



Analysis of the combinative effect of ultrasound and microwave power on *Saccharomyces cerevisiae* in orange juice processing



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ABSTRACT

High temperature in conventional method for juice pasteurization causes adverse effects on nutrients and nutritional value of food. The objective of this study was to examine the effect of microwave output power, temperature, ultrasound power, and ultrasonic exposure time on *Saccharomyces cerevisiae* in orange juice. Based on our findings, microwave output power, ultrasound power, ultrasonic exposure time orange juice temperature were the most effective factors to reduce *S. cerevisiae*. The results showed that the quadratic model included was the best model for account. The model showed that regarding decrease of *S. cerevisiae* account microwave-induced temperature was more effective than microwave output power. Also, compared to microwave power, the ultrasound power was more effective on *S. cerevisiae* reduction. The optimum processing condition was 350 W microwave power, 35 °C temperature, 778.2 W ultrasonic power, and 11 min of exposure. Based on our result, the consumption energy was 142.77 J/mL with no remaining of *S. cerevisiae*. The results showed that the given scores by panelists to the combinative and conventional methods for color and flavor indices were significant ($P < 0.05$).

Industrial Relevance: In order to reduce the adverse effects (loss of vitamins, flavor, and non-enzymatic browning) of the thermal pasteurization method, other methods capable of inactivation of microorganisms can be applied. In doing so, non-thermal methods are of interest, including pasteurization using high hydrostatic pressure processing (HPP), electric fields, and ultrasound waves. The ultrasound technology has been the main focus of studies in recent years. However, the main challenge facing the non-thermal technologies in food processing is the inactivation of pathogenic microorganisms and food spoilage agents, which can be achieved by various methods. The aim of present research was examined simultaneous effect of ultrasonic and microwave to remove microorganism. This research introduces new, innovative, and combined method for fruit juice pasteurization, and this method can benefit the food industry.

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1. Introduction

Today, with the advancement of knowledge and technology in food quality control, consumers' expectations have been increased for fresh food products with least amount of chemical preservatives and/or processing conditions. There is a remarkable trend among people towards the consumption of juices which are directly produced from fresh fruit (not in the form of concentrates), which distribute in refrigerator

conditions and having a relatively short shelf life (Timmermans et al., 2011). Since antioxidant and functional food products are of the top 10 topics in the food industry, producers tend to maintain the maximum quality characteristics in these types of products. The importance of fruit juice processing is inevitable, because unpasteurized juices have low shelf-time due to their high microbial load and low taste and favor, as compared with processed juices.

In order to reduce the adverse effects (loss of vitamins, flavor and non-enzymatic browning) of the thermal pasteurization method, other methods capable of inactivation of microorganisms can be applied. In doing so, non-thermal methods are of interest, including pasteurization using high hydrostatic pressure processing (HPP), electric fields, and ultrasound waves (Aronsson, Lindgren, Johansson, & Rönner, 2001; Mertens, 1992; Toepfl, Heinz, & Knorr, 2007). The ultrasound technology has been the main focus of studies in recent years,

Abbreviations: N_0 , Primary count; N , Secondary count; MP, Microwave power; T , Sample temperature; UP, Ultrasonic power; UI, Ultrasonic intensity; T , Ultrasonic exposure time; C_p , Heat capacity; D , Internal probe diameter; $\beta_0 \beta_j, \beta_{ij}, \beta_{jj}$, Regression coefficients for intercept, linear, interaction and quadratic coefficients; X_i and X_j , Coded independent variables; E , The error.

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which mainly uses the frequency range of 20 kHz to 10 MHz (Knorr, Zenker, Heinz, & Lee, 2004; Valdramidis, Cullen, Tiwari, & O'Donnell, 2010). However, the main challenge facing the non-thermal technologies in food processing is the inactivation of pathogenic microorganisms and food spoilage agents, which can be achieved by various methods. According to the literature, the non-thermal methods not only totally eliminate the microorganisms but also decrease or in some cases completely remove the heat required for the destruction of microorganisms (Piyasena, Mohareb, & McKellar, 2003). Moreover, different studies have reported on the capability of ultrasound in deactivating microorganisms and enzymes (De Gennaro, Cavella, Romano, & Masi, 1999; Hosseinzadeh Samani, Khoshtaghaza, Minaee, & Abbasi, 2015; Samani, Khoshtaghaza, Minaee, & Abbasi, 2013; Samani & Lorigooini, 2015).

A novel thermal processing method using a heating mechanism other than the direct thermal method is called microwave. Microwave heating is widely used in the food industry because of its reduced processing time and costs, enhancing product uniformity and yields, improving unique micro-structure and protecting food from surface browning and crusting (Acierno, Barba, & d'Amore, 2004; Badeka, Pappa, & Kontominas, 1999; Huang, Sheng, Yang, & Hu, 2007). Many studies have reported a faster eradication of *Saccharomyces cerevisiae*, *Lactobacillus plantarum*, and *E. coli* in vinegar and apple juice compared with conventional pasteurization practices (Cañumir, Celis, de Bruijn, & Vidal, 2002; Igual, García-Martínez, Camacho, & Martínez-Navarrete, 2010; Tajchakavit, Ramaswamy, & Fustier, 1998).

However, to date, no studies have been reported in the literature on simultaneous effect of microwave and ultrasound on *Saccharomyces cerevisiae* in orange juice. This study aims to develop a combined microwave-ultrasonic system and examine its effectiveness on *Saccharomyces cerevisiae* in orange juice.

2. Materials and methods

2.1. Preparation of orange juice samples

For this purpose, a certain amount of orange fruit of Thompson navel variety was purchased from local markets. First, the fruits were washed and sliced. The prepared fruits were then dewatered using an electric juicer. In order to separate pulp suspensions and tissue components, the extracted juice was poured into a centrifuge with the speed of 6000 rpm (4307 g) for 20 min. For complete separation of the remaining suspended particles, the transparent portion of the extract was passed through a Whatman filter paper using a vacuum pump. Afterwards, the samples were poured into a reactor with diameter and height of 80 and 50 mm, respectively. It is necessary to mention that the dimensions of the reactor were optimized during pretests.

2.2. Microbial testing method

Initially, the mediums and instruments used in microbial testing were sterilized by autoclave. In order to preserve the sterile conditions, microbial mediums were treated under the hood. Ampoules containing standard strains (desiccated by freezing method) were opened, and *S. cerevisiae* was cultured on agar malt extract medium. Later, it was transferred to the liquid medium following the incubation process. In so doing, a loop filled with microbial level grown on agar was inoculated to 25 ml malt extract agar for providing microbial suspension under sterile conditions. Subsequently, the substance was incubated (Gerhardt, Königswinter, Germany) for 24 h 37 °C. To provide dilution of equal microbial basis in different mediums, the resulting mediums were diluted by relevant liquid medium so that the turbidity would be similar in 600 nm wavelength. The liquid medium containing grown cells was centrifuged for 5 min by 8000/min (Sigma 3-30 K, Osterode am Harz, Germany) and the resulting cell mass was suspended in sterilized orange juice. 3 ml of this suspension was inoculated to 300 ml

of orange juice. Subsequently, prior to inactivation studies, this cellular suspension was preserved for adaptation to new context for 15–30 min (Gabriel & Nakano, 2011).

To determine the amount of living cells, processed samples with different treatments were diluted by phosphate buffer frequently and their culturing was conducted on specific medium. *S. cerevisiae* pellet were incubated in 30 °C and they were counted after 48–72 h (Gabriel & Nakano, 2009; Liao, Zhang, Hu, & Liao, 2010). It is worth noting that microbial level of *S. cerevisiae* (RITCC 1177) was obtained from Iran Industrial and Scientific Research Organization.

2.3. Microwave and ultrasound processing

The effect of microwave-ultrasound combination on pasteurization of orange juice was studied. In doing so, the destruction level of *S. cerevisiae* was considered as an indicator for the effectiveness of these waves on microorganisms. To this effect, the selected variables included microwave power, sample temperature, the ultrasound power, and exposure time of samples to ultrasound. The RSM using the central composite design (CCD) was employed for data analysis and optimization (Table 1). In order to obtain the optimum value using the RSM, Eq. (1) was used (Halim, Kamaruddin, & Fernando, 2009):

$$Y_i = \beta_0 + \sum \beta_i X_i + \sum \beta_{ij} X_i X_j + \sum \beta_{ij} X_i^2 + \varepsilon \quad (1)$$

A schematic of set up used for the experiment is shown in Fig. 1. In order to supply uniform ultrasonic waves, a 1000 W electric generator (Model MPI, Switzerland) working at 20 ± 1 kHz frequency was used. To complete the processing system, a microwave oven (Samsung, 800 W, Korea) was placed in line. It is noteworthy that the power of consumption was continuously recorded using a power analyzer (Lutron, DW-6090) throughout the experiment.

The actual input power from each device is converted to heat, which is dissipated in the medium. The actual ultrasound power was determined by calorimetry, calculated as shown in Eq. (2) (Fayyazi, Ghobadian, Najafi, Hosseinzadeh, & Montazeri, 2012; Hosseinzadeh, Khoshtaghaza, Minaei, Zareiforoush, & Najafi, 2013; J Mason, Chemat, & Vinatoru, 2011; Pingret et al., 2012):

$$P = m \times C_p \times \frac{dT}{dt} \quad (2)$$

where C_p is the heat capacity of the solvent at constant pressure ($J g^{-1} K^{-1}$), m is the mass of solvent (g), and dT/dt is the temperature rise per second. The C_p was calculated from the slope of the curve from internal energy variation as a function of temperature at constant volume using a calorimeter. The consequent ultrasonic intensity (UI) was calculated for each ultrasonic probe using the calculated power (from Eq. 2) as shown in Eq. (3).

$$UI = \frac{4P}{\pi D^2} \quad (3)$$

where UI is the ultrasonic intensity ($W mm^{-2}$), P is the ultrasound power (W) as calculated by Eq. 2, and D is the internal diameter (mm) at the tip of the probe.

Table 1
Various levels of independent variables with regards to the response surface method.

Independent variable	Range of level				
	−α 1	−1	0	1	α
Microwave power (W)	200	350	500	650	800
Temperature (°C)	20	30	40	50	60
Ultrasound intensity (W/mm ²)	0.11	0.16	0.20	0.24	0.30
Ultrasound exposure time (min)	3	6	9	12	15

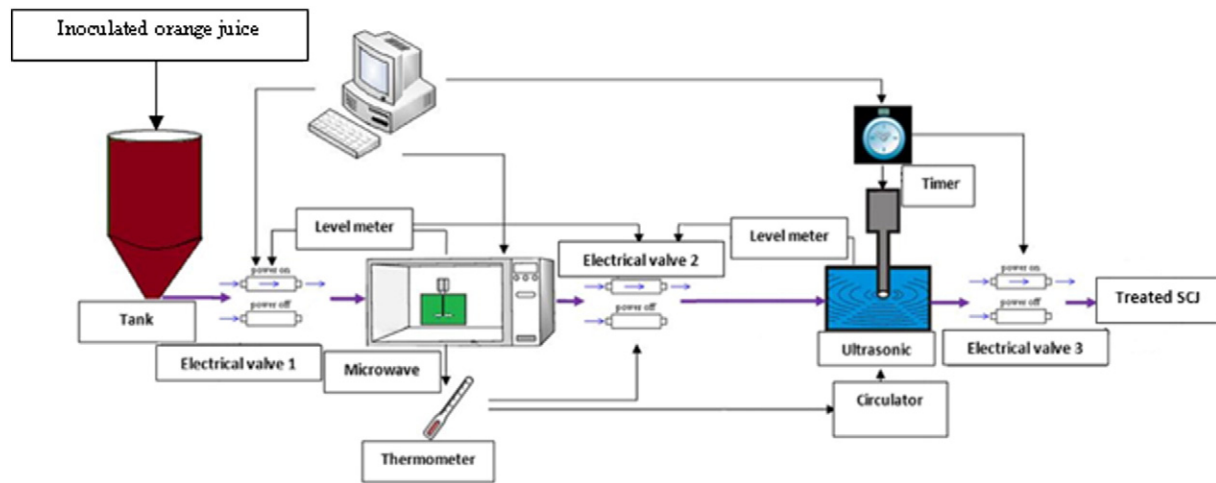


Fig. 1. Schematic of the designed microwave-ultrasound processing system.

First, the inoculated orange juice was poured into the system's tank. The experimental treatments were set according to Table 1. In this table, the UI values of 0.11, 0.16, 0.20, 0.24, and 0.30 W/mm² corresponded to ultrasonic power of 200, 400, 600, 800, and 100 W, respectively. Adjusting a level meter, 200 ml of orange juice was selected as the size of each sample. In this system, the samples were first heated in a microwave oven to a desired temperature and were then placed inside the ultrasonic reactor where they were exposed to ultrasonic waves. Laboratory circulating water (Sahand 1000i, Iran) was used to maintain the samples' temperature during sonication. Finally, the treated samples were removed from the system for the intended experiments.

2.4. Sensorial analysis

Consumer acceptability was studied by 40 (20 female, 20 male) untrained panelists ranging from 18 to 60 years of age. Prior to sensory evaluation, orange juice samples were refrigerated, randomly coded, and served (20 ml) at 15 °C together. The panelist seated separately in booths to allow an unbiased evaluation of the sensory attributes. Samples of orange juice processed by MW-US and conventional (heating treatment) pasteurization were compared in terms of product-specific color, odor, sweetness, acidity, flavor, and overall acceptability on a 9-point hedonic scale. When assigning scores to color, odor, flavor,

and overall acceptability, 1 had the lowest and 9 had the highest value. Conversely, optimum sweetness and acidity were graded at the midpoint of the scale.

3. Results and discussion

As the variance analysis of quadratic model indicates, the assumed model for the obtained data was significant, which were derived from reduction of *S. cerevisiae* within the juice sample. Also, non-significant status of lack of fit implies efficiency of the obtained model (Table 2). Adjustment determination coefficient and coefficient of variation (C.V) of model were 0.99 and 3.1, respectively. Variance analysis results (Table 2) show that other existing variables' coefficients were significant in 10% level in addition to the coefficient derived from multiplying ultrasonic power and time.

As it was observed, some of the coefficients of model were not significant and they were excluded from the studied model. Hence, the final equation was obtained as follows:

$$\log(N/N_0) = -0.14308 + 2.09108E-003 * MP - 0.091266 * T - 5.69899E-003 * UP + 0.070985 * t - 2.14998E-005 * MP * T + 1.49167E-006 * MP * UP - 5.46672E-005 * MP * t + 1.85749E-005 * T * UP - 1.07503E-003 * T * t - 1.87742E-006 * UP^2 + 7.75080E-004 * T^2 + 1.93270E-006 * UP^2 - 3.22356E-003 * t^2 \quad (4)$$

where N_0 and N were primary and secondary counts, MP was microwave power (W), T was the sample temperature (°C), UP was ultrasonic power (W), and t was the ultrasonic exposure time (min). Fig. 2 shows relation between experimental data and model data to reduction of *S. cerevisiae*.

The importance of orange juice final temperature derived from microwave power which caused such a temperature can be taken into account as contributing to reduction of *S. cerevisiae*. As Fig. (3) shows, increase of temperature has led to the reduction in slope of *S. cerevisiae*; however, increase of microwave power is characterized by slight slope compared to the temperature. In lower temperatures, the increase of microwave power does not affect the reduction trend of *S. cerevisiae*, but the increase of temperature to 50 °C results in reduction of *S. cerevisiae* trend due to higher rates of temperatures and lack of appropriate time for adapting the microorganisms to new contexts (Tajchakavit et al., 1998). Such derived results from Eq. (4) can be interpreted that multiplying microwave power and temperature gives a negative value and this indicated that the increase of each independent variable leads to higher negative values of Eq. (4) result so that it shows higher rates of reduction in *S. cerevisiae*.

Table 2

ANOVA results for first and second order coefficients using response surface method for *S. cerevisiae* values.

Source	Sum of squares	df	Mean square	P-value
Model	6.89	4	0.49	<0.0001
Microwave power (MP)	0.032	1	0.032	0.0018
Temperature (Temp)	3.56	1	3.56	<0.0001
Ultrasonic power (UP)	2.33	1	2.33	<0.0001
Ultrasonic exposure time (t)	0.31	1	0.31	0.0026
MP*temp	0.17	1	0.17	0.0108
MP*UP	0.031	1	0.031	0.0153
MP*t	0.009	1	0.009	0.0543
Temp*UP	0.022	1	0.022	0.0066
Temp*t	0.017	1	0.017	0.0153
UP*t	0.006	1	0.006	0.1145
MP ²	0.049	1	0.049	0.0003
Temp ²	0.16	1	0.16	<0.0001
UP ²	0.16	1	0.16	<0.0001
t ²	0.023	1	0.023	0.0057
Residual	0.033	15	0.002	
Lack of fit	0.015	10	0.001	0.8975
Pure/Error	0.019	5	0.003	
Total	6.93	29		

P-value < 0.10 denotes significant effect.

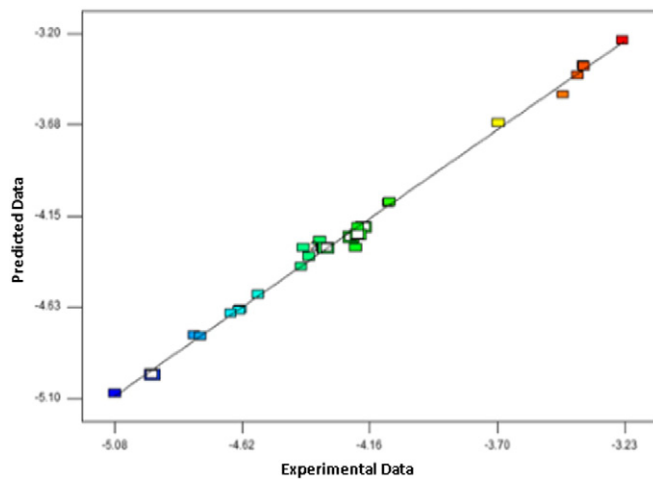


Fig. 2. Fitting of experimental data and model data in relation to reduction of *Saccharomyces cerevisiae*.

Compared to microwave power, ultrasonic power is more contributed to reduction of *S. cerevisiae* in orange juice (Fig. 3b). As it is seen in Fig. (3a, c, and e), the increase of ultrasonic power and exposure time leads to the fact that reducing slope of *S. cerevisiae* is more than the time that the microwave power increases. The increase of ultrasonic power results in increase of destruction effect of ultrasonic waves. One can contribute the reason of *S. cerevisiae* reduction to the increase of horn movement frequency of ultrasonic within the fluid. In addition, the increase of frequency leads to the increase in the number of formed bubbles in fluid which results in increasing cavitation (Vichare, Gogate, Dindore, & Pandit, 2001).

The increase in time of treatment with ultrasonic leads to reducing the number of *S. cerevisiae* in orange juice (Fig. 3c, d). This is due to the fact that the increase of ultrasonic exposure time leads to the increase of sonic stream in reactor and results in higher contributions of ultrasonic waves to *S. cerevisiae*. This is in line with the findings reported by researches in relation to other juices (Ugarte-Romero, Feng, Martin, Cadwallader, & Robinson, 2006; Wu, Gamage, Vilku, Simons, & Mawson, 2008).

Sample temperature and ultrasonic exposure time are taken into account as two important independent variables and effective elements to reduction in *S. cerevisiae*. Regarding the curve slope in line with temperature and time axis, one can say that temperature is more effective to reduction of *S. cerevisiae* when compared with the ultrasonic exposure time. Similar results have been proposed by other researchers (Ugarte-Romero et al., 2006; Wu et al., 2008).

Inactivation mechanisms of microorganisms are based on physical and chemical factors affected by ultrasonic wave on nutrition. Physical effects can be characterized by thinning of cellular membrane, generating local heating and changing of pressure (5500 °C and 50,000 kPa). In addition, chemical effects can be divided by producing free radicals such as hydroxyl and hydrogen radical in relation to sono-chemical reaction (Piyasena et al., 2003; Valdramidis et al., 2010). So, one can declare that reaching unsatisfactory level of inactivation is due to simultaneous effects if ultrasonic waves and generated temperature by ultrasonic (Lee, Zhou, Feng & Martin, 2009a).

Conditions of ultrasonic treatments such as the frequency, intensity, the positioning of horn in reactor, reactor geometric characteristics, method and place of sampling, sonic energy density and environmental characteristic play a great role in determining the rate of inactivation (H Lee, Zhou, Feng, & Martin, 2009b). Effectiveness and efficiency of ultrasonic in inactivating microorganisms are affected by physicochemical characteristic, nutrition amount, processing temperature, and ultrasonic intensity as well as duration (Piyasena et al., 2003). Hence, it is crucial that the details of experiment conditions are registered when it

comes to inactivating the microorganisms so that true comparisons of registered results are reported by research members.

The use of ultrasound on food products has proved to be advantageous in numerous processes. However, some modifications in the physicochemical parameters or structures of components and the degradation of some compounds have been increasingly reported. Studies show that the quality parameters of orange juice such as color, decrease of ascorbic acid, b-carotene contents and formation in hydroxyl radical can be changed by ultrasound treatment (Gómez-López, Orsolani, Martínez-Yépez, & Tapia, 2010; Pingret, Fabiano-Tixier, & Chemat, 2013; Vercet, Burgos, & López-Buesa, 2001; Valdramidis et al., 2010).

In a research work by Tiwari, O'Donnell, Muthukumarappan, and Cullen (2009), orange juice was treated with ultrasound at frequency of 20 kHz, power of 1500 W, sound energy density of 0.81–0.30 W/mm for 0–10 min in pulse mode. During the experiments, the temperature was controlled by water circulation at temperature of 25 ± 1 °C. Conventional thermal processing was also carried out at temperature of 98 °C for 21 s, and the samples were then kept in refrigerator (10 °C) for 30 days. They reported a significant reduction in the amount of ascorbic acid as a function of sound energy density and ultrasound exposing time. At the highest intensity and exposing time, the reduction in the amount of ascorbic acid was less than 5%. They also stated that the amount of ascorbic acid was better maintained in the ultrasound method, as compared with thermal treatments and control samples (Tiwari et al., 2009).

Vikram, Ramesh, and Prapulla (2005) compared the color appearance and vitamin C content of orange juice in four processing methods of infrared waves, ohmic process, microwave, and conventional treatment. They reported that the amount of molecular degradation in the microwave method was higher than those of the other processing methods due to uncontrolled generated heat during juice processing (Vikram et al., 2005).

Considering the kinetics of microbial inactivation, scientific discussions have been focused on non-thermal effects of microwave heating besides the deadly effects of heating (Vadivambal & Jayas, 2010). Basically, two approaches have been proposed for inactivation of microorganisms. The first suggests that the death of microorganisms is completely due to the heat which causes a change in the nature of proteins, enzymes abnormal activity, and membrane degradation (Heddleson & Doores, 1994). In the second approach, it is hypothesized that in addition to the heat, non-thermal effects are also involved in microorganisms inactivation. The developed theories for the heat effect strengthening include selective heating (effective heating of microorganisms by microwave radiation), electroporation (leaking due to pores creation in the cell membrane of microorganisms), destruction of cell membrane, and bacterial cell lysis (due to interaction of magnetic field and vital molecules) (Kozempel, Annous, Cook, Scullen, & Whiting, 1998). However, in most researches, eradication of microorganisms by microwave has been related to the thermal effects (Cañumir).

In a research work which had been carried out on apple juice, the eradication of *Saccharomyces cerevisiae* and *Lactobacillus plantarum* in the continuous-flow microwave treatment with applied power of 700 W, temperature range of 52.5–65 °C and flow rate of 174–230 mm/min was remarkably faster than that of conventional pasteurization method (Tajchakavit et al., 1998). Other researchers have reported that apple juice pasteurization and eradication of *Escherichia coli* could be done using microwave power in the range of 270–900 W with a time period of 40–90 s (Cañumir et al., 2002).

Cinquanta, Albanese, Cuccurullo, and Di Matteo (2010) studied the MW pasteurization of orange juice. The total of 7 carotenoid compounds were quantified during MW heating (at 70 °C for 1 min). They reported that zeaxanthin and beta-carotene content decreased by about 26%, while no differences ($P < 0.05$) were found for beta-cryptoxanthin. A slight decrease in vitamin C content was observed after MW heating. Results also indicated that MW heating with a fine temperature control could result in promising stabilization treatments (Cinquanta et al.,

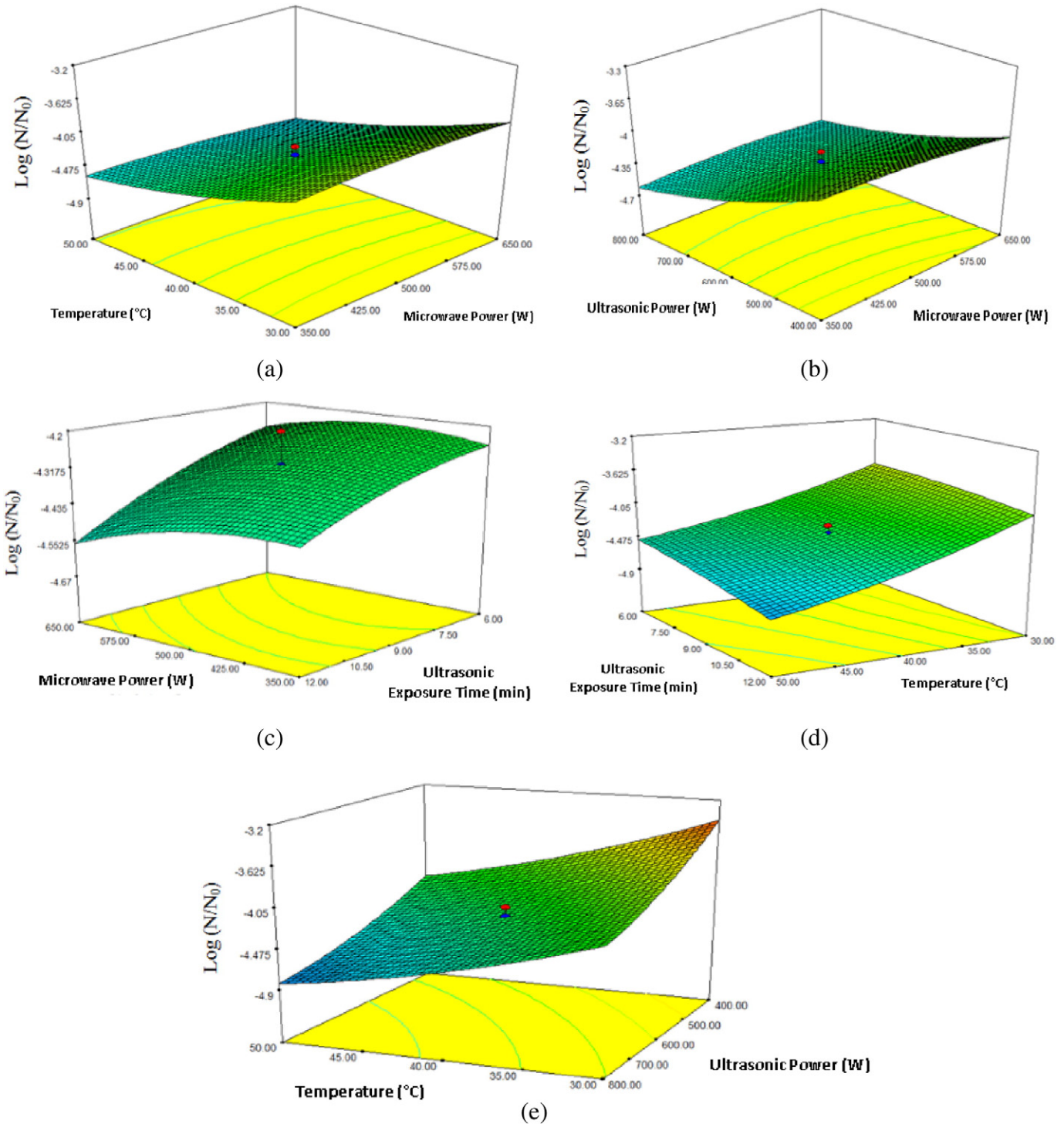


Fig. 3. Influence of treatment variables on *S. cerevisiae* reduction.

2010). The data were reported by Nikdel, Chen, Parish, MacKellar, and Friedrich (1993) showed that conventional heat pasteurization of orange juice sometimes results in an off flavor due to overheating of the juice at the heat-exchange surface. Heating with microwave energy heats the juice uniformly without changing the taste. Also, it caused complete inactivation of bacteria and pectin methyltransferase was obtained. There was no adverse effect on juice flavor. The results indicated that the continuous flow pasteurizer using microwave energy is effective for pulpy juice pasteurization. It offers a good alternative to conventional methods of pasteurization using steam-based heat exchangers (Nikdel et al., 1993).

Finally, optimization was conducted on the process. The target function was Eq. (2). The aim of optimization was to realize conditions pertinent to independent variables (microwave power, sample temperature, ultrasonic power, and duration of ultrasonic treatment) in which the amount of *S. cerevisiae* equals to zero in the sample using the least value of energy. Of the other boundary conditions of optimization can be regarded as the domain of independent variables which was experiment domain in the present study. Microwave power, sample temperature, ultrasonic power, and ultrasonic treatment duration in the optimum point were 350 W, 35 $^{\circ}\text{C}$, 778.2 W and 11 min, respectively. Having run the experiment in optimum point, *S. cerevisiae* was obtained

as zero and this indicates the confirmation of determined optimum point. It is worth noting that energy consumption of this process is 142.77 mJ/ml in which the energy consumption of ultrasonic system is negligible compared to that of the microwave. Energy consumption can be optimized through making variations in wave section of microwave which leads to concentration of waves.

3.1. Sensorial analysis of pasteurization process in combinative and conventional methods

The results showed that the given scores by panelists to the combinative and conventional methods for color (6.9 vs. 6.1) and flavor (4.0 vs. 4.9) indices were significant ($P < 0.05$), while in the case of acidity (4.9 vs. 5.1), odor (5.1 vs. 5.2), overall acceptability (5.3 vs. 5.4), and sweetness (5.3 vs. 5.3), the given scores were not significant ($P > 0.05$). One of the cases which was mentioned by all of the panel members, was to have a metal taste for the orange juice treated by the combinative method. This was possibly due to corrosion of the ultrasonic probe during the cavitation process and mixing the corroded particles with the juice. It should be mentioned that the appearance of the orange juice in the combinative method was better than those of conventional method (57% vs. 43%).

4. Conclusion

Microwave power, juice temperature, ultrasonic power, and ultrasonic treatment duration contribute to the amount of *S. cerevisiae*. The importance of final temperature of orange juice effect on reducing *S. cerevisiae* is considered higher than the importance of microwave power which leads to the temperature. Compared to microwave power, ultrasonic power has a higher impact on reducing *S. cerevisiae* in orange juice, increase of duration having to do with ultrasonic treatment results in reduction of *S. cerevisiae* in orange juice. This is due to the fact that increase of treatment duration results in increase of sonic cycles in reactor. Further, this results in higher impacts of ultrasonic waves on *S. cerevisiae*. Sample temperature and duration of ultrasonic treatment are taken into account as important and effective independent variables in relation to reducing *S. cerevisiae*. Achieving satisfactory inactivation level is affected by simultaneous effect of ultrasonic waves and generated temperature by ultrasonic. It should be mentioned that the appearance of the orange juice in the combinative method was better than those of conventional method (57% vs. 43%).

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