

Modeling the Simultaneous Effects of Microwave and Ultrasound Treatments on Sour Cherry Juice Using Response Surface Methodology

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ABSTRACT

This study aimed to examine the effectiveness of combined microwave-ultrasonic pasteurization system on *Escherichia coli* and vitamin C content in sour cherry juice (SCJ). Based on the findings, microwave output power, ultrasound power, and ultrasonic exposure time as well as the microwave-induced temperature were the most effective factors in reducing *E. coli* and vitamin C content. In addition, the microwave-induced temperature and ultrasonic exposure time, as independent variables, were both effective on *E. coli* removal. At higher temperatures, the effectiveness of ultrasonic waves as well as cavitation intensity declined. However, their combined effect (ultrasound and temperature) was more significant than their individual effect. It was also found that any increase in ultrasound power, ultrasonic exposure time, and microwave output power led to a significant reduction in vitamin C content, while the ultrasound power was the most effective. On the basis of RSM modeling, the optimum processing condition was: 352.21W microwave output power, 49.94°C temperature, 475.13W ultrasound power and 6 minutes of exposure time. On the basis of response surface methodology (RSM) modelling, the maximum vitamin C content was 142.5 mg per 100 mL with no remaining *E. coli*.

Keywords: Cavitation, Microwave output power, Pasteurization, Ultrasound power.

INTRODUCTION

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 (Toepfl *et al.*, 2007; Aronsson *et al.*, 2001; Mertens, 1992). The ultrasound technology has been the main focus of studies in recent years, which mainly uses the frequency range of 20 kHz to 10 MHz (Knorr *et al.*,

2004; Valdramidis *et al.*, 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 *et al.*, 2003). Moreover, different studies have reported on the capability of ultrasound in deactivating microorganisms and enzymes (Hosseinzadeh Samani *et al.*, 2013;

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Baumann *et al.*, 2005; De Gennaro *et al.*, 1999).

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 (Hosseinzadeh Samani *et al.*, 2013; Acierno *et al.*, 2004; Huang *et al.*, 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 *et al.*, 2002; Tajchakavit *et al.*, 1998). Using microwaves improved levels of ascorbic acid, total phenol content, and antioxidant activities compared to the conventional pasteurization method (Iguar *et al.*, 2010).

Utilization of microwave and ultrasonic is useful for inactivating *Alicyclobacillus* vegetative cells in laboratory medium. Wang *et al.* (2010) showed that there was a synergetic interaction in killing DSM 4006 between microwave and ultrasonic treatment while no such interaction with the other two strains was observed.

However, to date no studies have been reported in the literature on simultaneous effect of microwave and ultrasound on sour cherry juice (SCJ) pasteurization. This study aimed to develop a combined microwave-ultrasonic system and examine its effectiveness on *E. coli* and vitamin C content in SCJ.

MATERIALS AND METHODS

Preparation of SCJ Samples

Sour cherry fruit of Mashhad Champa variety was purchased from local markets and washed, cleaned, and cored. 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 6,000 rpm (4,307 g) for 20 minutes. 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 (Hosseinzadeh Samani *et al.*, 2013). 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.

Preparation of Microbial Suspensions

Once the lyophilized standard strain of *E. coli* vial was opened, the strain was cultivated on an agar nutrient culture (Micromedia, Hungary). Therefore, a loop of grown microbial strain on agar was inoculated to a 25 mL nutrient liquid culture under sterile conditions in order to prepare the microbial suspension. This was kept inside an oven for 18–24 hours at 37°C. The liquid culture containing the grown cells was centrifuged for 5 minutes at 8,000 rpm (7,656 g), so that the resulting cellular mass was suspended in sterile SCJ. A total of 3 mL of this suspension was inoculated to 300 mL of SCJ. For adaptation purpose, it was held for 15–30 minutes before the deactivation. It should be noted that the cultures used for primary activation and proliferation included nutrient solid/liquid cultures (Kuldiloke, 2002).

Microwave and ultrasound processing

The effect of microwave-ultrasound combination on pasteurization of SCJ was studied. In doing so, the destruction level of *E. coli* was considered as an indicator for the effectiveness of these waves on microorganisms. Moreover, the amount of remaining vitamin C was selected as a qualitative index. 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*, Equation (1) was used (Halim *et al.*, 2009; Mostafaei *et al.*, 2013):

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

A schematic diagram of the set up used for the experiment is shown in Figure 1. In order to supply uniform ultrasonic waves, a 1,000W electric generator (Model MPI, Switzerland) working at 20 ± 1 kHz frequency was used. To complete the processing system, a microwave oven (Samsung, 800 watt, Korea) was placed in line. It is noteworthy that the power consumption was continuously recorded using a power analyzer (Lutron, DW-6090) throughout the experiment.

First, the inoculated SCJ was poured into the system's tank. The experimental treatments were set according to Table 1. Adjusting a level meter, 200 mL of SCJ 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. (Figure 1)

E.coli Survival Counts

In order to determine the cells survival count, the treated and untreated samples were diluted using physiological serum and they were superficially cultivated on MacConkey sorbitol agar (Merck, Germany). *E.coli* plates were placed inside the oven at 35°C and the emerged colonies were counted after 48 hours (Kuldiloke, 2002).

Vitamin C Assay

Table 1. Various levels of independent variables with regards to the employed analysis method.

Independent variable	Range of level				
	$-\alpha$	-1	0	1	α
Microwave Power (W)	200	350	500	650	800
Temperature ($^\circ\text{C}$)	20	30	40	50	60
Ultrasound power (W)	200	400	600	800	1000
Ultrasonic exposure time (Min)	3	6	9	12	15

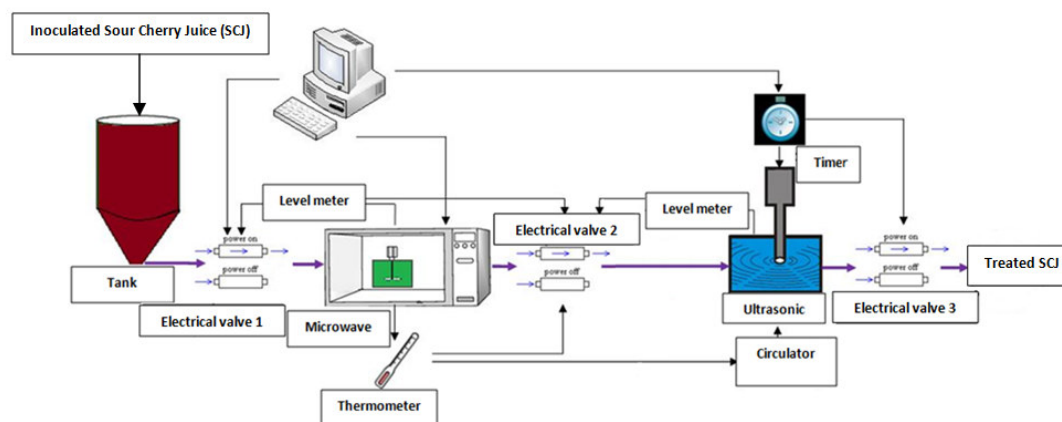


Figure 1. Schematic diagram of the designed microwave-ultrasound processing system.



Vitamin C content of SCJ was determined by 2,6-dichlorophenol-indophenol colorimetry using a Cecil spectrophotometer ($\lambda = 500$ nm) and reported as mg 100 mL⁻¹ SCJ (Burdurlu *et al.*, 2006).

RESULTS AND DISCUSSION

As the analysis of variance (ANOVA) results for the second order model show, the data from the surviving *E. coli* count was statistically significant. Also, the non-significance of the “lack of fit” indicates the effectiveness of the resulting model (Table 2). Except for the square of microwave power and temperature, all the other factors were significant ($P < 0.10$). The adjusted coefficient of determination and coefficient of variation (CV) of the model were 0.98 and 5.6%, respectively.

Table 3 shows the ANOVA results for the amount of vitamin C remaining after the process. The first and second order variables of microwave power, the interaction effect of microwave power and temperature, as well as

the interaction effect of temperature and the ultrasonic exposure time were not significant in the fitted model ($P < 0.10$). Since the “lack of fit” is non-significant and also the values for the coefficient of determination and coefficient of variation were 0.94 and 7.3%, respectively, it could be asserted that the accuracy of the developed model is acceptable.

As shown in Tables 2 and 3, some of the model coefficients were not significant and were excluded from the fitted model for simplification purposes. Therefore, the final equations were as follows:

$$\begin{aligned} \text{Log}(N/N_0) = & -4.36588 + 0.002734 \times \text{MP} + 0.171088 \times \text{Temp} - \\ & 0.0063 \times \text{UP} + 0.203052 \times t - 9.2\text{E-} \\ & 05 \times \text{MP} \times \text{Temp} + 2.08\text{E-}06 \times \text{MP} \times \text{UP} - \\ & 0.00022 \times \text{MP} \times t - 2.5\text{E-}05 \times \text{Temp} \times \text{UP} - \\ & 0.00458 \times \text{Temp} \times t + 0.000187 \times \text{UP} \times t - \\ & 0.00175 \times \text{Temp}^2 + 1.79\text{E-}06 \times \text{UP}^2 \end{aligned} \quad (2)$$

$$\begin{aligned} \text{Vitamin C} = & 219.4945 - 1.05621 \times \text{Temp} - \\ & 0.06348 \times \text{UP} - 2.41697 \times t + 4.51\text{E-} \\ & 05 \times \text{MP} \times \text{UP} + 0.003533 \times \text{MP} \times t + 0.001054 \times \text{Te} \\ & \text{mp} \times \text{UP} - 0.04605 \times \text{Temp} \times t + 0.00698 \times \text{Temp}^2 - \\ & 1.6\text{E-}05 \times \text{UP}^2 + 0.08426 \times t^2 \end{aligned} \quad (3)$$

Table 2. ANOVA results for first and second order coefficients using response surface method for *E. coli* values.

Source	Sum of squares	df	Mean square	F value	P-value ^a
Model	19.93928	14	1.424234	117.7054	< 0.0001
Microwave Power (MP)	0.096503	1	0.096503	7.975457	0.0128
Temperature (Temp)	11.95015	1	11.95015	987.6167	< 0.0001
Ultrasonic Power (UP)	5.600587	1	5.600587	462.859	< 0.0001
Time (t)	0.073639	1	0.073639	6.085841	0.0262
MP×Temp	0.302212	1	0.302212	24.97624	0.0002
MP×UP	0.062371	1	0.062371	5.154606	0.0384
MP×t	0.159693	1	0.159693	13.19781	0.0025
Temp×UP	0.039442	1	0.039442	3.259703	0.0911
Temp×t	0.302149	1	0.302149	24.97104	0.0002
UP×t	0.202106	1	0.202106	16.70303	0.0010
MP ²	0.021954	1	0.021954	1.814393	0.1980
Temp ²	0.836628	1	0.836628	69.1429	< 0.0001
UP ²	0.141173	1	0.141173	11.66721	0.0038
t ²	0.010794	1	0.010794	0.892069	0.3599
Residual	0.1815	15	0.0121		
Lack of Fit	0.1025	10	0.01025	0.648734	0.7383
Pure Error	0.079	5	0.0158		
Total	20.12078	29			

^a P-value < 0.10 denotes significant effect.

Table 3. ANOVA results for first and second order coefficients using response surface method for remaining vitamin C.

Source	Sum of squares	df	Mean square	F value	P-value ^a
Model	897.4866	14	64.10619	39.54719	< 0.0001
Microwave Power (MP)	3.24585	1	3.24585	2.002369	0.1775
Temperature (Temp)	115.3266	1	115.3266	71.14517	< 0.0001
Ultrasound Power (UP)	326.1697	1	326.1697	201.2146	< 0.0001
Time (t)	235.7016	1	235.7016	145.4047	< 0.0001
MP×Temp	0.525669	1	0.525669	0.324286	0.5775
MP×UP	29.32336	1	29.32336	18.08962	0.0007
MP×t	40.44025	1	40.44025	24.94765	0.0002
Temp×UP	71.15057	1	71.15057	43.89289	< 0.0001
Temp×t	30.53701	1	30.53701	18.83832	0.0006
UP×t	0.075683	1	0.075683	0.046689	0.8318
MP ²	2.262503	1	2.262503	1.395741	0.2558
Temp ²	13.36353	1	13.36353	8.243977	0.0117
UP ²	10.61319	1	10.61319	6.547291	0.0218
t ²	15.77374	1	15.77374	9.730842	0.0070
Residual	24.31507	15	1.621005		
Lack of Fit	15.56341	10	1.556341	0.88917	0.5929
Pure Error	8.751657	5	1.750331		
Total	921.8017	29			

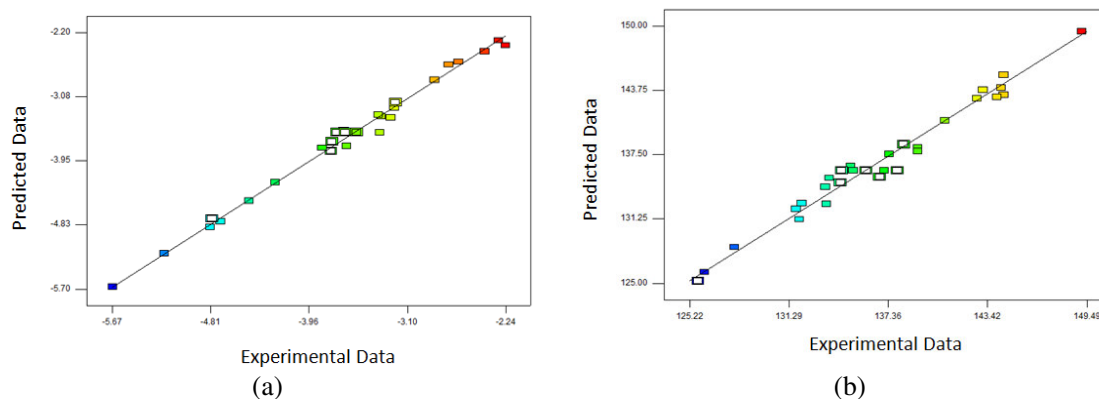
^a P-value < 0.10 denotes significant effect.

Where, N is *E.coli* count after treatment, N_0 is initial *E.coli* account, MP is microwave power (W), $Temp$ is temperature (°C), UP is ultrasound power (W), t is time (s).

Figure 2 presents the goodness of fit for the experimental and model predicted data.

It was found that the effect of final SCJ temperature on *E. coli* reduction was more significant than that of the microwave power and its resulting heat. According to Figure 3a, it is likely due to the steeper microbial

removal gradient caused by the increased temperature. However, increasing microwave output power had a milder incremental gradient than the microwave-induced temperature. At lower temperatures, increasing the microwave power had no effect on the reduction of *E. coli*. By increasing the temperature to 50 or 60°C and raising the microwave power, the *E. coli* reduction was enhanced possibly owing to the faster reaching to the distinct higher

**Figure 2.** Comparison of experimental and model predicted data: (a) *E. coli* survival, (b) Vitamin C.

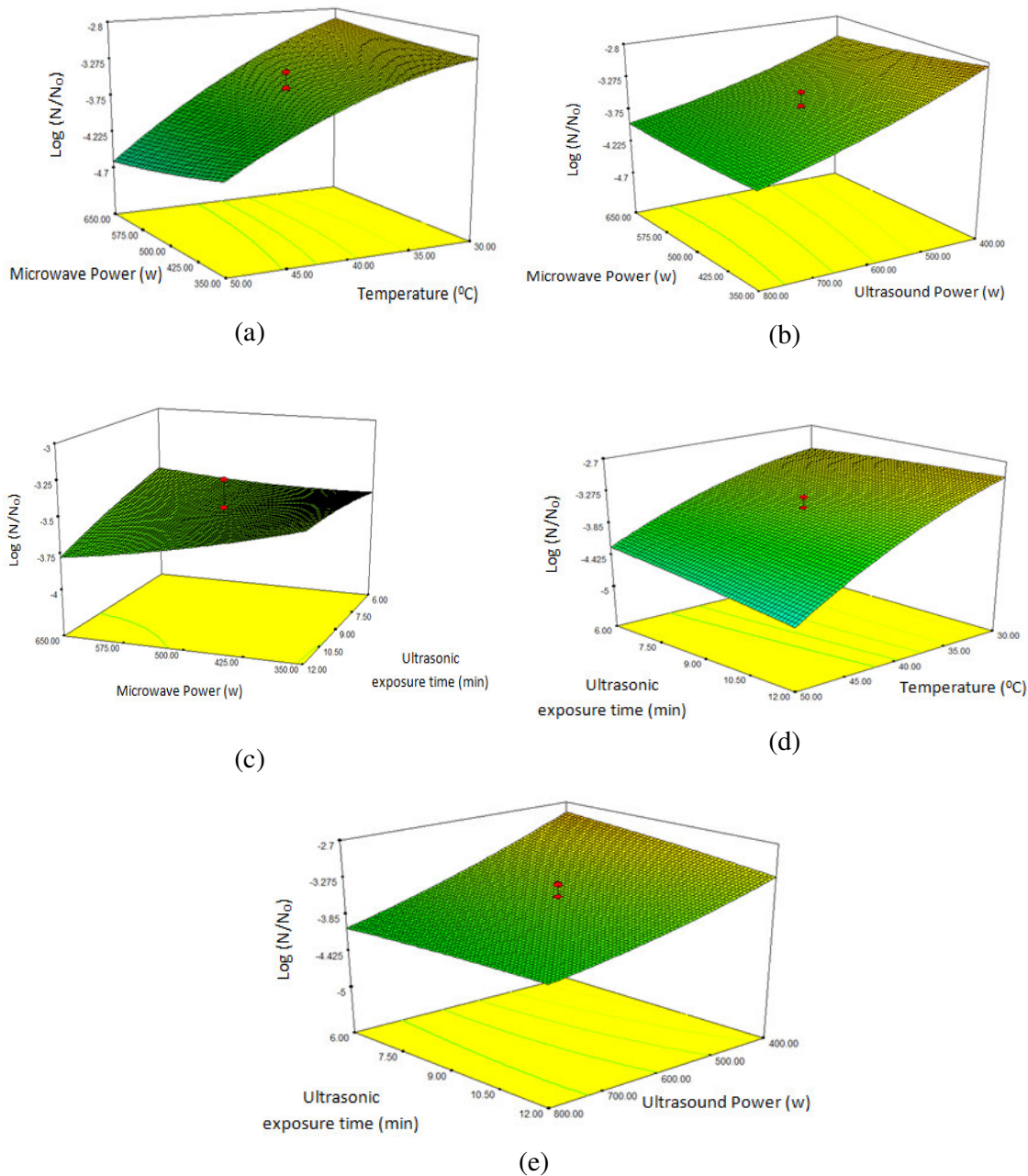


Figure 3. Influence of treatment variables on *E. coli* reduction.

temperatures and having insufficient chance for the microorganisms to adapt with the new conditions. These findings could be derived from Equation (1) as the product of microwave power multiplied by temperature had a negative coefficient. This shows that

any increase in the independent variable would result in a larger negative value in Equation (1); as well as more *E. coli* removal. Various researches have already indicated the effect of microwaves on removal of microorganisms (Tajchakavit et

al., 1998; Gentry and Roberts, 2005; Cañumir *et al.*, 2002).

The effect of ultrasound power on *E. coli* reduction was greater than that of the microwave power (Figure 3-b). As shown in Figures 3-e and -c, the *E. coli* reduction gradient was greater in regions where the ultrasound power and exposure time were increased, than in the regions where only the microwave output power was increased. The removal capability of ultrasonic waves was enhanced as the power increased. The reason for this reduction could be the wider movement range of the probe in the fluid. Wider probing range would result in more bubbles inside the fluid giving rise to cavitation (Vichare *et al.*, 2001). Ultrasonic waves remove the microorganisms by thinning the cell walls, concentrating heat, and developing free radicals inside the fruit juice. During the ultrasonic treatment, and when ultrasonic waves collide with the liquid medium, longitudinal waves are created which set the stage for pressure variation. This, in turn, produces bubbles inside the medium. The bubbles expand and attain larger cross-section area, contributing to the formation of gas and development of bubbles. At the critical point, ultrasound energy inside the bubble is not sufficient for turning into the gas phase and, therefore, the contraction process occurs. The contracted molecules collide with each other and create shock waves. The shock waves develop regions with extreme temperature and pressure which, in some cases, reach 5,500°C and 500 bar. The main bacterial effect of ultrasound is the pressure variations induced by these conditions. The hot points can kill some bacteria, but they are too concentrated and their influence area is not large (Kuldiloke, 2002).

Increasing the ultrasonic exposure time can decrease the survived number of *E. coli* due to providing higher sonic flow periods in the reactor chamber. Similar findings have been reported by other researchers (Wu *et al.*, 2008; Ugarte-Romero *et al.*, 2006).

The SCJ temperature and ultrasonic exposure time are both important

independent variables effective on *E. coli* removal. As shown in Figure 3-d, at high temperatures, effectiveness of ultrasonic waves and the cavitation intensity were both weakened. However, the combined effect of temperature and ultrasonic exposure time was more significant than their individual effects. It is believed that increasing temperature of the fruit juice can result in vapor pressure increase inside the collapsing bubbles which cushion the inward motion of the bubble wall, causing a drastically reduced cavitation effect (Wu *et al.*, 2008; Ugarte-Romero *et al.*, 2006).

According to Figures 4-a and -b, any increase in the ultrasound power, ultrasonic exposure time, and the microwave power would lead to a reduction in vitamin C content of the SCJ. The impact of ultrasound power was more effective than the microwave power. This is clearly shown by the increased vitamin C reduction gradient as the ultrasound power increased. This ultrasound-induced loss in vitamin C content was likely the result of ascorbic acid breakdown or interaction with other compounds such as anthocyanin. This, in turn, can lead to a loss of pigments and discoloration. Oxidative reactions can be the reason for anthocyanins and ascorbic acid breakdown during the ultrasound treatment. This process can be enhanced by the free radicals created through the ultrasound treatment. Moreover, the cavitation-induced thermolysis could be another reason for the breakdown of these compounds (Tiwari *et al.*, 2008). It was reported that microwave processing could increase the breakdown of vitamin C of orange juice, due to its thermal effects (Vikram *et al.*, 2005).

Vitamin C content of SCJ decreased with increasing temperature (Figures 4-c and -d). This clearly indicates that vitamin C is sensitive to heat, and the process temperature is directly correlated with the breakdown of these compounds. Thus, thermal processing adversely affects vitamin C breakdown at high temperature (Odriozola-Serrano *et al.*, 2009; Torregrosa *et al.*, 2006). In addition, as the temperature

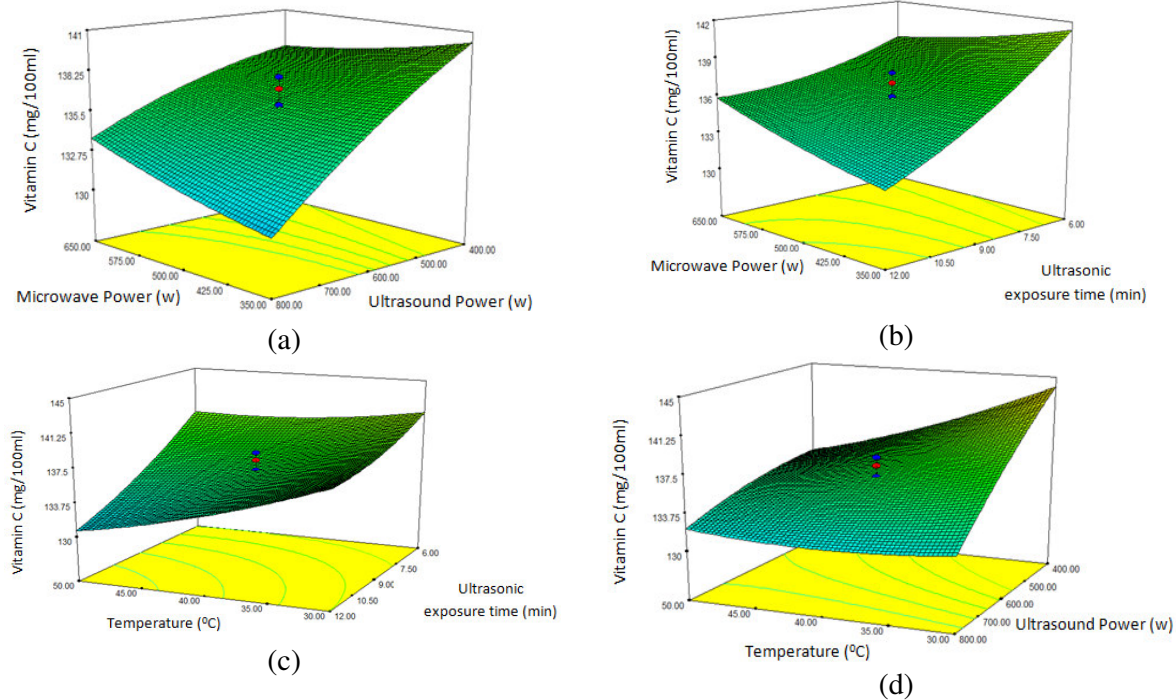


Figure 4. Effect of process variables on vitamin C content of SCJ.

reached 50°C, the effectiveness of ultrasonic waves was enhanced owing to the increased internal pressure of bubbles. However, with further temperature increase, temperature would become the leading factor in decreasing the vitamin C content since cavitation intensity decreases. This weakens the impact of ultrasonic waves.

On the basis of our findings, longer ultrasonic exposure times decreased the vitamin C content, more likely because of the increased duration of oxidative reactions and occurrence of thermolysis. Moreover, another research showed that the breakdown level of ascorbic acid in watermelon juice increased significantly with the ultrasonic amplitude, exposure time, and temperature (Rawson *et al.*, 2011).

The objectives for the process optimization were to obtain: minimum energy consumption, reduce the *E. coli* content to zero, and maximum vitamin C retention. Considering the priority of *E. coli* reduction, it was weighted 5 in the final optimization while the rest were weighted 3. The optimum

combination was obtained as 352.21W microwave power, 49.94°C temperature, 475.13W ultrasound power and 6 minutes of exposure time. Based on these values, vitamin C content would be 142.5 mg per 100 mL with zero *E. coli* count and 1,425.87 kJ of consumption energy. By implementing the optimal values on the device, the actual vitamin C content was obtained to be 144.2 mg per 100 mL with no *E. coli* remaining, while consuming 1,407.94 kJ of energy. This confirms the precision of the optimum condition determined by the developed model.

CONCLUSIONS

Ultrasound power, microwave power, and ultrasonic exposure time as well as sample temperature are the influential factors in the reduction of *E. coli* and retention of vitamin C contents of SCJ. When the ultrasound power was increased, the amount of *E. coli* was decreased due to the increased intensity of cavitation. This negatively affected the vitamin

C content because of increased oxidation. Microwave treatment reduced *E. coli* and destroyed vitamin C, due to the thermal effect. As the ultrasonic exposure time of SCJ increased, the *E. coli* and vitamin C reduction rates were first steep and then became slower over time. Moreover, the RSM-based optimization fitted well with the measured experimental results.

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چکیده

در این تحقیق هدف بررسی اثر امواج فراصوت و مایکروویو بر شمار اشیشیاکلی و محتوای ویتامین ث در پاستوریزاسیون آب آلبالو بوده است. یافته‌های آزمایش نشان دادند توان مایکروویو، توان فراصوت، زمان اعمال فراصوت و دمای نمونه بر تعداد اشیشیاکلی و میزان ویتامین ث تاثیر داشتند. دمای نمونه و زمان امواج دهی فراصوت هر دو از متغیرهای مستقل مهم و تاثیر گذار بر کاهش اشیشیاکلی بودند. با افزایش دمای نمونه، اثر امواج فراصوت و در نتیجه شدت کاویتاسیون کاهش یافت. هر چند که اثر ترکیبی امواج فراصوت و دما بیشتر از حالتی بود که هر کدام به تنهایی مورد استفاده قرار می‌گرفت. افزایش توان فراصوت، زمان اعمال فراصوت و توان مایکروویو موجب کاهش معنی‌دار ویتامین ث شد، به طوری که توان فراصوت نسبت به سایر متغیرها تاثیر گذار تر بودند. در بهینه سازی توسط روش *RSM*، نقطه بهینه شامل: توان مایکروویو $352/21$ وات، دمای $49/94$ °C، توان فراصوت $475/13$ W و زمان 6 min بوده است. با توجه به این مقادیر، بیشینه مقدار ویتامین ث برابر $142/5$ mg بر 100 mL و تعداد اشیشیاکلی برابر صفر حاصل گردید