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Effect of ultrasonic waves on pasteurization of sour cherry juice

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Abstract

The objective of this study was to explore the effects of ultrasound probe diameter, reactor diameter, and juice level in the reactor upon effectiveness of ultrasound waves on decontamination of sour cherry juice. Results showed that the effects of probe diameter, reactor diameter and reactor height were significant (P<0.01). In addition, by increasing the probe diameter from 30 to 40 mm no significant effect was seen in reactors with 65 and 75 mm diameter; however, for 85 mm diameter reactor, the effect of ultrasound waves diminished and, as a result, the total microbial count increased. Increasing the probe diameter from 20 to 30 and then 40 mm, on the average decreased the total microbial count by 15% and 5%, respectively. This effect was obvious at 85 mm diameter, and any increase in height steepened the slope of total microbial count. Finally, using the response surface method (RSM), optimum values were obtained for reactor diameter, reactor height, and probe diameter.

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Introduction

In order to decrease the adverse effects of the thermal pasteurization method (loss of vitamins, food flavor and non-enzymatic browning), other methods capable of inactivation of microorganisms could be applied. In doing so, non-thermal methods are of interest, including pasteurization using high hydrostatic pressure processing (HPP), electrical fields, and ultrasound waves (Mertens and Knorr, 1992; Arnsson *et al*., 2001, Alvarez-Lo´pez *et al*., 2003, Toepfel *et al*., 2007).

The HPP method has shown great potential in inactivation of microorganisms and enzymes in recent years. The combination of high pressures (700 to 1000 MPa) with temperatures (70 to 90˚C) has scored high successes for sterilization. Principally, high hydrostatic pressure damages bacterial membranes and enzymes (Chen and Tseng, 1996).

Electric fields have the potential to deactivate microorganisms. In this process, the destructive effects on tangible properties and nutrition value are quite lower than the thermal method. Studies have also reported the effectiveness of electric fields on deactivating bacteria, molds, and yeasts (Evrendilek *et al*., 2008).

In ultrasonic method, cavitations occurring in the liquid, lead to extermination of microorganisms and enzymes. High-power ultrasonic propagation in a medium creates micro-bubbles which increase temperature and ambient pressure bursting. This process imposes a shear force and sudden pressure variation which are destructive to microbial membranes. A doubling effect could be achieved when combining this method with the thermal method (Kuldiloke, 2002, Cheeke and David, 2002). Thus, ultrasounds decrease the physical tolerance of microorganisms by inflicting damages. Similarly, this is also true for enzymes deactivation (Valero *et al*., 2007).

The effect of ultrasonic waves and mild heat on active and deactivated microorganisms of orange juice during and after exposure has been explored in several studies. For instance, in a recently reported study, 500 kHz and 240 W waves were applied to orange juice for 15 minutes and it was studied for 14 days of storage at 5˚C (Valero *et al*., 2007). It was revealed that pulps increased the microbial resistance against ultrasounds. Moreover, the use of ultrasound in combination with other methods was suggested to decrease microbial activity and shelf life extension.

Another study showed the capabilities of a combination of ultrasound, heat, and high hydrostatic pressure for deactivation of pectin esterase (PE); which is responsible for cloud destabilization in citrus and other juices. Inherently, the existence of clouds gives a fresh look to products and ensures product desirability. In this regard, the combination of thermal (temperatures under 100 ˚C), pressure (100-300 kPa), and ultrasonic (24 kHz and high powers) treatments, called *Manothermosonication*, were used to make the effect of heat on enzyme inactivation more efficient. This method was tested on lemon, strawberry, and tomato juices. Results showed that the deactivation of enzymes using ultrasounds depends on exposure time, pressure, ultrasonic domain and pH of the juice. Furthermore, it was concluded that enzyme deactivation is irreversible and enzymes would not reactivate during storage (Kuldiloke and Eshtiaghi, 2008).

Reviewing of the literature showed that there is no result on the effect of reactor and probe dimensions on sour cherry juice properties during its pasteurization process. Hence, the aim of this study was to evaluate the effects of probe diameter, reactor diameter, reactor height, as well as ultrasound intensity on the total microbial count of sour cherry juice during the product pasteurization and optimization of the process using Response Surface Methjod (RSM).

Material and methods

Experimental procedure

In order to supply constant ultrasonic waves, an electric generator (Model MPI, Switzerland) with

1000 W power and 20±1 kHz working frequency was used. By conducting a preliminary test and using RSM method, optimum dimensions for ultrasonic probe and reactor were selected to intensify ultrasonic efficacy for micro-organism destruction in sour cherry juice. In the next step, reconstituted sour cherry juice was prepared and placed at room temperature for 48 hours to be contaminated with micro-organisms. Then, the infected juice was poured into three reactors having 65, 75 and 85 mm diameters and 30, 50 and 70 heights (juice levels) to conduct the experiments. All samples were treated at constant ultrasonic power (700 W) for 10 minutes (Kuldiloke, 2002). In order to investigate the effect of probe diameter on ultrasonic intensity and destruction of micro-organisms, three aluminum probes with diameters of 20, 30 and 40 mm were designed and developed using the appropriate formulae.

Microbial experiment

In this study, the total microbial count was measured using the pour-plate technique. First, in order to decrease the microbial population and isolate a single colony, the samples were diluted using physiological serum. Then, 1 ml of the diluted samples was poured into a 90 mm plate using a sterile pipette. Subsequently, 20 ml of Plate Count Agar culture which was cooled down to 45˚C was added to the mixture. In order to mix the sample with the culture, plates were gently moved. After the agar was hardened, plates were kept for 24 to 48 hours at 37˚C inside an oven (Valero *et al.*, 2007).

Optimization and Modeling using Response Surface Method

Response surface methodology (RSM) has an important application in the design, development and formulation of new products, as well as in the improvement of existing product design. It defines the effect of the independent variables, alone or in combination, on processes. In addition, to analyzing the effects of the independent variables, this experimental methodology generates a mathematical model which describes the chemical or biochemical processes (Anjum, *et al.*, 1997, Halim *et al.*, 2009).

In order to obtain the optimum value, Eq. (1) will be used:

$$Y_i = \beta_o + \sum \beta_i X_i + \sum \beta_{ij} X_i X_j + \sum \beta_{ij} X_i^2 + \varepsilon \qquad 1$$

where, β_o, β_j, β_{ij}, β_{jj} are regression coefficients for intercept, linear, interaction and quadratic coefficients, respectively, while X_i and X_j are coded independent variables and ε is the error. In the present study, Box-Behnken design with 3 central points was used. Coded values of the independent variables of the experiment for destroying microbes are given in table 1.

Results and discussion

As shown by the ANOVA results in table 2, the effects of probe diameter, reactor diameter, reactor height, and the interaction of reactor diameter and reactor height on microbial count were significant ($P<0.01$). The interaction effect of probe diameter and reactor diameter was significant ($P<0.05$). The sum of squares of reactor heights revealed that 59% of variations in data could be explained by this factor. This indicates that the reactor height is more important than the other factors.

Table 1. Experimental range and levels of independent variables.

independent variable	Range of level		
	-1	0	1
Probe Diameter (mm)	20	30	40
Reactor Diameter (mm)	65	75	85
Reactor Height (mm)	30	50	70

A regression model with 96.16 coefficient of determination and 0.0754 standard error, was developed to estimate the survival rate of micro organisms as a function of reactor and probe dimensions (Eq. 2).Fig. 1 shows the proper fitting of this parameter's actual data with the model.

$$N=5.6813 - 0.01898\times D_p + 0.00692\times D_r - 0.1079\times H + 0.0004\times D_p^2 - 0.00023\times D_r^2 + 0.0005\times H^2 + 0.00007\times D_p\times D_r - 0.00001 D_p\times H + 0.00099 D_r\times H \quad (2)$$

where, D_p, D_r, H and N are probe diameter (mm), reactor diameter (mm), height diameter (mm) and total microbial count (cfu ml^{-1}), respectively.

The reason for diminishing microbial count in the presence of ultrasonic waves could be due to the burst of very tiny bubbles developed by ultrasounds which expand quickly and burst in a short time. Due to this burst, special temperature and pressure conditions are developed which could initiate or intensify several physical and/or chemical reactions. One of the most well-known reactions in such circumstances is the sonolysis of water molecules into hydroxyl free radicals. These radicals are very strong oxidants and can easily react with many compounds, including amino acids. Therefore, the chemical structure of microorganisms is changed and their biological activity ceases. Meanwhile, the mechanical nature of ultrasonic waves, through bursting bubbles inside the juice, destroys microorganism cell walls. This is achieved in two steps. Firstly, cavitation process disperses the microbial colony and next, the cell walls are destroyed (Kuldiloke, 2002).

Table 2. ANOVA results for total microbial count.

Source of variation	df	Sum of Squares	Mean Squares
Probe Diameter	2	0.03178	0.01589**
Reactor Diameter	2	2.28631	0.14315**
Reactor Height	2	6.33820	0.16910**
Probe diameter × Reactor diameter	4	0.00529	0.00132*
Probe diameter × Reactor height	4	0.00538	0.00134 ns
Reactor diameter × Reactor height	4	1.65235	0.41304**
Probe diameter × Reactor diameter × Reactor height	8	0.00765	0.00096 ns
Error	54	0.14647	0.00271
Total	80	10.7343	

** and * significant at 1 and 5% level, respectively.
ns not significant.

Probe and reactor diameter effects on decreasing total microbial count

Results of analysis (table 2) indicates that microbial count was significantly affected by the interaction of probe diameter and reactor diameter ($P<0.05$). As shown in Fig. 2, increasing probe diameter (from 20 to 30 mm at all levels of reactor diameter), decreased microorganism survival. Increasing probe diameter, from 30 to 40 mm at 65 and 75 mm reactor diameters, showed no significant effect. However, at higher reactor diameter, the effect of ultrasound diminished, and the total microbial count increased. On average, increasing probe diameter, from 20 to 30 and 20 to 40 mm, decreased the total microbial count by 15% and 5%, respectively. This can be explained by increased cavitation due to the increase in probe diameter. However, further increase in probe diameter caused a decrease in probe vibration as a result of increasing probe weight and consequently reducing the force required for probe vibration. This caused the piezoelectric and electric power source to be overloaded. Also, with increasing the probe diameter, ultrasonic wave reflection perpendicular to the probe axis, increased and caused a decrease in cavitations. Therefore, it is suggested that probe diameter should be less than a quarter of probe length which confirms findings by Mcculloch, 2008. A similar research into the effect of probe diameter and reactor diameter on effectiveness of ultrasonic waves on salt dissolution time in water stated that increasing the ratio of probe diameter to reactor diameter decreased the effectiveness of ultrasounds, leading to decreased salt dissolution time in water (Vichare *et al.*, 2001).

As shown in Fig. 3, the optimal probe and reactor diameters are in the range 25-35 mm and 65-70 mm

providing the most effective ultrasound conditions for microbial control.

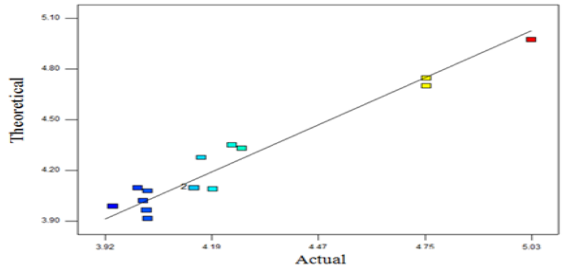


Fig. 1. Actual microbial count data against model outputs.

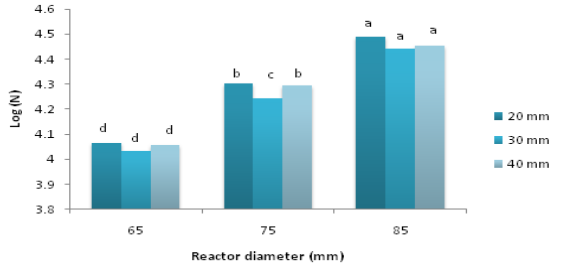
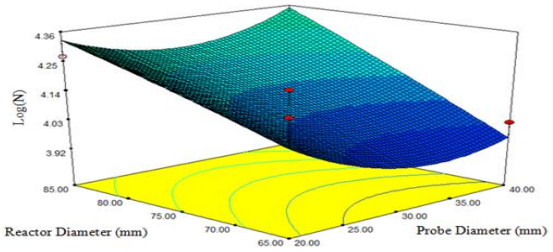


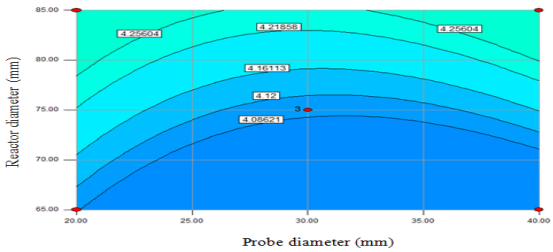
Fig. 2. Effect of probe and reactor diameter on remained microbial population (Different letters denote significant at the 5% level).

Effect of reactor dimensions on decreasing the total microbial count

Based on ANOVA results, the interaction effect of reactor diameter and height on microbial count was significant ($P<0.01$). As shown in Fig. 4, by increasing volume, the effectiveness of ultrasonic waves decreased. This effect was obvious at the 85 mm diameter where the total microbial count slope was increased as the reactor height increased. Increasing the height from 30 to 50 mm for 65, 75, and 85 mm reactor diameters, increased the total microbial count by 14.4, 29.6, and 85%, respectively. Moreover, increasing the height from 50 to 70 mm, at three reactor diameters, increased the total microbial count to 42, 403, and 636%, respectively. On average, increasing the reactor height from 30 to 70 mm and diameter from 65 to 85 mm increased the total microbial count by 428 and 593%. Overall, it appears that larger reactor size decreases effectiveness of ultrasonic waves, because, in addition to the cavitation effect, the sonic movement effect is also developed within the reactor. This helps to agitate the sample which causes multiple exposures of the sample particles to the ultrasonic waves and cavitations. Whereas, with higher reactor diameter and height, movement course of a particle to complete one cycle is also increased, this decreases the chance of its exposure to ultrasonic waves during that cycle (Vichare *et al.*, 2001).



(a)



(b)

Fig. 3. Illustration of a) response surface and b) contour plot on variation of total microbial population *versus* probe and reactor diameter.

It should be noted that the course and period of each sample cycle inside the reactor is obtained from equations (3) and (4) (Vichare *et al.*, 2001; Benitez, 2004).

$$L=D_r+2H \quad (3)$$

$$t_{mix}=\frac{5\times L}{v_c} \quad (4)$$

where, L, is the course of each cycle, t_{mix} is the time period of a sample cycle inside the reactor and v_c is the cycle speed of the sample inside the reactor.

Additionally, Eq. (5) is also available for calculating the time period of each cycle. By comparing equations (4) and (5) and using Eq. (3) sample volume may be determined (Benitez, 2004; Vichare *et al.*, 2001).

$$t_{mix}=\frac{5\times V_L}{v_c\times\frac{\pi}{4}d_r^2} \quad (5)$$

$$(Dr + 2H) \times \frac{\pi}{4} d_r^2 = V_L \qquad 6$$

where, V_L is sample volume inside the reactor.

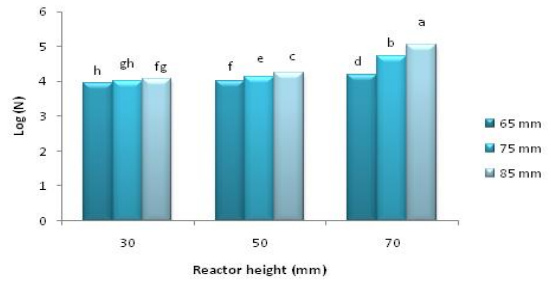
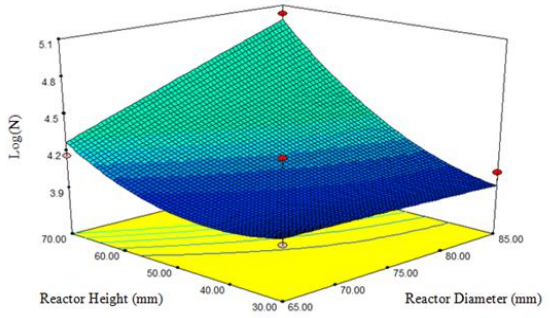
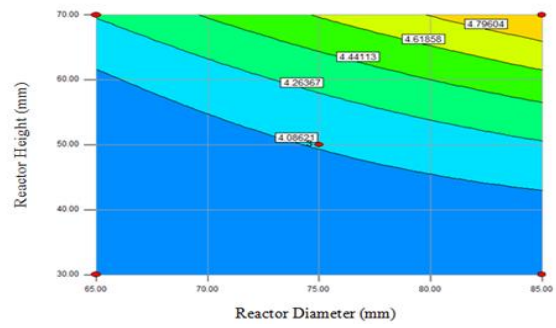


Fig. 4. Effect of reactor diameter and height on remained microbial population (different letters denote significant at the 5% level of significance).

As shown in equations (4) and (5), the time period of each cycle is a function of the reactor's height and diameter. Since the reactor height is multiplied by 2, it has a larger effect in the equation, and height variations are more influential than reactor diameter. This is the reason for the value of height variable's sum of squares in the ANOVA table (table 2); as mentioned earlier, height variations explain 59% of the changes of data in total microbial count.



(a)



(b)

Fig. 5. illustration of a) response surface and b) contour plot on variation of total microbial count *versus* reactor diameter and height diameter.

As shown in Fig. 5, the optimal reactor diameter and height values for maximum effectiveness of ultrasound on reducing the total microbial count is within the 65–70 mm and 30–50 mm range, respectively.

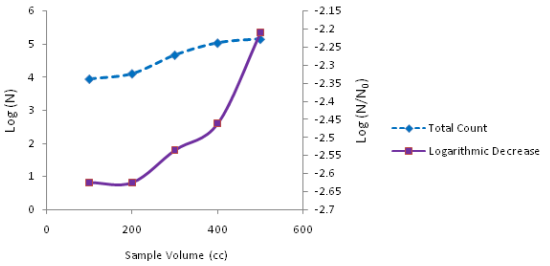


Fig. 6. Effect of volume on the effectiveness of ultrasonic waves on total microbial count (N_0 is initial microbial counts).

Another factor affecting the time of agitation, in other words, duration of exposure of each juice particle to ultrasonic waves inside the reactor, is the volume (Fig. 6). As shown by Eq. (5), fluid cycle duration inside the reactor increases with volume. Equation (6) indicates the relationship between the desired volume and probe size, as the effectiveness of ultrasonic waves has a direct relationship with the probe diameter, provided the volume is constant. Thus, as far as designing parameters allow (i.e. generator power, piezoelectric, probe material, modal analysis, and stresses exerted on the probe), developing probes with larger diameters agitate larger portions of the fluid. However, in this study, increasing the probe diameter from 30 to 40 mm increased the probe's weight thus limiting its ability to vibrate along the used apparatus as well as the 30 mm diameter. However, this effect was also noticeable when diameter increased from 20 to 30 mm.

In order to determine the optimum conditions, two dependant variables (sample size and total microbial count) were considered. The objective was to maximize the product volume and minimize the total microbial count. In optimization, considering the higher importance of minimizing total microbial count rather than reactor size, the total microbial count and volume were given weights of 2 and 1, respectively. Given the target function (Eq. 1) and

volume, the obtained values for reactor diameter, reactor height, and probe diameter were 80, 45.2, and 29.6 mm, respectively. For the mentioned independent variables, the obtained volume and logarithm of the total microbial count were 227cc and 4.04, respectively. Finally, in order to evaluate the obtained values, reactor diameter, reactor height, and probe diameter were taken to be 80, 45, and 30 mm, respectively. Using this setting, the obtained total logarithm of microbial count was 4.11 which indicates a proper estimate of the model and suggested optimal point.

Conclusions

Dimensions of the reactor had an important role in performance intensity of ultrasonic waves. Height of the reactor had more effect as compared to reactor diameter. Increasing the probe diameter from 20 to 30 mm caused a decrease in the total microbial count while further increase from 30 to 40 mm resulted in elevated total microbial count. However, further increase in probe diameter caused a decrease in cavitations due to increasing ultrasonic wave reflection perpendicular to the probe axis. Use of RSM method for optimization of reactor designing parameters indicated that this method has considerable ability for application in the similar scientific researches.

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