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THERMOSONICATION ASSISTED EXTRACTION OF BLOOD FRUIT (*HAEMATOCARPUS VALIDUS*) JUICE AND PROCESS OPTIMIZATION THROUGH RESPONSE SURFACE METHODOLOGY

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ABSTRACT

In this study, simultaneous optimization of various ultrasound power intensity, processing temperature and processing time was performed to extracted blood fruit juice with retention of highest amount of bioactive compounds by the novel approach of thermosonication. Face centered central composite design of response surface methodology (RSM) was used to obtain optimum conditions while performing this extraction approach in an ultrasonic treatment chamber with lower temperatures as compared to the conventional extraction methods. The following combination of parameters including ultrasonic power at 118, 224 and 330 Watt /cm^2, ultrasonic time at 1, 3 and 7 min and processing temperatures at 42, 54 and 64ºC were taken and evaluated for the following responses: Peroxidase activity, juice yield, lethality of *E.coli*, cloud value, Ascorbic acid and anthocyanin content. Optimum process condition was achieved at a temperature, ultrasound power, and treatment time of 59.9ºC, 118 Watt /cm^2 and 7 min, respectively.

Keywords: *Haematocarpus validus*, physicochemical, phytochemical, anthocyanins, thermosonication

INTRODUCTION

Haematocarpus validus Bakh.f. Ex Forman (Menispermaceae) (India possessing voucher number 51014) is commonly known as blood fruit/tépattang/garo grapes/ khoonphal/ raktaphal, contains high amount of bioactive compounds mainly anthocyanins, ascorbic acids, carotenoids, iron, polyphenols, antioxidant properties etc. and so it has been utilized in the traditional medicine system **(Shajib *et al*., 2013)**. Although a rich source of nutrients, its contents are highly thermosensitive and may lead to heavy loss of nutritional and functional compounds **(Sasikumar *et al*., 2018)**. Conventional processing has positive effects on rapid microbial deduction and enzymatic inactivation, but subsequently shows negative effects like heavy loss of nutrients, bioactive compounds and the original colour of juices, thus leading to poor consumer acceptability (**Walkling-Ribeiro *et al*., 2009).**
Nowadays, in the food industry, the application of power ultrasound has become an attractive and innovative processing tool. It has a lot of merits over thermal treatment like minimal loss of flavour, increased homogeneity and significant energy saving (**Abid *et al*., 2014**; **Chemat *et al*., 2011)**. Thermosonication has been used as a replacement of thermal treatment for processing of fruit juices such as that of blackberries, oranges and strawberries for production of superior quality fruit juices sans additives having an extended shelf life **(Tiwari *et al*., 2008; Tiwari *et al*., 2009).** Several investigators **(Abid *et al*., 2014; Bhat *et al*., 2011; Erkaya *et al*., 2015; Sasikumar *et al*., 2018)** revealed that the employment of thermosonication (TS) process treatment at lower processing temperature (44–55°C) for shorter times, gives the same intensity of microbial lethality and enzymes residual activity as that in case of using conventional thermal processing.
Literatures describe the positive effects of thermosonication treatment over conventional thermal methods only but limited research have reported on the positive effects on organoleptic quality and original freshness of the juice keeping in mind the market value of high-quality fruit juices. However, the effects of thermosonication on juice yield, cloud value, ascorbic content, peroxidase, anthocyanins, lethality of *E.coli* have not been studied, therefore, the main objective of this paper was to evaluate quality attributes of blood fruit juice treated by manipulated TS parameters. The novel concept is to produce superior quality blood fruit juice, retain the maximum nutritional and therapeutic values and extend the shelf life through optimized TS treatment using response surface methodology.

MATERIALS AND METHODS

Blood fruit juice preparation and treatments

Freshly matured blood fruits (*Haematocarpus validus*) (India possessing voucher number 51014) were selected, which were free from external injuries and procured from Tura bazar, West Garo Hills, Meghalaya, India (25.467ºN, 91.3662ºE). The fruits were washed thoroughly and pulp was separated from peel and seed manually using a stainless steel knife. The fruit pulp was fed to a motorized juice extractor (Model-250JE; Philips, India) and filtered using muslin cloth to obtain homogenous juice. Adequate amount of deionized distilled water was added and this was used for several treatments without any additives**. Dabir *et al.* (2017)** reported that when 75, 118, 224, 330 and 373 Watt/cm^2 ultrasound power was applied to soursop juice for short processing times (2-10 min) , then 330 Watt/cm^2 power intensity was optimal at processing time of 9 min.
The extracted juice sample was subjected to sonicator (Q-1500; Qsonica, Newtown, CHR, USA) at ultrasound power 118, 224 and 330 Watt/cm^2, with constant frequency of 20 kHz at 80% amplitude for a time interval of 1 , 4 and 7 min using a titanium probe of 1/5 inch length. The treatment temperature, processing time and sonication power load combinations were established from previous study reports **(Caminiti *et al*., 2011).** The sample was quantified to 200 ml per treatment. Sonication treatment was carried out using a thin plastic container of 4.0 cm diameter and height of 5.0 cm placed in water bath at 42, 54 and 66°C with a thermostat controller. The sonicator probe tip was submerged 3 cm into the juice sample. TS process configuration condition was adjusted according to previous studies **(Mayuoni Kirshenbaum *et al*., 2017; Seshadri *et al*., 2014; Anaya-Esparza *et al*., 2017)** and treated samples were categorized and coded. The combinations of these experiments were optimized by using response surface methodology and the optimized data set were confirmed later by performing experiments at those conditions.

Juice Yield (%)

The percentage of juice yield (% w/w) was calculated as the difference between the initial mass and the weight of the pellet after centrifugation divided by the initial mass as follows:

$$Juice\ yield(\%) = \frac{Mass\ of\ the\ fruit - Mass\ \ after\ extraction(waste\ portion)}{Mass\ of\ the\ fruit} \times 100 \quad (1)$$

Vitamin C (Ascorbic acid) content

Ascorbic acid was determined according to Alex ***et al.*** **(2017)** with slight modifications and the crude blood fruit extract and thermosonicated fruits extracts were analyzed by adding 0.5 mL sample into 9.5 ml of 4% oxalic acid and titration were done against 2, 6-dichlorophenolindophenol sodium salt solution. On the basis of amount of dye reduced vitamin C content was determined by the given formula
Amount of vitamin C (mg/100gm) edible portion is,

$$= \frac{0.5\ mg \times V_2 \times total\ extracted\ volume\ (ml)}{V_1 \times 0.5\ ml \times wt\ of\ the\ sample\ (g)} \times 100 \quad (2)$$

where,
V_1= volume of dye solution required for titrating standard vitamin C solution.
V_2= volume of dye solution required for titrating sample solution.
The vitamin C content was expressed as mg/100 g blood fruits.

Anthocyanins content

Anthocyanins were determined according to Chorfa *et al.* (2016) with minor modifications. The extracted fresh juice sample (1 ml for each treatment) was mixed in a solution which contained (HCl-MeOH-H_2O in mixer ratio 1:3:16) and was allowed to settle for 72 hrs at 4°C. The absorbance was measured at two different wavelengths of 653 and 530 nm. Results were expressed as milligrams of cyanidin-3- glucoside equivalent (mg C_3GE/100 g) of juice sample in dry basis.

Cloud value

Cloud value is one of the physical quality parameter of juice. It is attributed to the visible suspended particles mainly composed of complex mixture of insoluble fraction of proteins, pectin, carbohydrates, lipids and other minor components that give characteristic aroma and mouth feel to the juices. Cloud value can be determined using a spectrophotometer as reported by Dabir *et al.* (2017). Supernatant of 5 ml of centrifuged blood fruit juice samples (centrifuged at 1512 G value for 5min at 25°C) were collected and checked for absorbance at 660 nm using 96 well ELISA plate reader.

Peroxidase assay

The oxidative enzymes like peroxidase have high thermal resistance and their activity leads to yellow, brown or even pink coloring during storage, even under refrigeration. Peroxidase (POD) activity was determined using the method described by **Kwak *et al*. (1995)** with minor modifications. The reaction mixture was 3 ml, which contained 2.2 ml of centrifuged blood fruit sample, 0.32 ml of 100 mM (w/v) potassium phosphate buffer (pH 6) and 0.32 ml of 5% pyrogallol and 0.16 ml of 0.147 M H_2O_2. The increase in absorbance upon addition of H_2O_2 was recorded at 420 nm in 3 minutes. For calculating residual activity Equation (3) was used

$$\text{Residual activity RA (\%)} = \frac{RA_t}{RA_0} \times 100 \quad (3)$$

RA_t=Residual activity after treatment at t time
RA_0=Residual activity before treatment

Lethality of *E.coli*

Cell suspension and inoculation of *E.coli* in blood fruit juice was performed, as recommended by **Brinez *et al.* (2006); Anaya-Esparza *et al.* (2017).** Previously, cryobeads were prepared by inoculating strain culture separately (*E. coli*) in tryptone soy broth and stored at −20°C for future experimental use. Before each experiment, 2 ml cryobead was inoculated into 10 mL of tryptone soy broth and was incubated at 37°C for 24 ± 2 h. After incubation, the broth was spread using a sterilized disposable loop in a Petri dish containing tryptone soy agar (TSA) and was incubated at 37°C for 24 ± 2 h.
Subsequently, cell suspension was prepared adding enough inoculum of bacteria (E. coli) in 11 mL of sterile peptone water (0.1%). The final concentration (9.0 ± 0.1 log CFU/mL) of cell suspension was determined using a UV visible spectrophotometer (CECIL 7400, 700 series, Aquarius) at an absorbance of 405 nm. The blood fruit juice was inoculated with cell suspension (10 mL/L) and homogenized by mixing and was subjected to thermosonication treatment. Lethal and sub-lethal injuries in bacterial strains were assessed by serial dilution using pour plating methods. 10 ml of each treatment were placed into 90 mL of sterile peptone water and homogenized. Serial dilutions (up to 10^{-7}) were made in peptone water (9 mL) which was previously sterilized with samples (1 mL) taken before TS treatment; then, 1 mL of diluted aliquots was pour plated and incubated at 37°C for 24 hrs, and the results obtained was expressed in terms of log CFU/mL. The difference between the logarithms of colony counts before and after treatments was used to calculate the lethality. Also, to detect the bacterial cell injury, dilutions of TS samples were pour-plated in TSA and to it 2% sodium chloride was added and incubated at 37°C for 24 h **(García *et al*., 2003).**

$$Lethality\ E.coli = \text{logarithms of colony counts in TSA before} - \text{after TS treatment} \quad (4)$$

Experimental Design and Statistical analysis

Response surface methodology (RSM) was used in present study to optimize process conditions for thermosonication process. **Sasikumar *et al.* (2010)** mentioned that in face centered central composite design (FCCD), only three levels are used (α=±1), which indicates less number of experiments and also it provide relatively high quality predictions over the entire design range and so, FCCD was chosen for this work. However, it has poor precision for estimating pure quadratic coefficients. A face centered central composite rotatable design **(Gamboa-santos *et al*., 2013)** was used for designing the experiments and for the optimization of thermosonication of blood fruit. The FCCD experimental design consists of 8 factorial, 6 axial and 6 rotatable centre runs. Temperature (X_1), ultrasound power (X_2) and time (X_3) was taken as independent parameters and its range are shown in Table 1. Total combination of 20 experiments were designed in which 6 experiments were performed at centre point. The design of the experiments in terms of coded and actual values is shown in Table 2. The effect of three independent variables *viz.*, temperature, ultrasound power and time was studied on the following responses- juice yield, ascorbic acid, anthocyanin, cloud value, POD activity and lethality of *E. coli*. Analysis of variance (ANOVA) was conducted to determine the level of significance. A second order polynomial equation (5) was used to predict the responses. The response (Y_i) is the function of linear components, quadratic components and interaction components **(Sahin *et al*., 2013).**

Table 1 Actual and coded values of the experimental variables used in the central composite design.

Experimental Variables	Code	Coded levels		
		-1	0	1
Temperature (°C)	X_1	42	54	66
Ultrasound Power (Watt/cm^2)	X_2	118	224	330
Time (min)	X_3	1	4	7

Table 2 Experiment with coded and actual values

	Coded Values			Actual Values		
Run	X1	X2	X3	X1	X2	X3
1	0	0	-1	54	224	1
2	-1	0	0	42	224	4
3	1	-1	-1	66	118	1
4	-1	1	-1	42	330	1
5	0	0	0	54	224	4
6	-1	-1	1	42	118	7
7	0	-1	0	54	118	4
8	0	0	0	54	224	4
9	1	-1	1	66	118	7
10	1	0	0	66	224	4
11	0	0	0	54	224	4
12	0	0	0	54	224	4
13	0	0	0	54	224	4
14	1	1	-1	66	330	1
15	0	1	0	54	330	4
16	-1	1	1	42	330	7
17	1	1	1	66	330	7
18	0	0	1	54	224	7
19	0	0	0	54	224	4
20	-1	-1	-1	42	118	1

X_1: Temperature (°C), X_2: Ultrasound Power (Watt/cm^2), X_3: Time (min)

$$Y = \beta_o + \sum_{i=1}^{k} \beta_i X_i + \sum_{i<j}^{k} \beta_i X_i X_j + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \ldots.. \quad (5)$$

Where Y represents the response, βo represents intercept of the graph, βi represents regression coefficients and X_i represents the independent variables. All the analytical measurements were carried out in triplicates (n = 3) and mean value with standard deviation was expressed. Analysis of variance ($p < 0.05$) was used to analyze the data using STATISTICA v.7 (Stat Soft, Tulsa, OK, USA) software and

differences between means of the analytical measurements were compared using the Turkey test ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Table 3 Experimental results obtained by FCCD

	Experimental Conditions			Experimental Response					
Run	X_1	X_2	X_3	Y_1	Y_2	Y_3	Y_4	Y_5	Y_6
1	54	224	1	63.43	38.65	350.12	0.123	10.721	3.707
2	42	224	4	68.87	41.88	368.77	0.115	15.444	2.977
3	66	118	1	70.55	39.68	359.78	0.131	5.765	4.041
4	42	330	1	69.79	40.12	349.44	0.117	15.128	2.976
5	54	224	4	72.23	38.96	358.66	0.123	8.768	3.732
6	42	118	7	70.05	43.65	366.78	0.117	14.679	2.808
7	54	118	4	67.32	39.76	347.87	0.121	11.212	3.587
8	54	224	4	71.77	37.65	354.78	0.123	9.123	3.731
9	66	118	7	68.97	39.67	372.88	0.131	5.385	4.112
10	66	224	4	78.28	34.22	370.89	0.132	6.106	4.121
11	54	224	4	70.58	38.78	370.63	0.127	8.54	3.824
12	54	224	4	78.54	40.77	361.76	0.124	9.122	3.547
13	54	224	4	72.65	36.75	368.19	0.119	9.589	3.73
14	66	330	1	77.56	40.95	360.43	0.131	5.896	4.103
15	54	330	4	78.65	36.11	369.11	0.128	8.012	3.906
16	42	330	7	73.65	35.32	371.88	0.119	13.983	2.989
17	66	330	7	80.11	35.35	376.89	0.137	4.012	4.123
18	54	224	7	82.66	33.34	376.67	0.129	7.975	3.896
19	54	224	4	72.23	38.96	358.66	0.123	9.988	3.57
20	42	118	1	61.87	42.65	342.76	0.112	17.045	2.822

Here, Y_1: Juice yield , Y_2: Ascorbic acid , Y_3: Total anthocyanin content , Y_4: Cloud value , Y_5: POD activity and Y_6: Lethality of *E. coli*

Experimental results and Modelling (or optimization of process parameters)

The present study optimized the process parameters in a multivariable system in order to evaluate the fitness of response function in the experimental set up. Linear model was fit in the juice yield, total anthocyanin content and cloud value and quadratic model was fit for ascorbic acid, POD activity and lethality of E. coli as shown in the Table 5.

Table 4 Regression coefficients of the RSM

Parameters	Juice Yield %	Ascorbic acid (mg/100ml)	Anthocyanin (mg/100ml)	Cloud Value (660 nm)	POD activity (RA%)	Lethality of E. coli (log CFU/ml)
Constant	72.490	37.920	362.820	0.120	9.350	3.721
X_1	3.120	-1.380	4.120	0.008	-4.910	0.593
X_2	4.100	-1.760	3.770	0.002	-0.710	0.073
X_3	3.220	-1.470	10.260	0.001	-0.850	0.028
X_1X_2	-	0.980	-0.890	-	0.170	-0.033
X_1X_3	-	-0.230	-2.110	-	0.160	0.012
X_2X_3	-	-1.420	0.220	-	-0.035	-0.003
X_1^2	-	1.220	5.940	-	1.180	-0.221
X_2^2	-	1.100	-5.400	-	0.016	-0.023
X_3^2	-	-0.840	-0.490	-	-0.250	0.032
R^2	0.691	0.738	0.808	0.918	0.978	0.972

RA- Residual activity, POD- Peroxidase, CFU- colony forming unit

Table 5 ANOVA results of process variables against each response

Responses	Model	Sum of square	Mean square	F-value	P-value
Juice yield	Linear	369.640	123.210	10.090	0.0006
Ascorbic acid	Quadratic	109.040	12.120	3.130	0.04510
Anthocyanin	linear	1364.11	454.70	13.570	0.0001
Cloud value	Linear	0.00075	0.00025	59.316	<0.0001
POD activity	Quadratic	259.500	28.830	50.510	<0.0001
Lethality of *E. coli*	Quadratic	3.820	0.420	38.190	<0.0001

The analysis of variance (ANOVA) was done for each responses and the value of various statistical parameters F-value, p-value, mean square, SSE for respective responses are shown in Table 5. Linear and quadratic effect of the independent variables i.e. X_1, X_2, X_3, and their interactions were analyzed for the regression coefficients in the RSM study (Table 4). However, fitness and adequacy of the model was judged by the coefficients of determination (R^2) and lack of fit test.

Effect of Thermosonication on Juice yield

Among the 20 experiments as represented in Table 3.,the experiment run no. 18 led to the highest juice yield (82.66%, temperature of 54ºC, ultrasound power of 224 watt/cm^2, time of 7 min) and the lowest juice yield was obtained in experimental run no. 20 (61.87%, 42ºC, 118 watt/cm^2 and 1 min). This confirms that for higher juice extraction a moderate temperature, ultrasound power and greater time is required for pre-treatment prior to juice extraction. When the juice was extracted without re-treatment with ultrasound, the yield was found to be 68.98%. This proves the advantages of ultrasonication prior to juice extraction. Temperature at 54°C, sonication time at 7 minutes and power at 224 watt/cm^2 were the parameters in combination which gave maximum predicted response of blood fruit juice with 82.66% yield, which is 13.68% more than the yield value of untreated juice. Similar results were reported by **Altemimi *et al*. (2016)**, in which temperature at 74°C and treatment time of 13 minutes gave the maximum yield of extracted grape juice (82.3%), which was 12.9% higher than the yield of the untreated juice. In general, juice yield increases to an optimum temperature before decreasing as heat leads to increase in viscosity and a weak intensity of bubble collapse at higher vapour pressure of a thermosonication process. Thermosonication treatment process is generally optimized in terms of temperature for sufficient violent cavitation bubbles for juice extraction **(Patist *et al*., 2008; Holtung *et al*., 2011)**. At optimal thermosonication temperature of 55ºC more than 45% juice yield was achieved for blackcurrant fruit.

To study the relationship between the input parameters and the output responses, second order polynomial equations were best fitted using multiple regression analysis and the coefficient for each term of the equation was determined. After pre-treatment with ultrasonication, the extraction yield of the juice of different runs ranged between 61.87-82.66%. The p-value and F-value of the model are 0.0006 and 10.09 respectively and the lack of fit shows non-significant (p>0.2). The first order polynomial equation was obtained as follows:

Juice Yield (%) = $72.49 + 3.12X_1 + 4.10X_2 + 3.22X_3$ (6)

The effect of pre-treatment *viz.*, temperature, ultrasound power and time on the total yield of juice extraction has been shown in Figure 1.

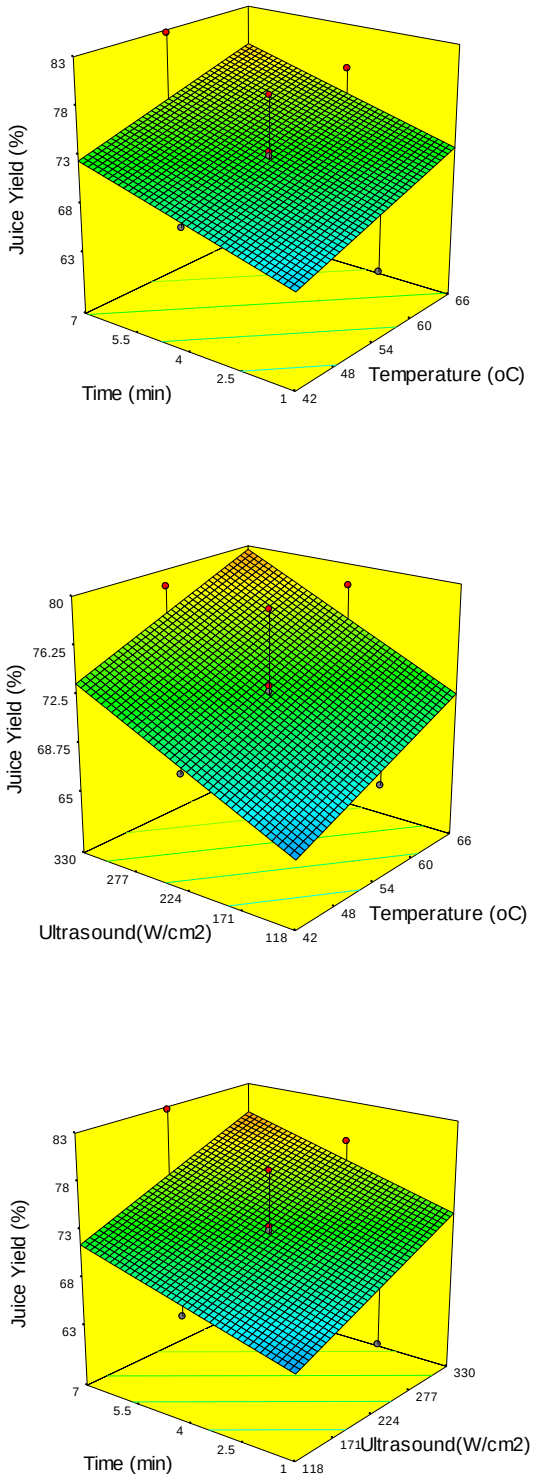


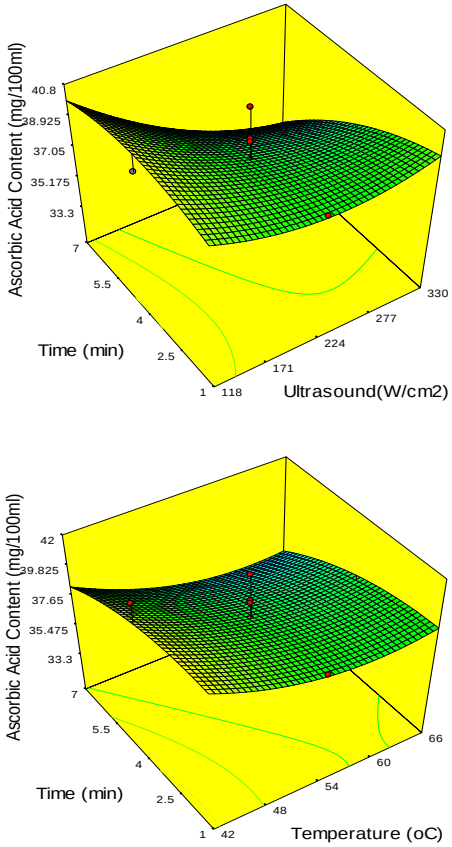
Figure 1 Response surface curve of juice yield as a function of temperature, ultrasound power and time**.**

Effect of Thermosonication on Ascorbic acid

Considering the ascorbic acid concentration, the maximum amount was observed in the experiment run no. 6 (43.65 mg/100ml) when treated at 42ºC with ultrasound power of 118 watt/cm^2 for 7 min and the lowest content (33.34 mg/100ml) was observed in the experiment run no. 18 when treated at 54ºC with an ultrasound power of 224 watt/cm^2 for 7 min. This suggests that higher temperature and higher ultrasound power has negative effect on the ascorbic acid retention. **Cruz *et al.* (2007)** reported that rapid loss of ascorbic acid by increasing treatment temperature, processing time and sonication power are crucial factors to be considered with respect to fruit juice quality and shelf life. The higher retention of ascorbic acid in fruit juices at lower processing conditions agrees with the present experimental result but with a shorter shelf life. **Gamboa-Santos *et al.* (2013)** reported that effect of thermosonication process is due to efficient removal of obstructed oxygen from the juice and influencing the better retention of ascorbic acid. However, the negative effect of conventional (68°C) and nonconventional (55°C with thermosonication) process with increased temperatures is that the degradation of ascorbic acid increased and resulted in its poor retention in the end product. Both the process treatments at elevated temperature (55 and 68°C) resulted in the degradation of ascorbic acid at at a rapid rate and this might be due to severe cavitational collapse of bubbles attributed to severe physical conditions **(Erkaya *et al.*, 2015)**. When TS processing of the samples were carried out at constant temperature for different power load and exposure time combinations, the loss of ascorbic acid content was increased.

The ascorbic acid content of juices of different experiment runs ranged between 33.34 - 43.65 mg/100 ml. The p-value of the model was found to be significant (p<0.05) and lack of fit was non-significant (p>0.1). A second order quadratic polynomial was found to fit the model. A developed model of coded process variables for ascorbic acid with only significant terms are shown as follows:

Ascorbic Acid Content (mg/100ml) = $38.66 – 1.38X_1 – 1.76X_2 - 1.47X_3 - 1.42X_2X_3$ (7)



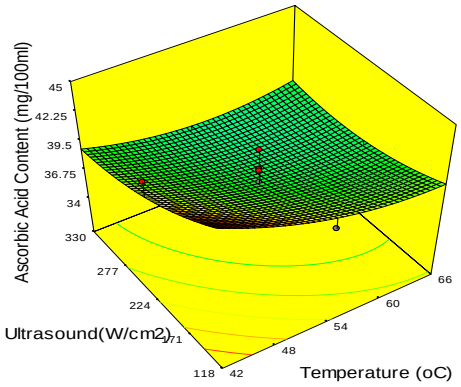


Figure 2 Response surface curve of ascorbic acid as a function of temperature, ultrasound power and time**.**

All the input variables like temperature, ultrasound power and time along with the combined effect of time and ultrasound power have negative effect on the ascorbic acid content. The negative effect shows that with the increase in the values of these variables, ascorbic acid decreases and vice versa. The effect of temperature, ultrasound power and time on ascorbic acid content is shown in Figure 2.

Effect of Thermosonication on Anthocyanin

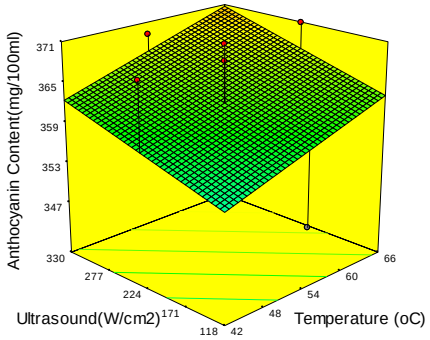
With regards to extraction of anthocyanin, the highest value (376.89 mg/100ml) and lowest value (342.76 mg/100ml) was observed in the experiment run no. 17 and 20 respectively. This suggests that higher ultrasound power and treatment time is required for the better extraction of anthocyanin. However the bioactive compound reduction rate was directly influenced by processing temperature, exposure time and ultrasound power load. Thermosonication treatments produces cavitation bubbles that exerts mechanical force enhancing the disruption of the fruit cell wall and the sudden expulsion of bioactive compounds **(Martínez-Flores *et al.*, 2015)**. The extraction yield of the bioactive compounds increased with the increasing ultrasound power which agrees with our results. The increase in ultrasound power facilitates more cavitation which results in higher disruption of the peel's cell walls, increasing the solubility of the compounds present in the peel resulting in the enhancement of the extraction yield **(Ying *et al.*, 2011)**. This enhancement in extraction of bioactive compounds with ultrasound could be attributed to the ultrasonic effects such as micro jet formation and acoustic streaming **(Sivakumar *et al.*, 2009).** Since the cavitational force of ultrasound causes sonocapillarity and sonoporation, it helps in better penetration of solvents and easy extraction of anthocyanins **(Chemat *et al.*, 2017)**. Therefore, thermosonication can be used as an extraction technique for positive influence on bioactive components with controlled processing condition such as temperature, time, and amplitude level.

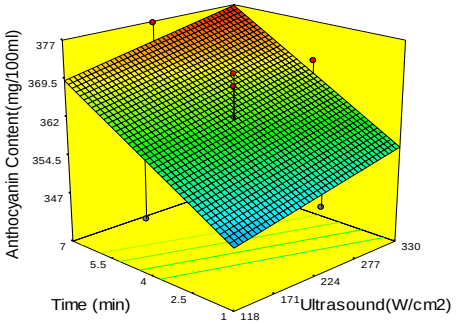
The total anthocyanin content of the juice ranged between 342.76 - 376.89 mg/100 ml. The p-value of the model was found significant ($p<0.005$) with F-value of 13.57 and the lack of fit was non-significant ($p>0.4$). The first order linear model of temperature and time has positive effect on the total anthocyanin content while the ultrasound power was found to be non-significant.

The first order polynomial model which the anthocyanin's follows

$$\text{Total Anthocyanin Content (mg/100 ml)} = 362.85 + 4.12X_1 + 10.26X_3 \quad (8)$$

The response surface curve for anthocyanin content as a function of temperature, ultrasound power and time is shown in Figure 3.





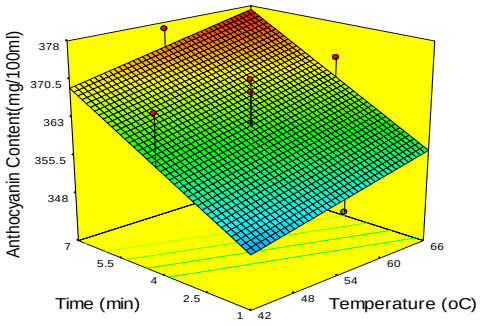


Figure 3 Response surface curve of total anthocyanin content as a function of temperature, ultrasound power and time.

Effect of Thermosonication on Cloudiness

Highest value of cloudiness (0.137), POD activity (17.045 RA%) and lethality of *E. coli* (4.123 log CFU/ml) was observed in the experiment run 17, 20 and 17 respectively and the lowest value of cloudiness (0.112), POD activity (4.012 RA%) and lethality of *E. coli* (2.808 log CFU/ml) was observed in the experiment run 20, 17 and 6 respectively. The cloudiness value was higher when the treatment parameter *viz.*, temperature, ultrasonication and time was higher and the least value of cloudiness was obtained when these treatment parameter was kept lower which was similar to the results reported by **Dabir *et al.* (2017).** Cloud value in sonicated juices increased with increase in sonication power and it may be due to the generation of high pressure gradient resulting in higher cavitation which may lead to colloidal disintegration, dispersion and breakdown of macromolecules into smaller counter micro molecules. **Seshadri *et al.* (2003)** suggested that the application of ultrasound breaks the linear pectin molecule, reducing its molecular weight resulting in weaker network formations.

For cloud value of juice, the experimental values ranged between 0.112 - 0.137 with p-value of the model is found to be significant ($p<0.0001$). The F-value is 59.316 and lack of fit is non-significant ($p>0.5$). The cloud value increases with increase in temperature and ultrasound power as they have positive effect on the cloud value. Also the time has a positive effect and the cloud value increases with the increase in treatment time. The linear model is found to be fit for the experimental design and the first order polynomial model in terms of coded values for cloud value is as follows:

$$\text{Cloud Value} = 0.12 + 0.0082X_1 + 0.002X_2 + 0.0019X_3 \quad (9)$$

The response surface curves for cloud value that shows the effect of temperature, ultrasound power and time is shown in Figure 4.

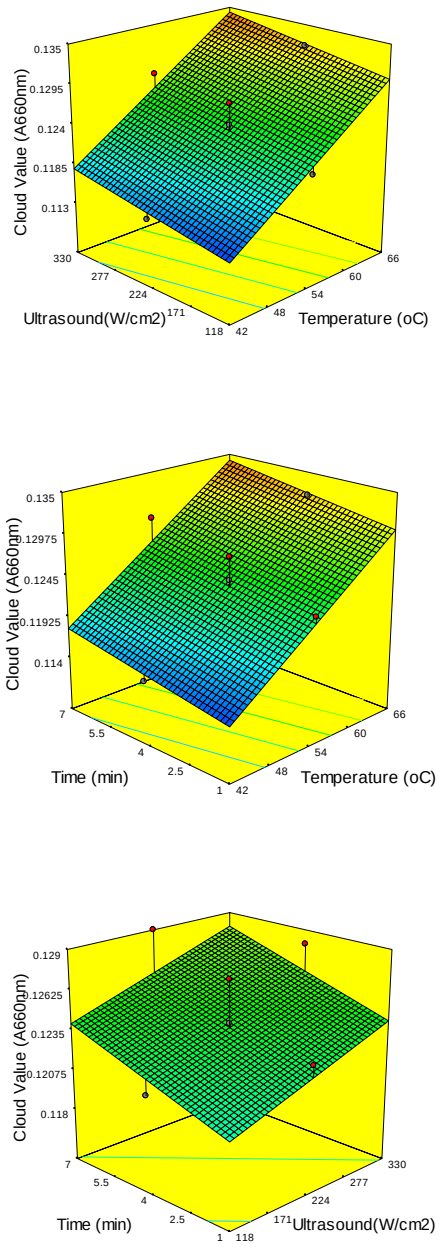


Figure 4 Response surface curve of cloud value as a function of temperature, ultrasound power and time.

Effect of Thermosonication on POD Inactivation

POD activity was found to be highest when the treatment parameters were kept at the lower side and lowest when these parameters was kept at higher side which were in agreement with **Dabir *et al*. (2017).** The key advantage of the TS treatment over conventional pasteurization is the possibility of enzymes inactivation at a slightly higher temperature and shorter time. The thermosonicated juice samples exhibits better quality **(Sasikumar *et al*., 2018)** and that is why the combined effect of thermal and sonication-treated samples shows excellent results of enzymes inactivation at lower temperatures and shorter time combinations. The combined effect of thermosonication in treated juice samples initially ensured thermal and mechanical shock into micro streaming. These cause sudden damage to protein structures of endogenous enzymes and so, this severe physical condition causes higher inactivation of enzymes in juice samples **(Islam *et al*., 2014)**. The mechanical force exercised by cavitation leads to sudden burst of air bubbles produced through thermosonicated process with acoustic field that can cause enzyme inactivation in fruit juice. Free radicals, intrinsic and extrinsic parameters are important factors to be considered and optimized level causes inactivated enzymes during thermosonicated process **(Koshani *et al*., 2015).**

The linear model coefficient viz. temperature, ultrasound power and time has negative effect on the POD activity and so with the increase in these parameters, the POD activity also increases and vice versa. The quadratic coefficient of temperature has positive effect and so with the increase in this value, the value of POD activity also increases and vice versa. A second order polynomial equation of the quadratic model of the POD activity was developed with only significant terms as shown below:

$$\text{POD Activity (\%RA)} = 9.30 - 4.91X_1 - 0.71X_2 - 0.85X_3 + 1.04X_1^2 \quad (10)$$

The effect of temperature, ultrasound power and treated time on the POD enzymes activity of the juice are shown in Figure 5

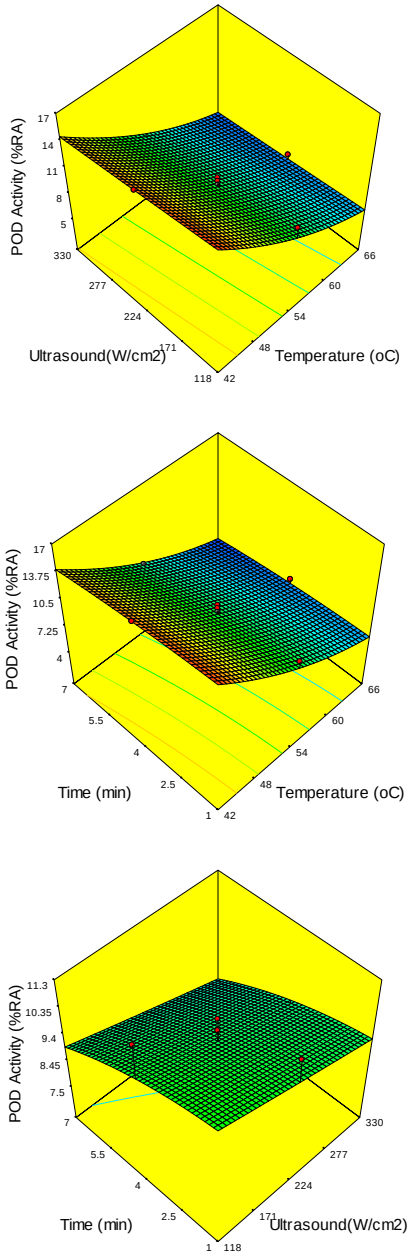


Figure 5 Response surface curve of POD activity as a function of temperature, ultrasound power and time.

Effect of Thermosonication on Lethality of *E.coli*

Lethality of *E. coli* was found to be highest when treatment parameters were set at higher end and vice versa. **Altemiml *et al*. (2016)** reported that as recorded for cranberry, grape fruit and pineapple juices, thermosonication process cause the highest inactivation of harmful microorganism and pathogens. Ultrasound has been proven to decrease the microbial resistance and increase microbial sensitivity to low pH, high osmotic pressure and heat due to cavitations and other characteristics changes in the outer membrane of the cell structure **(Wordon *et al*., 2012; Wong et al., 2008).** At 66ºC, 330 watt/cm^2 and 7 min processing time, a reduction of 4.123 logs for *E.coli* was observed. Similar results were reported by **Muñoz *et al*. (2012)** at conditions of 24 kHz, 50°C for 5 and 2.9 min in orange and apple juices respectively where they achieved a reduction of 5.1 and 4.9 logs, respectively, using E. coli as an indicator. **Walkling-Ribeiro *et al*. (2009)**, in orange juice (30 kHz, 30 min and 55°C), attained a reduction of 5.5 logs in *S. aureus*. **Ying *et al*. (2011)** mentioned that some microorganisms might be more sensitive than others

to thermosonication treatment. Also, shape or size of the microorganisms may affect the treatment efficiency, probably due to an increase in surface area. **Sasikumar *et al.* (2018)** reported that there were no differences on reduction range between gram-negative and gram-positive organisms when ultrasound treatment was applied. **Silva (2016)** have suggested that bacterial cells generally become more sensitive when they undergo TS, mainly due to the absorption of energy by the membranes providing a cumulative effect on the basic functions of the microorganism causing a weakening and/or disruption of the cell membrane, leading to cell lysis.

Similarly for the lethality of *E. coli*, the linear coefficient viz. temperature and ultrasound has positive effect and its value increases with the increase in these values and vice versa. The quadratic coefficient of temperature has negative effect on the lethality of *E. coli*. A generalised second order polynomial equation of the quadratic model for the lethality of E. coli was obtained with only significant terms as shown below:

$$\text{Lethality of } E.\ coli \text{ (log CFU/ml)} = 3.72 + 0.59X_1 + 0.073X_2 - 0.22X_1^2 \quad (11)$$

Figure 6 shows the effect of temperature, ultrasound power and time on the effect of lethality of *E. coli*.

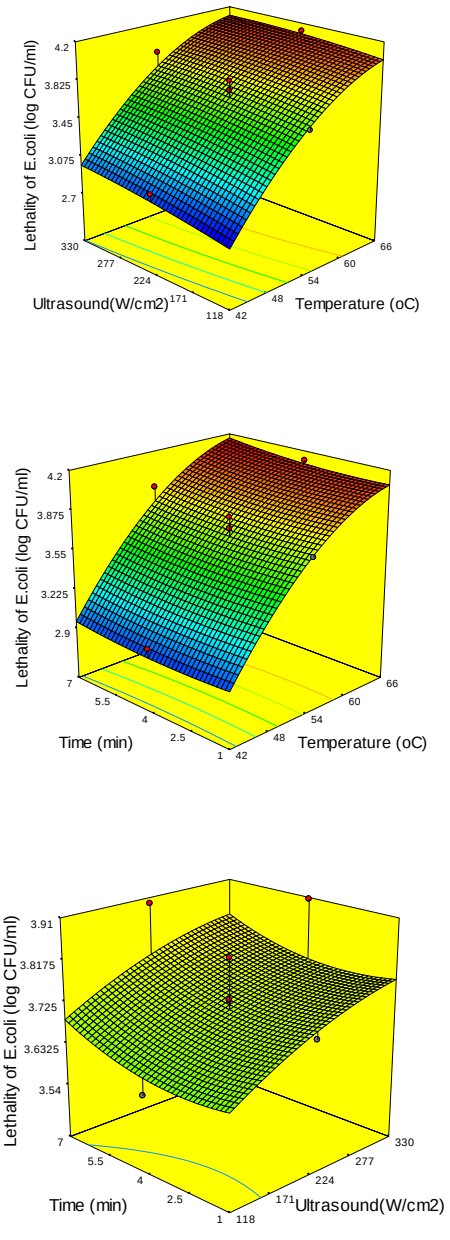


Figure 6 Response surface curve of lethality of *E. coli* as a function of temperature, ultrasound power and time.

CONCLUSION

In the present study, we have tried to implement an ongoing thermosonication extraction technique to produced better quality blood fruit juice. For this purpose, an experimental design was constructed which was based on the RSM face centred central composite design conditions to investigate the effect of various operating conditions including ultrasonic power (118-330 Watt/cm^2), ultrasonic time (1-7 min) and processing temperature (42- 64ºC) which were evaluated for the following responses: Peroxidase activity, juice yield, Lethality of *E.coli*, Cloud value, Ascorbic acid and anthocyanin content. This work indicated that RSM, conventional graphical representation and desirability function are one of the effective tools for identifying the optimum condition within the experimental region. The advantages of using RSM in optimization of process parameters for getting highest juice yield, ascorbic acid, anthocyanin content, lethality of *E. coli* and for minimum cloud value and POD activity has been discussed in this study. Using numerical optimization method, the process variables of temperature at 59.9ºC, ultrasound power at 118 Watt/cm^2 and time of 7 minutes gave maximum juice yield of 73.15 %, ascorbic acid 38.92 mg/100ml, total anthocyanin content of 371.32 mg/100ml, lethality of *E. coli* 3.95 log CFU/ml, minimum cloud value of 0.128 and POD activity of 6.86 %RA.

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