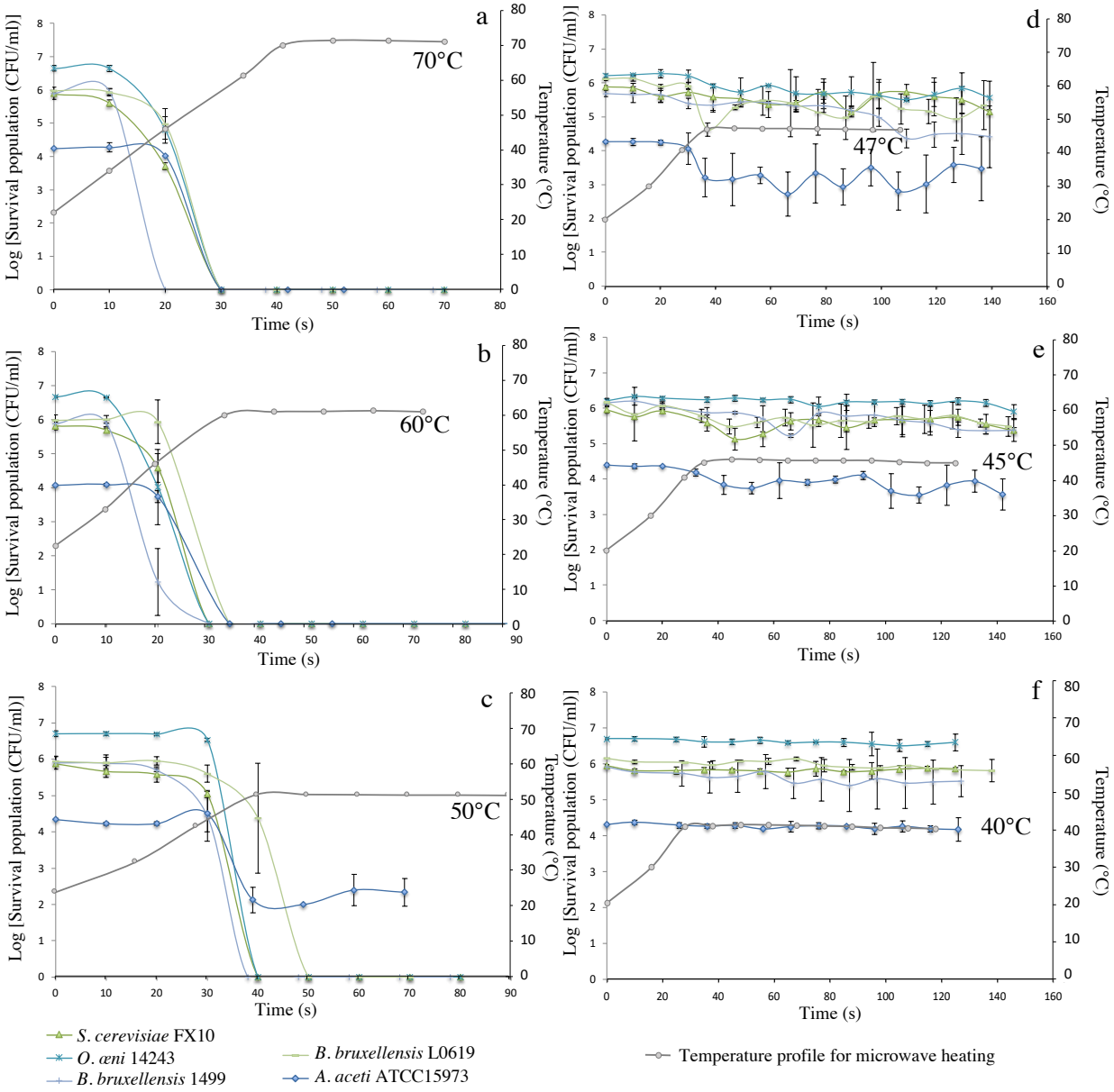


FIGURE 2. Thermal death curves at (a) 70 °C, (b) 60 °C, (c) 50 °C, (d) 47 °C, (e) 45 °C, and (f) 40 °C generated by microwave treatments (150 W for a and b, 100 W for c, d and e, and f for *S. cerevisiae* (FX10), *O. œni* (14243), *B. bruxellensis* (1499 and L0619) and *A. aceti* (ATCC15973). The monitoring of temperature over time is indicated by a grey line from 22 °C to the set-point temperature 70 °C.

for which the differences between the replicates (high standard deviation) indicate variability in strain behaviour.

These results show that the occurrence of microbial inactivation during heating by MW is not always linear, especially in non-isothermal conditions. Therefore, we investigated the goodness of fit of the experimental data in a mathematical model. The Weibullian model has been extensively applied to describe the inactivation of microorganisms in several types of food over the years for non-liner kinetics (Boekel, 2002; Mafart *et al.*, 2002; Peleg, 2021). The Weibullian model integrates stress as temperature, a_w or acidity to biological variation and could be described as a statistical model distribution of inactivation times (Boekel, 2002). The model has two parameters: the scale parameter (α) and the shape parameter (β).

In Figure 3, the experimental data and the prediction of the inactivation curves are shown. A good fit between the experimental data and the Weibullian model survival curves can be observed. Only *A. aceti* and *S. cerevisiae* (Figure 3e and a) at 50 °C require more experimental data in order to evaluate if the population survival drops after 60 sec or after intermediate sampling.

Despite the empirical nature of the Weibull model, a link can be established between the physiological effects and the observed Weibull parameters. The Weibull parameters (α and β) are summarised in Table 2, along with the calculated

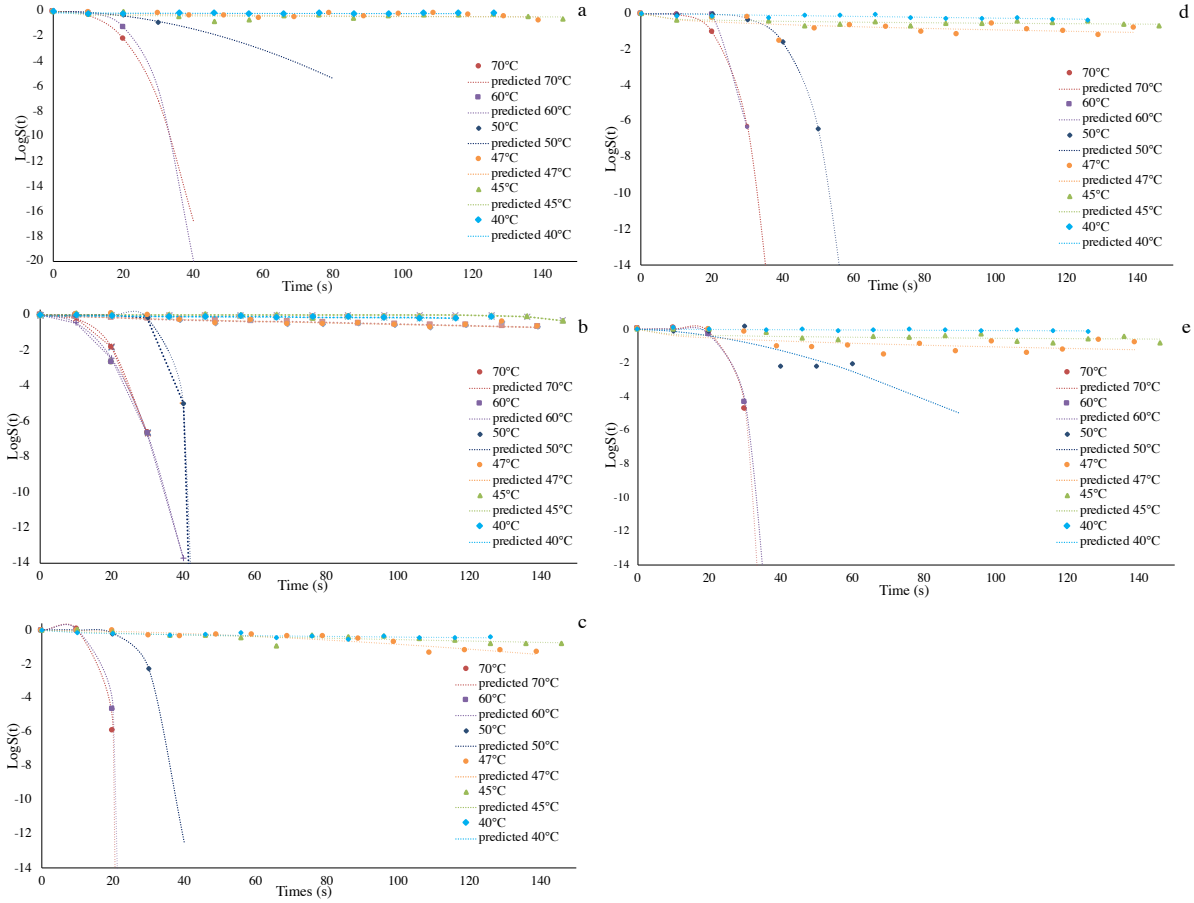


FIGURE 3. Inactivation curves for experimental data (dote) and the predictions made by the Weibullian model (dashed lines) at 70 °C, 60 °C, 50 °C, 47 °C, 45 °C and 40 °C generated by microwaves for (a) *S. cerevisiae* (FX10), (b) *O. œni* (14243), (c) *B. bruxellensis* (1499), (d) *B. bruxellensis* (L0619) and (e) *A. aceti* (ATCC15973).

coefficient of determination R^2 associated with these parameters and for the linear model.

For all the species studied, we observed that above 50 °C, β was superior to 1, which indicates that the remaining cells become increasingly damaged as a result of the applied stress (Boekel, 2002). On the other hand, below 50 °C, β was lower than 1, except for *O. œni at* 45 °C, *Brettanomyces bruxellensis* 1499 at 47 °C, and *A. aceti at* 40 °C; this could be due to the physiological state of the cells, the composition of the media or the application of MW through the test tube. Moreover, for the other conditions, $\beta < 1$ indicates that the probability of the cells dying decreases and they may have a higher ability to adapt to the applied stress.

Table 2 shows that β does not equal 1 in any of the test conditions. This indicates that first-order kinetics (which is usually used for D-value calculation) is not adapted to thermal inactivation kinetics studies and is only one particular case of the Weibull model ($\beta = 1$). Thus, this is similar to the results of several studies in the field of food protection (Arioli *et al*., 2019; Kaya & Unluturk, 2019; Liu *et al*., 2019; Tahi *et al*., 2021). In the present study, the β parameter seems to be dependent on the temperature, which contrasts with the results of data analyses carried out by Boekel (2002). However, the author hypothesised that a temperature dependence can occur, if more than three different temperatures are applied in the study, which is the case here.

Regarding the coefficient of determination obtained for the two models, we can see that in all cases except one (*O. œni* at 45 °C) Weibull R^2 is higher than linear R^2, which indicates that the Weibull model is more adapted to MW heat treatment.

When taking all the data into account, especially the mathematical analyses with the Weibull model parameters, *A. aceti* ATCC_15973 showed the highest resistance to MW heat treatment. Of the yeasts, *S. cerevisiae* FX10 was the most difficult to stabilise. Moreover, when comparing both strains of *B. bruxellensis*, CRBO_L0619 which belongs to the genetic group CBS2499-like (Wine 2N) is more resistant to heat treatment, whereas *B. bruxellensis* AWRI_1499 which belongs to the genetic group AWRI1499-like (Wine 3N) is more sensitive to MW heat treatment (47 °C). A recent study has shown differing levels of resistance to SO_2 addition to wine (Avramova *et al*., 2018). Indeed, in this study the authors have shown that strains belonging to the genetic group Wine 2N are more sensitive to SO_2 whereas strains from Wine 3N group resist up to 0.6 mg/L mSO_2. However, regarding the application of ultraviolet radiation (UV-$_C$), which is another type of physical stabilisation treatment, sensitivity has been shown to be reversed and triploid strains (Wine 3N) to be more sensitive to UV-$_C$ stabilisation (Pilard *et al*., 2021).

TABLE 2. Calculated parameters for Weibullian model and log linear model of the studied species at different temperatures and power.

Species	Strains	Power (W)	Temperature (°C)	α	β	R^2 Weibull	R^2 Linear
Saccharomyces cerevisiae	FX10	100	40	2.00E+22	0.03	0.4944	0.0069
			45	178.28	0.15	0.2242	0.0078
			47	152.37	0.38	0.3633	0.2764
			50	21.66	1.92	0.9537	0.8219
		150	60	15.40	4.01	0.9988	0.9493
			70	11.57	2.95	1.0000	0.9663
Œnococcus œni	14243	100	40	490.52	0.77	0.7382	0.7123
			45	332.02	1.83	0.3496	0.4852
			47	80.38	0.79	0.7420	0.7135
			50	32.51	11.78	0.9999	0.7677
		150	60	9.95	2.48	0.9939	0.9856
			70	12.90	3.24	0.9908	0.9381
Brettanomyces bruxellensis	AWRI_1499	100	40	113.47	0.46	0.7299	0.6554
			45	71.70	0.77	0.6608	0.6466
			47	68.53	1.65	0.8967	0.8554
			50	22.71	5.93	0.9999	0.9514
		150	60	15.07	8.4	1.0000	0.8977
			70	15.30	9.73	0.9999	0.7434
	CRBO_ L0619	100	40	185.82	0.94	0.6405	0.6648
			45	46.69	0.24	0.5580	0.346
			47	17.75	0.43	0.4705	0.3589
			50	32.48	6.24	0.9998	0.9052
		150	60	23.95	11.89	0.9999	0.5787
			70	16.63	4.53	1.0000	0.8668
Acetobacter aceti	ATCC_15973	100	40	377.69	1.18	0.5740	0.5851
			45	65.05	0.63	0.6482	0.5944
			47	14.02	0.45	0.4912	0.3515
			50	21.39	1.70	0.7305	0.4995
		150	60	20.89	6.38	0.9999	0.8133
			70	21.83	7.51	0.9999	0.795

The authors suggest that polyploidy was involved in the differences in UV-$_C$ sensitivity between the *B. bruxellensis* strains. The strains belonging to the AWRI1499-like genetic group are all triploid and the strains of the 2499-like genetic group are diploid, which suggests they may have less efficient repair systems due to the combination of divergent genomes, resulting in accrued UV-$_C$ or MW sensitivity in our case.

Furthermore, other authors have found that microbial inactivation (*Dekkera/Brettanomyces)* begins at 50 °C and depends highly on ethanol content (Couto *et al*., 2005). However, there are no data concerning MW treatment or conventional heat treatment on other main wine's microorganisms in the literature. Thus further studies on a large number of strains would be required in order to validate the inter- and intra-specific variability of sensitivity to heat treatment.

The morphological consequences of MW heat treatment on naturally contaminated red wine are shown in a TEM in Figure 4, in which the heterogeneous effect of heat on the cells can be observed. At 50 °C, some of the cells

showed intracellular leakage due to wall and membrane disorganisation, whereas the other cells are intact. These observations are also valid for 60 °C and 70 °C, but with a higher quantity of cells in the death state. This is in line with the utilisation of Weilbull model, because the microbial population was polydispersed and sensitivity to the heat treatment varied.

Same samples were also analysed by SEM (Figure 5). The results show that the number of cells with hollows, wall or membrane intussusception increases with temperature increase. The cell surfaces also become rougher. As in the TEM, a population heterogeneity can be observed in terms of sensitivity to temperature. The effect of MW treatment on cell morphologies has been documented in the past few years, especially on pathogens (Arioli *et al*., 2019; Liu *et al*., 2019). Several authors observed the apparition of damage on the membrane or the intracellular components after MW treatment (Woo *et al*., 2000; Wu & Yao, 2010). They suggest that the thermal effects resulted from the denaturation of enzymes, proteins and nucleic acid, as well as the disruption of membranes when the temperature reaches 50–60 °C.

Furthermore, during the MW treatment, the application of the electric field influences the ions, charged molecules and dipole moments. Translational and rotational motion is dependent on the electric field strength, while rotation is dependent on both dipole moment-intensity parameters. Motion at the submolecular atomic level occurs as a result of these two forces. From a microbiological point of view, the electric field has also been found to destabilise the cell membrane, increasing membrane permeability, but with no

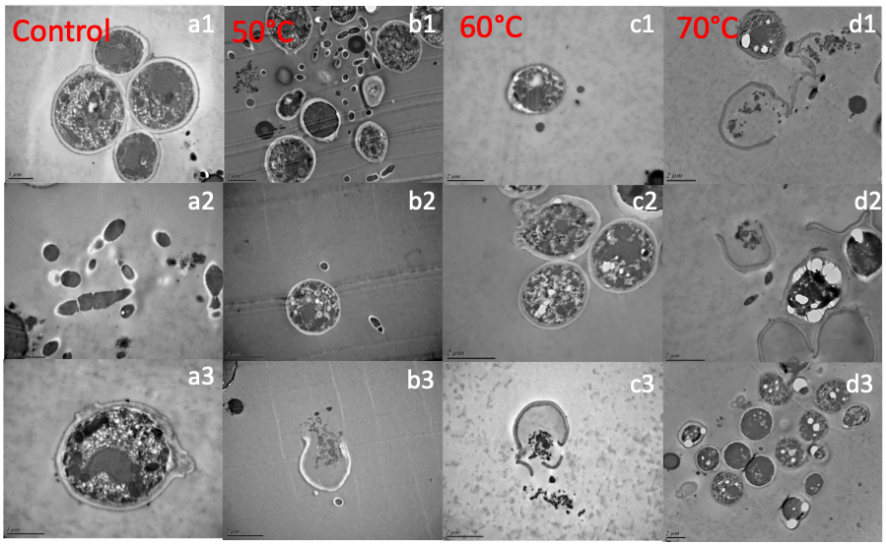


FIGURE 4. Transmission electron micrographs of microorganisms before MW treatment (a1, a2, a3) and after MW treatment at 50 °C (b1, b2, b3), 60 °C (c1, c2, c3) and 70 °C (d1, d2, d3) for 20 s. Each micrograph in a column corresponds to the same sample for a specific condition.

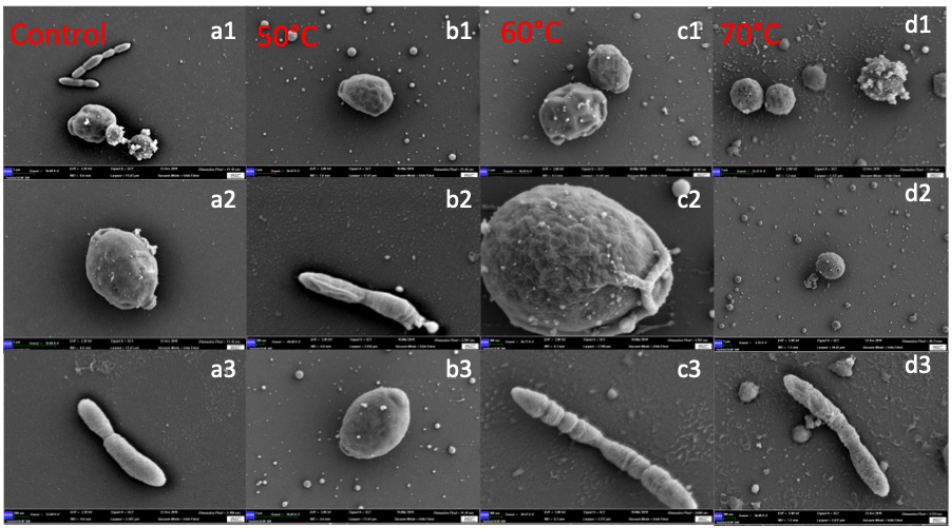


FIGURE 5. Scanning electron micrographs of microorganisms before MW treatment (a1, a2, a3) and after MW treatment at 50 °C (b1, b2, b3), 60 °C (c1, c2, c3) and 70 °C (d1, d2, d3) for 20 s. Each micrograph in a column corresponds to the same sample for a specific condition.

significant effect on viability at room temperature (Ahortor *et al*., 2020). In contrast, in the same study, magnetic fields had no impact on cell viability or membrane permeability.

Finally, based on previous studies on yeast, additional stresses can be considered to influence heat lethality (Couto *et al*., 2005; Leão & Uden, 1982). The presence of polyphenols, especially phenolic acids of which ferulic acid, is found to have the strongest effect on *D. bruxellensis*. Thus, the synergic effect of ethanol and temperature result in membrane disorder and higher alcohol concentration within the membrane (Adams *et al*., 1989; Leão & Uden, 1982). In lactic acid bacteria, especially *O. œni,* there is a huge variability in resistance to pH and polyphenols, which can have an impact during heat treatment (Breniaux *et al*., 2018).

These synergic effects should be taken into account in further MW treatment studies.

CONCLUSIONS AND PERSPECTIVES

The inactivation of microorganisms by MW is highly dependent on time and temperature treatment. Only a few seconds at 50 °C was sufficient to stabilise the wine-like medium, except in the case of *A. aceti* ATCC_15973. However, further investigations are needed to investigate the variability of the inter and intra -sensitivity of wine microorganisms to MW radiation. Regarding the mathematical model, the use of first order kinetics is not adapted to MW heat treatment studies and is only a particular case of the Weibull model.

The main advantage of MW treatment is the homogeneity of the temperature, in contrast to classical thermal treatment, which tends to be inhomogeneous and can result in the occurrence of cold spots and thus less efficient stabilisation. The optimisation of the MW treatment process is essential for obtaining a safe and high-quality product. Due to the potential benefits of reducing thermal exposure in order to deactivate spoilage microorganisms while maintaining high quality, microwave pasteurisation systems have stirred the interest of the beverage industry; however, the impact of the treatment on organoleptic quality needs to be studied, especially in terms of wine aromas.

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