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Mild Heat and Microwave Inactivation of *Escherichia coli* and *Saccharomyces cerevisiae* in Pomegranate Juice

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ABSTRACT

Pomegranate juice is a rich source of functional ingredients which thermal processing significantly affects its functional compounds. In this study, the effect of mild heat and microwave processing on the inactivation of inoculated microorganisms in pomegranate juice was studied. The juices were processed with mild heat treatment (52, 54, 56, 58 and 60°C for 0 to 120 s) and also with a domestic microwave at 600 W (for 0, 45, 60, 67.5, 75, 80, and 90 s) and 900 W (for 0, 30, 40, 45, 50, and 60 s). Mild heat (at 60°C for 120 s) and microwave (600 and 900 W for 90 and 60 s, respectively) treatments led to a reduction of approximately 6.65 log cycles in *E. coli* and 5.06 log cycles in *S. cerevisiae*. The mild temperature of 58°C for 120 s reduced *S. cerevisiae* to below the detection limit, while the population of *E. coli* was inactivated at 60°C for 120 s. Also, the *S. cerevisiae* load at 600 W/80 s and 900 W/50 s was less than the detection limit. But, the *E. coli* load at 600 W/90 s and 900 W/60 s were less than detection limit. The type of pomegranate juice did not have a significant effect on microbial inactivation in both mild heat and microwave processes. Similar to mild heat treatment, *S. cerevisiae* was more sensitive than *E. coli* at both microwave powers.

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INTRODUCTION

Pomegranate (*Punica granatum* L.) and its products have been used for centuries as a rich source of bioactive compounds such as flavonoids, ellagitannins, mainly punicalagins, ellagic acid, and punicalins (Akhavan et al., 2015). In recent years, the pomegranate has been known as one of the new superfoods with health promoting effects (Ciccone et al., 2023). This fruit is one of the most important commercial fruits in Iran and its total production in the year 2024 was ~ 1,350,000 tons. The pomegranate is consumed as fresh fruit, juice, paste, jams and jellies, and pomegranate supplements throughout the world. The average consumption of pomegranate in Iran is 7-8 kg per person per year, which is mainly consumed as fresh fruit and juices (obtained from pomegranate arils or whole fruits). Traditionally, ready-to-drink pomegranate juice (as similar as possible to that produced at home) is sold in grocery stores in Iran and contains no preservatives and is offered to the customers without any pasteurization, even though such products have a limited shelf-life (Neggazi et al., 2025).

The most serious concern in unprocessed fruit juice is microbial contamination such as acid-tolerant bacteria, fungi (yeasts and molds), and pathogenic bacteria especially *E. coli* O157:H7, *Salmonella* spp. and *Listeria monocytogenes* (Neggazi et al., 2025; Shojaei et al., 2025). The aforementioned microorganisms can survive in acidic conditions for extended periods during storage (Neggazi et al., 2025). Microbial growth of fruit juices causes deterioration of nutritional and sensorial properties such as loss of functional ingredients, color, flavor, and odor, and can also cause human illness in un-pasteurized fruit juices due to pathogenic bacteria or toxigenic fungi (Parish, 1997; Shojaei et al., 2025). Consequently, because of cases of illness from consumption of contaminated unpasteurized fruit juices the United States Food and Drug Administration (USFDA), enacted the 5-log pathogen reduction standard in fruit and

vegetable juices to ensure the safety of juice products (FDA, 2004).

According to the increasing demand for freshly squeezed fruit juices with minimal alteration of nutritional and sensorial properties, minimally processed techniques for fruit juices aimed at reducing microbial populations and inactivating pathogenic bacteria have been developed. Typically, fruit juices processed at 80–90°C for 10-60 s to inactivate spoilage microorganisms and enzymes to enhance the shelf-life of refrigerated juices (Maghsoudi et al., 2023; Wójcik et al., 2024); however considerable alterations in the organoleptic properties of fruit juices have been observed (Patras et al., 2010). But, to achieve a 5-log reduction of pathogenic bacteria in high-acid products the minimal processing can be applied around 70°C for 6 s (Kato et al., 2003). Therefore, replacing conventional pasteurization with milder pasteurization processes in fruit juices should be considered.

Also, the destruction of microorganisms using clean technologies, for example microwave processing has been developed on semi-industrial and commercial scales particularly in citrus juice pasteurization. Microwave pasteurization methods have effects similar to conventional processing, but they can improve product quality and reduce processing time (Wójcik et al., 2024). The lethality of microorganisms using radio frequency and microwaves has been studied and discussed (Canumir et al., 2002; Kozempel et al., 1998; Tajchakavit et al., 1998; Wójcik et al., 2024).

Thermal inactivation of microorganisms in various fruit juices and vegetables has been reported by several authors (Gomes et al., 2023; Liao et al., 2010; Noci et al., 2008; Wójcik et al., 2024). However, little information is available on the inactivation of yeasts and pathogenic bacteria using mild heat and microwave treatments specifically in pomegranate juice. Pomegranate juice is a matrix rich in bioactive compounds that may affect microbial resistance and heating behavior. This study was designed to investigate the effects of mild heat and microwave treatments

on the inactivation of *E. coli* and *S. cerevisiae* inoculated into pomegranate juice from two commercial Iranian cultivars.

MATERIALS AND METHODS

Chemicals and microbiological media

Malt extract agar and nutrient agar (Difco Laboratories, Detroit, USA), sorbitol MacConkey agar (Liofilchem, Teramo, Italy), dichloran rose bengal chloramphenicol agar (Merck, Darmstadt, Germany) were used in this study. KH_2PO_4 , NaOH and NaCl were purchased from Merck (Darmstadt, Germany). Phosphate-buffered saline (PBS) was prepared based on the formulation recommended by Gabriel and Nakano (Gabriel & Nakano, 2011).

Preparation of pomegranate juice

Fresh and commercially matured pomegranate cultivars of Malase Momtaze Saveh and Alak Saveh were bought from the Agricultural Research Center of Saveh, Iran. After deleting defective ones, each fruit was washed, drained, peeled, and then cut into pieces to separate the arils manually. Next, their juices were extracted by means of a hand press device. The juices of Malase Momtaze Saveh arils (MMSA) and Alak Saveh arils (ASA) were immediately centrifuged at 10,000 rpm for 2 min at 4°C with a refrigerated centrifuge (Sigma 3-30K, Osterode am Harz, Germany) and subsequently refrigerated until analysis. The experimental procedure of microbial inactivation by mild heat and microwave treatments is schematically illustrated in Fig. 1.

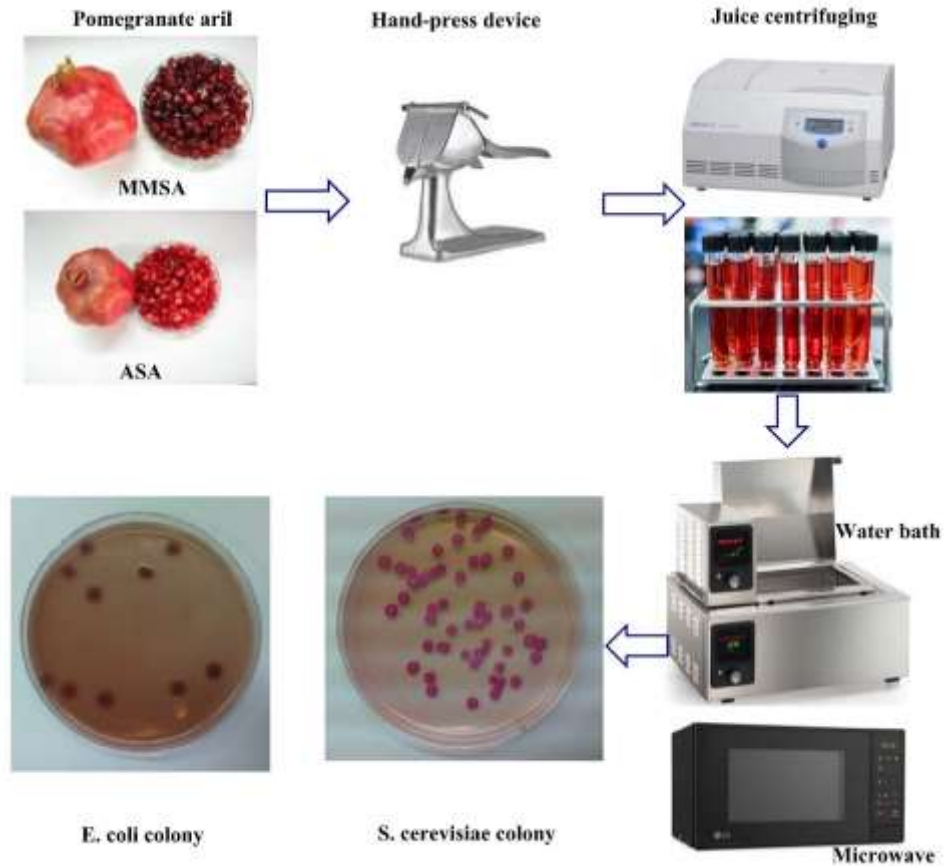


Figure 1. Schematic illustration of pomegranate juice preparation, including manual aril pressing and centrifugation, and subsequent microbial inactivation by mild heat and microwave treatments.

Preparation of inoculums and inoculation of test samples

According to recommendation of previous researchers, in this study *E. coli* and *S. cerevisiae* were nominated as spoilage and human illness microorganisms (Neggazi et al., 2025; Tournas et al., 2006). *E. coli* (RITCC 1177) and *S. cerevisiae* (PTCC 5052) were purchased from the Razi Institute Type Culture Collection and the Persian Type Culture Collection (Tehran, Iran), respectively. Lyophilized *E. coli* and *S. cerevisiae* were revived on nutrient agar and malt extract agar, and subsequently maintained at 4°C. The cells of *E. coli* and *S. cerevisiae* were separately loop-inoculated in nutrient broth (pH 7.0) and malt extract broth (pH 7.0), and then incubated at 35°C and 27°C, respectively for nearly 24 h in order to obtain cells in the early stationary phase (Gabriel & Nakano, 2011; Guerrero et al., 2001).

The resulting inoculated broth media were centrifuged at 8000 rpm for 5 min, and the precipitated cells were aseptically re-suspended in a 50-mL sterile phosphate-buffered saline (PBS, pH 7.2) solution and used as the inoculums. The acclimatization time of the re-suspended cells was limited to a maximum of 20 min prior to inactivation (Gabriel & Nakano, 2011). Then, 1 mL aliquot of *E. coli* and *S. cerevisiae* in PBS was re-suspended in 100 mL sterile pomegranate juice.

Mild heat treatment of inoculated juices

The clarified pomegranate juices (MMSA and ASA) were sterilized by autoclaving at 121°C for 15 min to eliminate native microbiota prior to inoculation. The sterilized juices were then used for microbial inactivation studies. The inactivation of *E. coli* and *S. cerevisiae* carried out using mild heat as a thermal processing. For the heat inactivation studies, 9.9 mL of sterilized pomegranate juices in glass test tubes (24 mm i.d.) were preheated to 52, 54, 56, 58 and 60°C on a shaking water bath (Memmert, WNE 14, Germany). The microbial cells (0.1 mL) were inoculated into each of the tubes. Then, the tubes with agitating were heated at considered

temperatures for 0, 30, 60, 90 and 120 s. After heat treatments, tubes were immediately immersed and kept in an ice bath until survivor enumerations.

Microwave treatment of inoculated juices

The 99 mL of sterilized pomegranate juices inoculated with 1 mL of *E. coli* and *S. cerevisiae* cells in the Schott-Duran bottles under sterile conditions. A 2450 MHz domestic microwave oven (LG, 180-900 W) was used in microwave inactivation tests. Pomegranate juices were batch pasteurized into microwave oven at two microwave powers for different times, base on receiving same energy at each power. Samples were treated at 600 W for 0, 45, 60, 67.5, 75, 80, and 90 s; and 900 W for 0, 30, 40, 45, 50, and 60 s. Treatment times were selected based on preliminary experiments to achieve similar total energy delivery at each power level while preventing sample boiling. After microwave treatments, tubes were immediately immersed and kept in an ice bath until survivor enumerations.

Survivor enumeration and decimal reduction times calculations

Tubes containing the inactivated cells were serially diluted with PBS and then, the number of surviving *E. coli* were determined by surface-plating on sorbitol MacConkey agar and incubating at 35°C for 48 h. The number of surviving *S. cerevisiae* determined by surface-plating on dichloran rose bengal chloramphenicol agar and incubating at 25°C for 72 h. Then, the log reduction and D-values (decimal reduction) of the heat and microwave treated juices were estimated. Survival curves were drawn on a semi log graph by plotting the logarithmic number of colony-forming units per milliliter against heating times of the treatments. D-value is the negative inverse slope of the plot represented the heating time of the treatments required to inactivate 90% of the microbial population (Guerrero et al., 2001). The log counts of colony-forming units for each power and heating time

were averaged, mean values plotted and curves were fitted by linear regression.

Statistical analysis

All experiments were performed in triplicate using a completely randomized design and data were analyzed with SAS software version 9.2 (SAS Institute, Inc.). Data were analyzed using analysis of variance (ANOVA), followed by Duncan's multiple range test for mean separation ($P < 0.05$).

RESULTS AND DISCUSSION

The results of some physicochemical characteristics of the studied pomegranate juice obtained from two pomegranate cultivars of MMSA and ASA are presented in Table 1.

Table 1. Some physicochemical quality parameters of untreated pomegranate juices*

	MMSA	ASA
Soluble solids content (°Brix)	16.7±0.2b	17.2±0.1a
pH	3.56±0.01a	3.09±0.02b
Titrateable acidity (g/100mL)	0.81±0.01b	1.61±0.00a

* Values with different letters within a similar row are significantly different ($P < 0.01$). (Malase Momtaze Saveh arils (MMSA); Alak Saveh arils (ASA)).

Mild heat inactivation of inoculated microbes in juice

Survival curves of *E. coli* and *S. cerevisiae* inoculated into pomegranate juice at mild heat treatment (52, 54, 56, 58 and 60°C for 0 to 120 s) are shown in Fig. 2. Survival curves plotted the number of microorganisms against heating time. In general, microbial load reduction by increasing the temperature from 52 to 60°C was significant. Mild heat treatment of juice samples reduced the *E. coli* and *S. cerevisiae* population by 1.20-6.65 and 0.43-5.05 log cycles, respectively. Using a linear model at the studied temperatures/times (52-60°C for 0-120 s), the D-values of *E. coli* were in the range of 17.15 s at 60°C and 107.95 s at 52°C. Also, in the same temperatures/time conditions, the D-values of *S. cerevisiae* were in the range of 18.62 s at 60°C and 375.02 s at 52°C. The z-values of *E. coli* and *S. cerevisiae* were 13.69 and 31.25°C, respectively. D-values at higher temperatures were significantly lower than those at lower temperatures.

Based on our results, it was found that inactivation of microorganisms at high temperatures is independent of juice types, but the temperature and residence time had a significant effect on the inactivation of these microorganisms. The D-values indicated that *E. coli* was more heat-sensitive than *S. cerevisiae* at lower temperatures and was inactivated more rapidly. However, at higher temperatures, *S. cerevisiae* was more sensitive to heat than *E. coli*. Thus, the *S. cerevisiae* population fell below the detection limit after treatment at 58 °C for 120 s, while *E. coli* required 60 °C for 120 s to reach the same level.

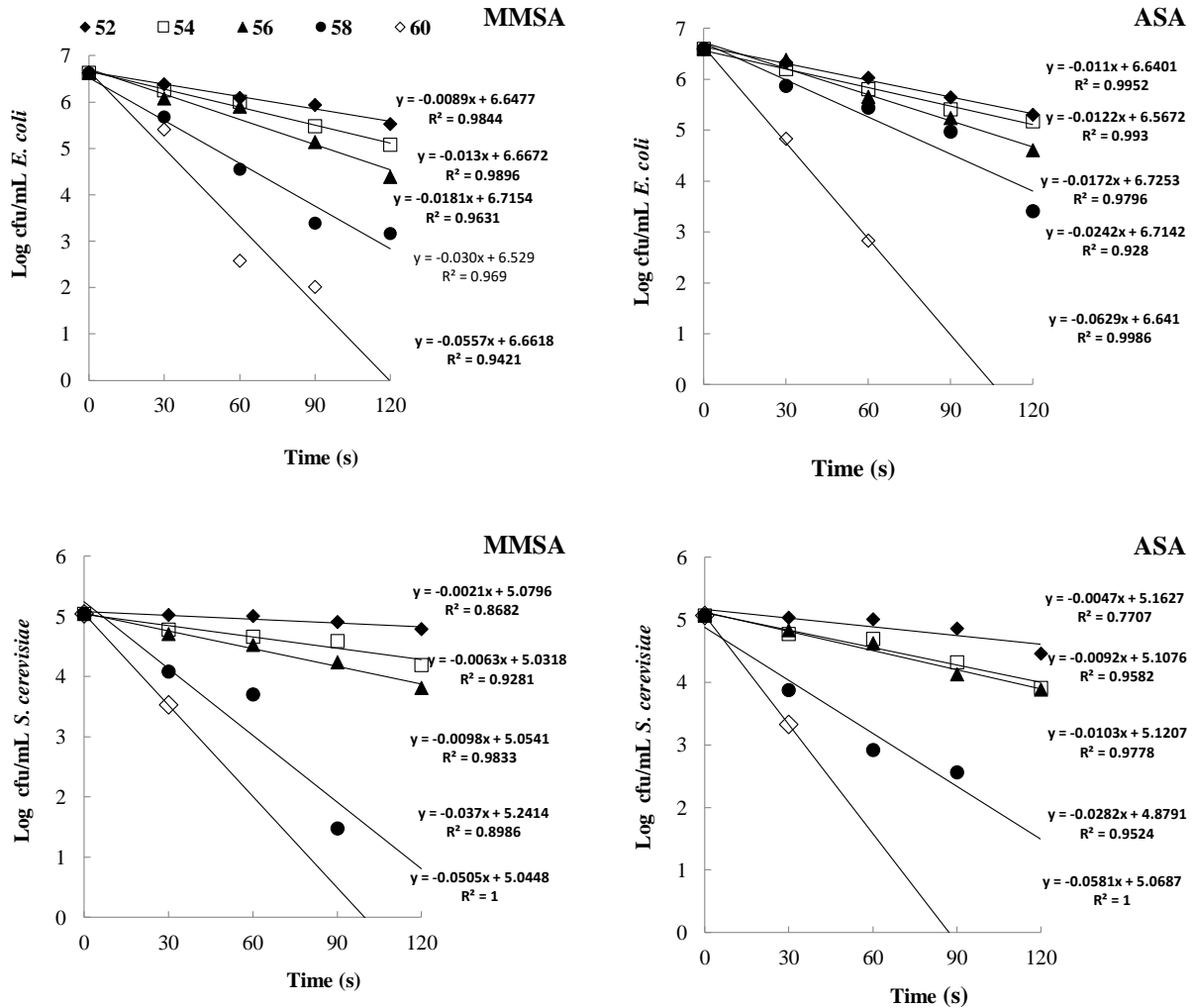


Figure 2. Effects of temperatures/times combination (mild heat at 52-60°C for 0-120 s) on survival of inoculated *E. coli* and *S. cerevisiae* in the pomegranate juices (Malase Momtaze Saveh arils (MMSA); Alak Saveh arils (ASA)).

Several studies reveal that mild heat treatments (40-60°C) can effectively reduce microbial populations through targeted cellular disruption. Chen et al. showed dramatic *E. coli* reduction in broccoli sprouts (Chen et al., 2022). Sado Kamdem et al. demonstrated enhanced Salmonella inactivation when combining essential oils with mild heat (Sado Kamdem et al., 2015), and Zhang et al. observed effective microbial inactivation in liquid systems using mild heat (40-50°C) combined with plasma-activated water against *S. cerevisiae*, achieving a 4.40 log reduction (Zhang et al., 2020). Liao et al. (2010) reported that aerobic bacteria survived

>26.3% at 37–42°C, with significant reduction at 47°C and complete inactivation (3.9 log cycles) at 62°C. Molds and yeasts exhibited greater temperature sensitivity than aerobic bacteria, achieving complete inactivation (3.9 log cycles) at 57–62 °C. At 57 °C, aerobic bacteria were reduced by only 2 log cycles, whereas molds and yeasts were entirely eliminated. These results are in accordance with our findings. Furthermore, the reduction of 6 and 6.7 log cycles of aerobic bacteria with an initial population of 109 cfu/mL at temperatures of 72°C and 94°C for 26 s was reported (Noci et al., 2008). In other research *E. coli* (K-12 and 0157:H7), Salmonella (enteritidis

and typhimurium) and *Listeria monocytogenes* (AS-1 and M24-1) were inoculated to the phosphate buffer saline and apple juice separately and were exposed to UV and heat processing (55°C). The D-values showed that *Listeria monocytogenes* and *E. coli* 0157:H7 had the highest resistance when inoculated into phosphate buffer (41.4 min) and apple juice (43.4 min), respectively (Gabriel & Nakano, 2009). Also, the heat resistance of *Listeria monocytogenes* was relatively higher than *Salmonella* and *E. coli* 0157:H7 in cantaloupe juice at heating temperature of 57°C (Sharma et al., 2005). However, similar study was found the lower heat resistances of *Listeria monocytogenes* than *Salmonella* and *E. coli* inoculated into watermelon juice (Gabriel & Nakano, 2009).

The observed microbial inactivation in pomegranate juice follows log-linear survival curves indicating first-order kinetics, where temperature and holding time serve as primary determinants of lethality (Zhang et al., 2020). Higher D-values at lower temperatures (e.g., 52°C) demonstrate slower inactivation rates, while increased temperatures accelerate the thermal destruction process. The kinetic parameters reveal distinct z-values between microorganisms (13.69°C for *E. coli* vs. 31.25°C for *S. cerevisiae*), indicating species-specific temperature sensitivity patterns. Heat treatment induces oxidative stress and membrane permeabilization, resulting in leakage of critical intracellular compounds including ATP, nucleic acids, and proteins (Davidson et al., 1996). Growth rate significantly influences thermal vulnerability, with faster-growing *E. coli* cells demonstrating increased susceptibility to heat damage compared to *S. cerevisiae* (Berney et al., 2006). These cellular response differences explain the temperature-dependent reversal in sensitivity observed between the two microorganisms. In acidic pomegranate juice, *E. coli* benefits from RpoS-mediated general stress responses that confer cross-protection, enhancing heat resistance at lower temperatures through acid-induced adaptive mechanisms (Berney et al., 2006). Conversely, *S. cerevisiae* relies primarily

on antioxidant enzyme systems (catalase, superoxide dismutase, cytochrome c peroxidase) rather than robust heat shock protein cross-protection (Davidson et al., 1996). This mechanistic distinction accounts for *S. cerevisiae* increased vulnerability at higher temperatures, where its oxidative stress defense systems become overwhelmed by thermal stress.

Microwave inactivation of inoculated microbes in juice

In this study, the effect of microwave treatment on the survival of *E. coli* and *S. cerevisiae* in pomegranate juice was investigated using a 2450 MHz domestic microwave oven. The juice samples were processed at microwave powers of 600 W (for 0, 45, 60, 67.5, 75, 80, and 90 s) and 900 W (for 0, 30, 40, 45, 50 and 60 s) based on achieving approximately the same energy for each sample. The survival curves of *E. coli* and *S. cerevisiae* are presented in Fig. 3, plotting microbial counts against microwave exposure time. The results showed that microwave treatment led to a considerable reduction in the inoculated microbial population. The rate of inactivation showed no significant difference between the two power levels (600 and 900 W) when adjusted for equivalent total energy delivery, indicating that microbial reduction depended primarily on total energy input rather than power level alone.

Microwave pasteurization at 600 W (90 s) and 900 W (60 s) achieved reductions of approximately 6.65 log cycles for *E. coli* and 5.06 log cycles for *S. cerevisiae*. The inactivation rate was initially low but increased with longer exposure times, resulting in non-linear survival curves. Thus, as shown in Fig. 3 the survival curves for both microorganisms at the two microwave powers did not follow a linear model ($R^2 < 0.80$). Based on these results, microbial inactivation was unaffected by juice type but strongly influenced by exposure time. The results also indicated that *S. cerevisiae* was more sensitive than *E. coli* at both microwave powers and was inactivated more rapidly. Thus, the *S. cerevisiae* population fell below the detection

limit at 600 W (80 s) and 900 W (50 s), respectively. However, the *E. coli* population was

fully inactivated at 600 W (90 s) and 900 W (60 s), respectively.

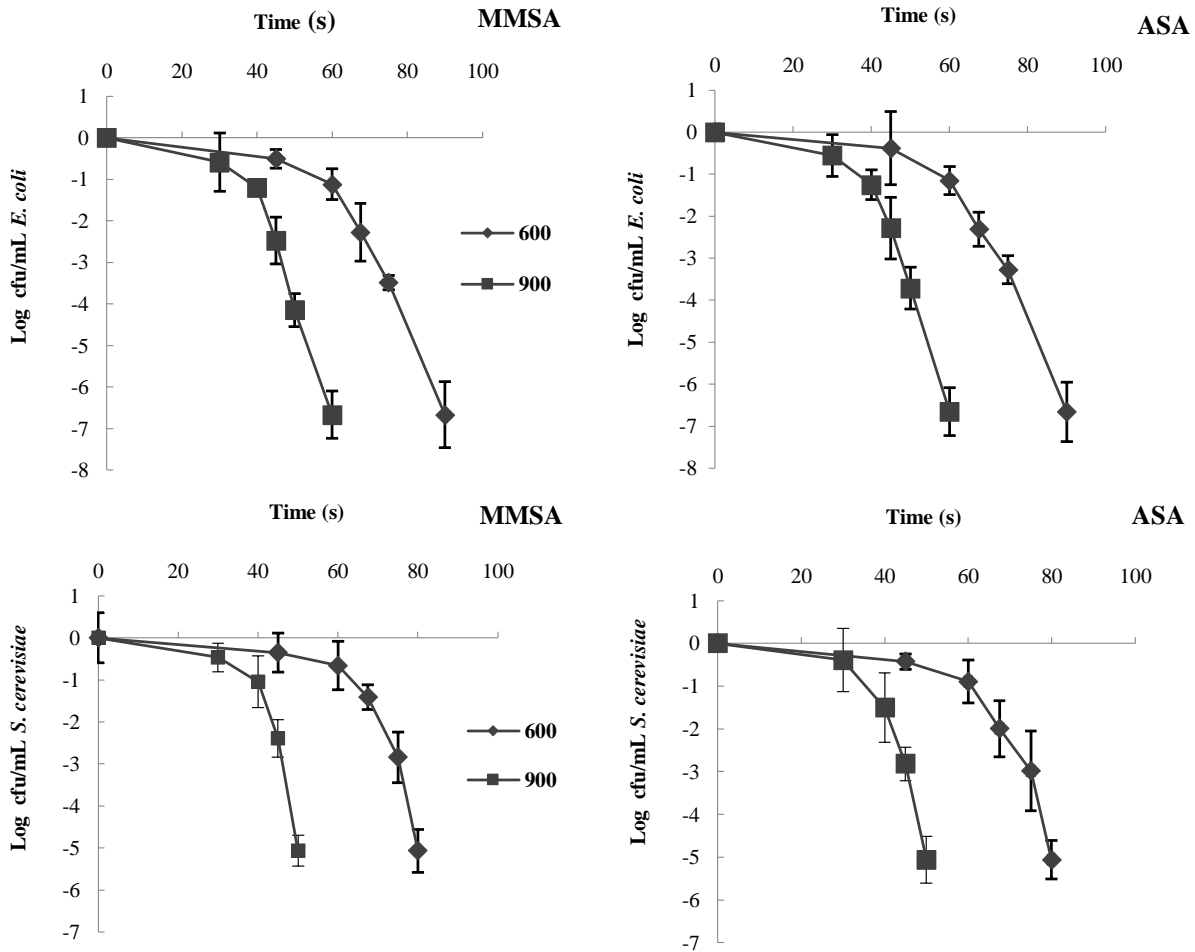


Figure 3. Effects of microwave powers/times combination (600 and 900 W for 0-90 and 0-60 s, respectively) on survival of inoculated *E. coli* and *S. cerevisiae* in the pomegranate juices (Malase Momtaze Saveh arils (MMSA); Alak Saveh arils (ASA)).

Microwave pasteurization of fruit juices shows variable microbial inactivation efficacy. Mendes-Oliveira et al. achieved up to 7-log reductions of *E. coli* O157:H7 and *S. typhimurium* in apple juice at 80–90°C for 5–25 s, with effectiveness increasing with power, temperature, and duration (Mendes-Oliveira et al., 2020). Kernou et al. reported complete 8-log *E. coli* inactivation using combined microwave-ultrasound treatment (Kernou et al., 2023). Canumir et al. obtained 2–4 log reductions of *E. coli* in apple juice (2450 MHz, 270–900 W, 60–90 s), equivalent to conventional pasteurization (83°C, 30 s), with no

significant difference in D-values between higher powers, indicating primarily thermal mechanisms (Canumir et al., 2002). Tajchakavit et al. found that continuous-flow microwave heating (2450 MHz, 700 W, 52.5–65°C) inactivated *S. cerevisiae* and *L. plantarum* ~6 times faster than conventional heating, with lower D-values despite similar first-order kinetics (Tajchakavit et al., 1998). Gentry and Roberts reported ≥ 5.2 -log reductions of *E. coli* in apple cider using continuous-flow microwave (900–2000 W) (Gentry & Roberts, 2005).

The debate on microwave microbial inactivation mechanisms focuses on thermal versus potential non-thermal effects (Heddleson & Doores, 1994; Kozempel et al., 1998; Shaw et al., 2021; Vadivambal & Jayas, 2010), with proposed non-thermal mechanisms including selective heating, electroporation, membrane rupture, and cell lysis. However, decades of research have established that inactivation is predominantly thermal. Early studies demonstrated that microorganisms are inactivated only in the presence of water, while dry or lyophilized cells remain unaffected even after prolonged exposure (Vela & Wu, 1979). Fujikawa et al. showed predictable thermal destruction patterns in *E. coli* with three-phase linear survival curves (Fujikawa et al., 1992). More recent studies reinforced this: Gedikli et al. attributed lethality to generated heat (Gedikli et al., 2008), and Canumir et al. confirmed heat-driven *E. coli* inactivation via D-values (Canumir et al., 2002). However, Atmaca et al. suggest non-thermal contributions, the overwhelming evidence supports thermal mechanisms as primary, with calls for more precise experiments to resolve the ongoing controversy (Atmaca et al., 1996).

CONCLUSIONS

Both mild heat and microwave treatments effectively achieved at least 5-log₁₀ reductions ($\geq 5D$) of *E. coli* and *S. cerevisiae* in pomegranate juice under the studied conditions. *S. cerevisiae* was generally more sensitive than *E. coli* to both mild heat and microwave treatments. Microwave processing provided significantly faster microbial inactivation than conventional mild heat treatment at equivalent lethality levels. Furthermore, the type of pomegranate juice had no significant influence on the inactivation efficacy of either microorganism in both mild heat and microwave treatments.

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