



OPTIMIZATION OF PROCESS PARAMETERS FOR MICROWAVE ASSISTED UV STERILIZATION SYSTEM FOR ORANGE JUICE

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ABSTRACT

A microwave assisted ultraviolet light sterilization system (MWUV) was developed to study the synergistic effect in the sterilization of orange juice. This study used Response surface methodology (RSM) based on Box-Behnken design to get the optimum sterilization condition of MWUV and to analyse its effect on viable bacterial count and biochemical properties. Three independent variables; microwave power (200–500 W), flow rate (120–200 mL/min) and treatment time (0–20 sec) were taken for this study. The optimized processing parameters such as total plate count for bacterial load (1.26 log CFU/mL), total phenols (641 mg GAE/L), L* (57.63), a* (6.37), b* (53.81) and Vitamin C (264.2 mg/L) were found at the microwave power (500 W), flow rate (166 mL/min) and treatment time (9.51 sec). The fresh untreated sample was taken as control. The results showed MWUV could be a fast and effective method for sterilization of orange juice and other liquid foods without negotiating the quality of the sample.

1. Introduction

Liquid foods such as milk, vegetables and fruits juices are globally accepted as nutritious. The Dietary Guidelines for Americans 2010 recommend us to make one-half of our plate fruits and vegetables. Diets high in such liquid foods are widely recommended for their health-promoting properties. From the early time, fruits and vegetables play a key role in our daily diets because of their richness in vitamins (mainly C and A), minerals, natural antioxidants and abundant flavonoids. Moreover, they are considered as a rich source of dietary fibre. Nowadays in the highly demanding global market, the consumers demand for fruit juices are becoming more because of their health consciousness; therefore, is high requirement for new value added and properly processed products to meet

consumer demand for convenience, nutrition, and health.

Citrus fruits and their products are widely consumed because of their health beneficiaries due to high content of vitamin C and other biologically active compounds such as polyphenols, flavonoid, limonoid, carotenoid and fibre. Orange (*Citrus cinensis*) belongs to the genus citrus of the *Rutaceae* family. These juices are consumed as non-alcoholic beverages and their demand in the market continues to rise due to increasing awareness of their health benefits. On the other hand, there is still a problem and risk of pathogenic infections that are related with the consumption of these juices. Different processing and preservations techniques are adopted to reduce this health risk. However, conventional heat (thermal) and prolong microwave sterilization

processing of these citrus juices may destroy the bioactive compounds present in them, thus reducing beneficial health effects (Plaza et al., 2006). To reduce the unwanted effects (loss of nutrients and natural qualities) of the thermal processing methods, other alternative methods that are capable of microbial inactivation can be used. For this, non-thermal methods for processing juices such as high pressure processing (HPP), pulse electric field (PEF), ultrasonic waves, ultraviolet radiation (UV) and their combinations to get a synergistic effect are emerging technologies that are becoming more popular these days (Aronsson et al., 2001; Mertens, 1992; Toepfl et al., 2007). Therefore, to ensure the safety for consumption, to maintain natural fresh quality of the juices, there is an utmost need for an alternative treatment method for longer storage life, microwave assisted UV treatment may also be a positive solution of these problems.

Many researchers had showed the effects of microwave treatment for processing and preservation on food products (Hayet et al., 2010; Polydera et al., 2003). Microwave heating works superior to conventional heating over slow thermal diffusion that results in slow cooking and burning of outside layer which is not found in microwave heating. The microwave pasteurization and sterilization for liquid food retain better quality due to lower thermal exposure i.e. require less processing time to inactivate enzymes and most heat resistant microbes. Sterilization by conventional methods may not be possible without altering the bio-chemical properties of orange juice (Handwerk & Coleman, 1998; Lee & Nagy, 1998). Combination of both microwave and UV reduced the microbial load exponentially in waste water treatment (Mishra et al., 2010).

For processing of food materials by UV light, a specific range of wavelength is used

which varies from 100 to 400 nm and is classified as UV-A (320–400 nm), UV-B (280–320), and UV-C (200–280 nm). Short-wavelength UV radiation UV-C (200-280 nm) is regarded as the germicidal region fatal to most of microorganisms (Bintsis et al., 2000; Sizer & Balasubramaniam, 1999). Since 1980s, disinfection of water by chlorination process has been replaced by UV radiation in many countries (Gibbs, 2000). UV irradiation treatment in foods has been approved and recommended by the US Food and Drug Administration (FDA) and US Department of Agriculture (USDA). It may be a suitable method for preserving various liquid foods such as milk, fruit juices and other beverages. During the UV treatment, there is no known toxic and substantial non-toxic by-products are formed that will harm the human health. But it may not be considered as a standalone method for complete sterilization of liquid foods because it works effectively on the surface and limited to bulkiness, organic solutes, suspended particles and colour of the juice (Koutchma, 2008; Falguera et al., 2011). UV radiation can be generated by an electrodeless lamp powered by the electromagnetic waves generated by microwaves at the frequency of 2450 MHz. When microorganisms are exposed to UV radiation, cellular DNA absorbs the energy by purines and pyrimidine bases, and adjacent thymine molecules links together that damages the cellular metabolism and kills the microorganisms (Reisz et al., 2014; Billmeyer, 1997; Giese, 1997).

Therefore, the primary aim of this study is to optimize the processing parameters involved in the microwave assisted UV sterilization system of liquid foods. The system was developed to combine both the microwave and UV radiations and to study the synergistic effect on microbial load and quality parameters of orange juice by

developing a lab scale microwave assisted ultraviolet sterilization (MWUV) system.

2. Materials and methods

2.1. Materials

Fresh orange fruit was procured from Rourkela market, Odisha, India. The raw samples were immediately taken to the laboratory for cleaning, juice extraction and then for microbial and quality analysis. A household microwave oven of frequency 2450 MHz (1200 Watts output) was used for the treatment in which mercury gas filled electrodeless lamps (Albatross UV, Part No. 558432, H-Type, USA) with dimensions (length - 152.4 mm, diameter - 9 mm) and power output of 300 watts per inch were added as per design required for treatment.

2.2. Extraction of fruit juices

The quality parameters like shape, size, colour and scratch-free were taken into account for choosing the fruits. The selected ones were sorted and washed thoroughly under tap water to get remove the surface microbes and contaminations. The peel was removed by using a stainless steel knife and the rinds and seeds were taken out from the juicy pulp manually to avoid bitterness of the extracted juice. Then the pulps were blended using a grinder (Bajaj Mixer, India) and filtered with the help of muslin cloth. The filtrate was immediately packed and kept in sterilized airtight glass bottles at 5 °C for further experimentations.

2.3. Optimization of sterilization process

The liquid samples were treated to microwave alone as well as microwave assisted ultrasound treatments according to different power or time combinations given by experimental design at a particular treatment time. Microwave irradiation power, A (200-500 W), flow rate, B (120-200 mL/min) and treatment time, C (0-20 sec) were taken as independent variables.

Untreated raw sample was taken as a control. All the experiments were done in triplicate to get precise results. After the treatments, the treated samples were immediately taken for microbial and biochemical analysis.

2.4. Experimental setup

Box–Behnken Design was used to optimise the MWUV treatment parameters viz. microwave power, flow rate and treatment time with respect to the responses such as microbial count (total plate count), and biochemical properties; colour (L^* , a^* , b^* values), total phenols and vitamin C. Analysis of data and model creation were executed by using the Design Expert Software (Version 10.0.7.0, Stat-Ease, Inc., Minneapolis, MN 55413) for optimisation of variables processing parameters. Table 1 shows the range and centre point values of the three independent variables (microwave power, flow rate and treatment time). Through the design software, a total of 17 (seventeen) experiments at 5 (five) replications at the centre point were designed. A second order polynomial equation can be used to show the effect of the dependent variables on the independent variables and also acts as a function of independent variables (Equation 1).

$$Y = b_0 + \sum b_1X_1 + \sum b_{11}X_1^2 + \sum b_{12}X_1X_2 \quad (1)$$

where Y is the experimental responses; X_1 and X_2 the levels of the variables; b_0 is the constant; b_1 the linear coefficient; b_{11} the quadratic term; and b_{12} the coefficient of the interaction terms. Analysis of variance (ANOVA), regression analysis and surface plotting (Figures 1 to 4) were performed to establish optimum condition for microwave assisted UV treatment on fruit juices. Three-dimensional response surface plots were achieved by changing the variables; keeping one variable constant at the centre point and changing the remaining two variables in the experimental range.

Table 1. Independent variables and their level used for central composite design

Independent variables	Units	Level		
		-1	0	+1
Microwave irradiation power (A)	W	200	350	500
Flow rate (B)	mL/min	120	160	200
Treatment time (C)	sec	0	10	20

2.5. Microbial analysis

Total plate count is the most common method used to quantify the total number of viable bacteria in foods, water and all processed foods. Bacterial cells present in food form colonies when nutrient medium is provided, which can be counted to find the number of cells in the sample. The results are expressed as the number of Colony Forming Units (CFU) per ml of the sample (CFU/ml). Nutrient agar was taken as a nutrient medium which supported growth of different types of bacteria. Molten and autoclaved nutrient agar was transformed to Petri plates to form agar plates. On solidifying of agar, inoculation of bacteria was done from the diluted samples. The plates were kept in incubator at 37 ± 1 °C (Anderson et al., 2011; Das et al., 2015). Bacterial colonies were counted in digital colony counter, after 24 hours of incubation.

2.6. Biochemical analysis

Both microwave assisted UV treated and raw juices were subjected for the biochemical analysis. Colour values (L^* , a^* , b^*), total phenolic content and vitamin C content were determined as response parameters for biochemical properties of the fruit juice. A Colorimeter (HunterLab Color Flex EZ spectrophotometer, USA) was used to measure the colour values that gave precise of values of L^* - lightness / darkness, a^* - redness / greenness, and b^* - yellowness / blueness of the samples. Determination of vitamin C was done by titration method explained by Mazumdar and Majumder using

2, 6 - dichloroindophenol (DCIP) dye solution (Mazumdar B.C. & Majumder K., 2003). The amount of total phenolic compounds was found out by using the Folin-Ciocalteu method; using gallic acid as standard (Abdullakassim et al., 2007). Absorbance values of the samples were measured at 765 nm wavelength using a Spectrophotometer (Perkin Elmer Lambda 25-UV/VIS, USA). The total phenolic compounds of the samples were expressed as milligrams per liter Gallic acid equivalents (mg GAE/L).

3. Results and discussions

Tables 2 and 3 show the Box-Behnken design matrix and dependent variables with their respective coefficients of determination (R^2), coefficient of variance (C.V.) and standard deviation (Std. Dev.) respectively. Statistical analysis indicated that the proposed model was adequate, possessing no significant lack of fit and with satisfactory values of the R^2 for the total phenol, colour values (L^* , a^* , b^*), vitamin C and total plate count. Table 4 shows the actual and predicted values of all the responses generated by Box-Behnken design. Generally, a higher value of coefficient of variances shows that difference in the mean value is high and does not satisfactorily develop an adequate response model (Ravikumar et al., 2006).

Table 2. Box-Behnken design matrix

Expt. No.	Microwave assisted UV (A)	Feed rate (B)	Treatment time (C)	Total phenols (mg GAE/L)	L*	a*	b*	Vitamin C (mg/L)	Total plate count (log CFU/mL)
1	-1	-1	0	628.56	58.00	6.35	52.6	275.44	3.49
2	1	-1	0	650.78	57.33	6.43	53.53	261.56	2.63
3	-1	1	0	592.89	60.00	6.28	54.66	293.78	4.50
4	1	1	0	624.44	57.00	6.36	54.01	270.00	3.10
5	-1	0	-1	590.00	60.00	6.28	54.00	285.00	7.00
6	1	0	-1	631.00	59.00	6.36	54.17	278.89	3.00
7	-1	0	1	616.00	58.33	6.35	53.97	265.56	5.22
8	1	0	1	615.00	56.33	6.44	53.47	230.00	1.10
9	0	-1	-1	606.11	59.00	6.36	54.08	260.00	3.22
10	0	1	-1	570.34	60.67	6.26	54.71	295.11	1.26
11	0	-1	1	615.00	57.33	6.41	53.38	249.55	2.60
12	0	1	1	620.00	57.00	6.51	54.00	260.00	8.00
13	0	0	0	630.44	58.67	6.32	54.28	279.44	4.89
14	0	0	0	620.00	59.00	6.33	54.00	267.11	2.33
15	0	0	0	631.00	58.33	6.41	53.50	267.00	1.13
16	0	0	0	625.00	59.00	6.35	54.00	269.00	6.00
17	0	0	0	631.00	59.33	6.30	54.28	267.00	1.30

3.1. Total plate count

The linear terms microwave assisted UV power and treatment time were found to be significant at $p < 0.001$. As the microwave power and treatment time increases the total plate count decreases. The interaction terms between microwave assisted UV and

treatment time were found to be significant at $p < 0.05$. As the combined effect of microwave power and treatment time decrease the total plate count and is shown in Figure 1. The quadratic term of treatment time is significant at $p < 0.001$. The coefficient of determination and adjusted

coefficient of determination was 96.90 and 93.11 (Table 3) and were extremely fitting the data obtained from total plate count. The reason for this better sterilization may be because of the extra stress added by the UV radiation given to the microorganisms against their growth (Koutchma, 2008; Steed et al., 2008). The results obtained from this technique of sterilization met the World Food Programme Standard.

3.2. Colour values (L^* , a^* , b^*)

From the Figure 2 (c-h), it can be observed that the effect of independent process parameters on dependent variables and their responses. The linear terms of L^* values i.e. microwave assisted UV and treatment time showed significant negative values at $p < 0.001$ while flow rate showed insignificant difference at $p > 0.10$. The interaction terms microwave assisted UV and flow rate; flow rate and treatment time showed significant negative values at $p < 0.001$ and $p < 0.05$ respectively. Some quadratic terms such as microwave assisted UV and flow rate showed significant negative difference at $p < 0.05$ and $p < 0.10$. The coefficient of determination and adjusted coefficient of determination found to be very high for L^* values. There was a slight decreased in L^* value (untreated sample - 61.82; optimized value - 57.63); Table 5. This shows the orange juice turned little bit dark due to the heating effect of microwave treatment. Similar findings were reported by different researchers (Wibowo et al., 2015; Cortés et al., 2008; Cserhalmi et al., 2006; Lee & Coates, 2003). The linear term flow rate showed significant negative difference on a^* values. Also, the interaction terms between flow rate and treatment time showed significant positive difference on a^* values. The data points do not fit extremely well because the coefficient of determination (R^2) and adjusted coefficient of determination (Adj R^2) were found to be low

i.e. 81.82 and 58.46 respectively (Table 3). There was a slight increased in a^* value with the increased in flow rate and microwave power whereas reverse was the case for treatment time. This indicates the juice became little bit reddish the treatment (Wibowo et al., 2015; Koka et al., 2004).

The linear terms microwave assisted UV and flow rate were found to be significant at $p < 0.001$ and $p < 0.10$. b^* values decreased as the microwave power and flow rate increased. The interaction term between microwave assisted UV power and flow rate were found to be significant at $p < 0.05$ and the b^* value decreased as this interaction term values increased. The coefficient of determination and adjusted coefficient of determination were found to be 82.56 and 60.15 respectively (Table 3). This decrease may be due to partial precipitation of unstable, suspended particles in the treated orange juice (Rivas et al., 2006; Genovese et al., 1997).

3.3. Vitamin C

All the linear terms i.e. microwave assisted UV power, flow rate and treatment time were found to be significant at $p < 0.001$. Vitamin C increases as the flow rate increases whereas decreases when microwave power increases Figure 3 (i and j). The reason for decrease in the vitamin C content may be because of the effect of heat generated by microwave since it is very heat sensitive (Cinquanta et al., 2010; Vikram et al., 2005). The interaction terms between microwave assisted UV power and treatment time; flow rate and treatment time were found to be significant at $p < 0.05$ and 0.10 respectively. The data fits well because the coefficient of determination (R^2) and adjusted coefficient of determination (Adj R^2) were found to be 94.23 and 86.83 respectively Table 3. The quadratic term of treatment time found to be significant at $p < 0.05$.

Table 3. Regression coefficients, standard deviation (Std. Dev.), R², and CV values for six dependents variables for the microwave assisted UV treatment

Coefficient	Total phenols	L*	a*	b*	Vitamin C	Total Plate Count
Intercept	627.49	58.87	6.340	53.900	269.91	12.87
A	11.72***	-0.83***	0.041	0.0063***	-9.92***	-9.79***
B	-11.60***	0.38	-0.017***	0.470*	9.04***	-0.20
C	8.57**	-1.21***	0.056	-0.270	-14.24***	-18.95***
A ²	3.41	-0.43**	-0.0073	-0.230	1.99	2.60
B ²	-6.73	-0.35*	0.020	-0.086	3.29	3.80
C ²	-17.90***	-0.01	0.023	0.120	-7.04**	9.84***
AB	2.33	-0.58***	0.001	-0.390**	-2.47	0.0075
AC	-10.50**	-0.25	0.0025	-0.170	-7.36**	5.20**
BC	10.19*	-0.50**	0.050**	-0.0025	-6.17*	-1.40
Std. Dev.	8.72	0.33	0.042	0.300	5.70	4.36
C.V. (%)	1.42	0.57	0.660	0.560	2.12	21.27
R ²	90.75	96.67	81.820	84.17	94.23	96.90
Adj R ²	78.85	92.39	58.460	60.15	86.83	93.11

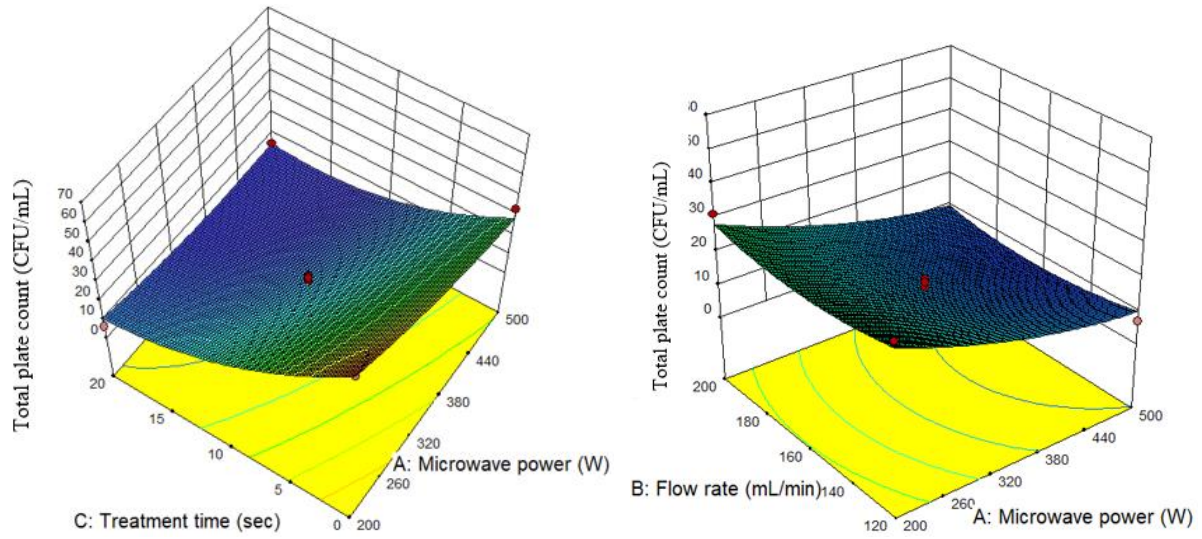
*** Significant at p < 0.001

** Significant at p < 0.05

* Significant at p < 0.10

Table 4. Box–Behnken design: Actual and predicted values of the responses

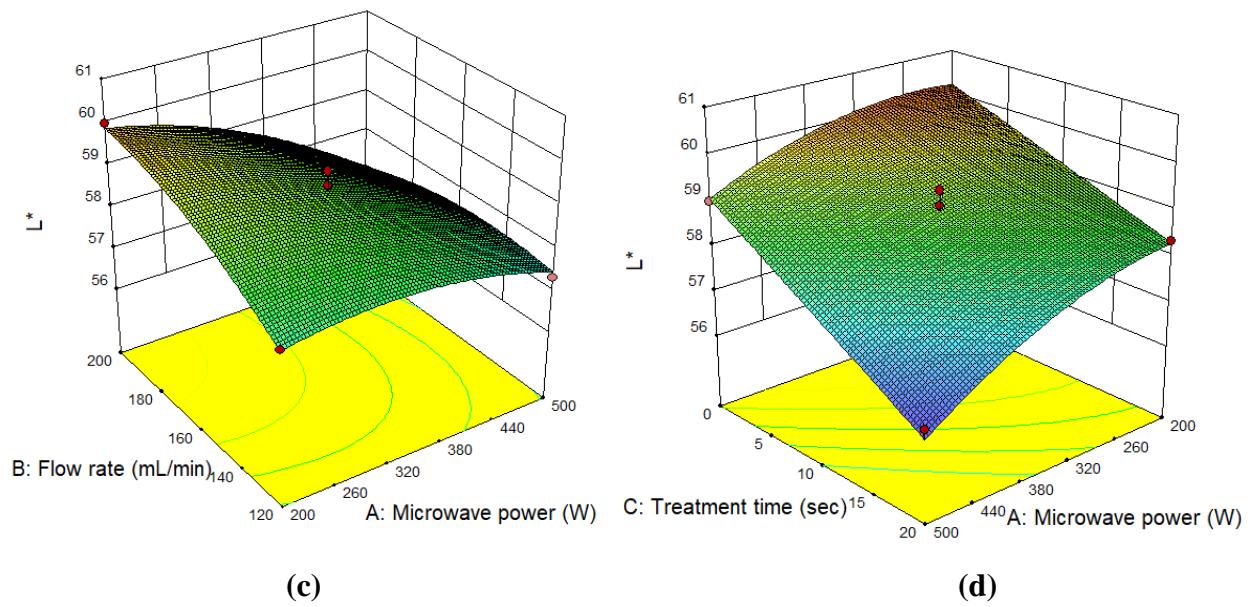
Expt. No.	Total phenols (mg GAE/L)		L*-value		a*-value		b*-value		Vitamin C (mg/L)		Total plate count log(CFU/mL)	
	Act	Pre	Act	Pre	Act	Pre	Act	Pre	Act	Pre	Act	Pre
1.	628.56	626.38	58.00	57.96	6.35	6.33	52.60	52.84	275.44	273.59	3.49	1.68
2.	650.78	645.15	57.33	57.45	6.43	6.41	53.53	53.62	261.56	258.71	2.63	1.52
3.	592.89	598.52	60.00	59.88	6.28	6.30	54.66	54.58	293.78	296.63	4.50	4.85
4.	624.44	626.62	57.00	57.04	6.36	6.38	54.01	53.77	270.00	271.85	3.10	2.89
5.	590.00	582.21	60.00	60.21	6.28	6.26	54.00	54.01	285.00	281.65	7.00	7.58
6.	631.00	626.65	59.00	59.04	6.36	6.34	54.17	54.33	278.89	276.55	3.00	3.27
7.	616.00	620.35	58.33	58.29	6.35	6.37	53.97	53.81	265.56	267.91	5.22	4.95
8.	615.00	622.79	56.33	56.12	6.44	6.46	53.47	53.46	230.00	233.35	1.10	1.76
9.	606.11	616.08	59.00	58.83	6.36	6.40	54.08	53.83	260.00	265.19	3.22	3.27
10.	570.34	572.50	60.67	60.59	6.26	6.26	54.71	54.79	295.11	295.61	1.26	1.66
11.	615.00	612.84	57.33	57.41	6.41	6.41	53.38	53.30	249.55	249.05	2.60	1.16
12.	620.00	610.03	57.00	57.17	6.51	6.47	54.00	54.25	260.00	254.81	8.00	5.95
13.	630.44	627.49	58.67	58.87	6.32	6.34	54.28	54.01	279.44	269.91	4.89	3.64
14.	620.00	627.49	59.00	58.87	6.33	6.34	54.00	54.01	267.11	269.91	2.33	2.82
15.	631.00	627.49	58.33	58.87	6.41	6.34	53.50	54.01	267.00	269.91	1.13	2.67
16.	625.00	627.49	59.00	58.87	6.35	6.34	54.00	54.01	269.00	269.91	6.00	5.82
17.	631.00	627.49	59.33	58.87	6.30	6.34	54.28	54.01	267.00	269.91	1.30	2.22



(a)

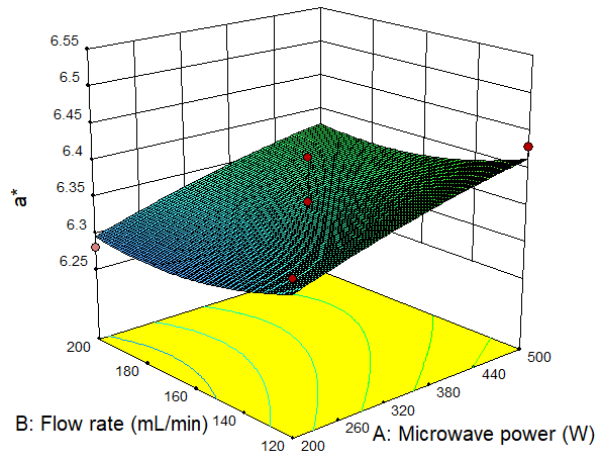
(b)

Figure 1 (a, b). Response surface plots (3D) for total plate count as function of microwave power, flow rate and treatment time.

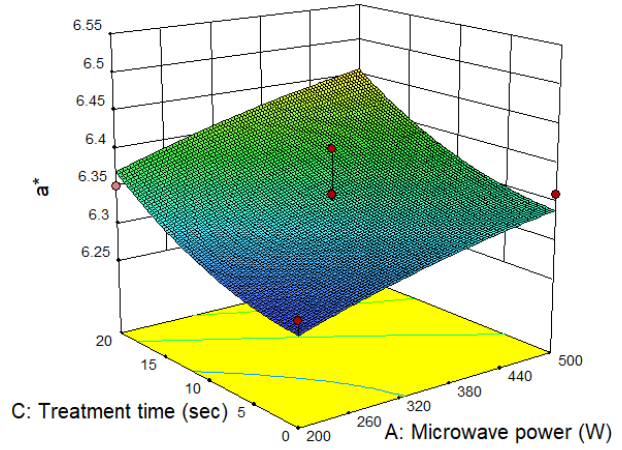


(c)

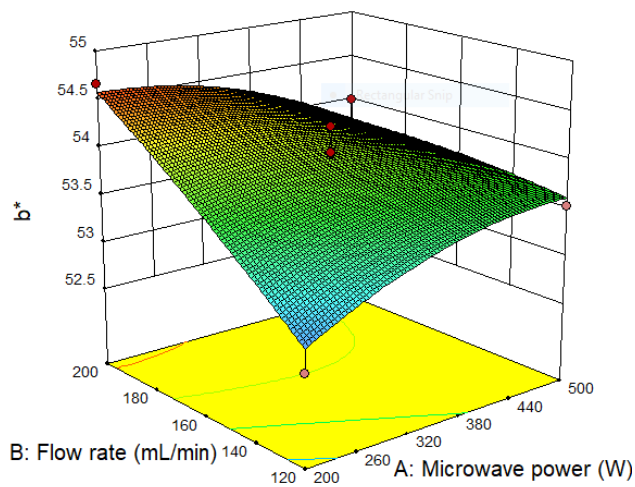
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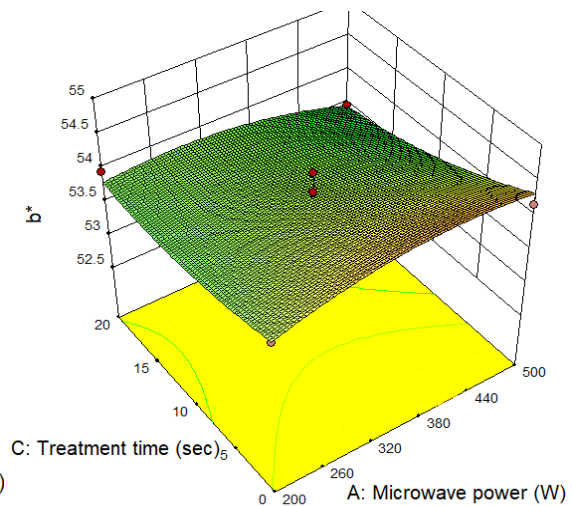
(e)



(f)



(g)



(h)

Figure 2 (c-h). Response surface plots (3D) for colour values (L^* , a^* , b^*) as function of microwave power, flow rate and treatment time.

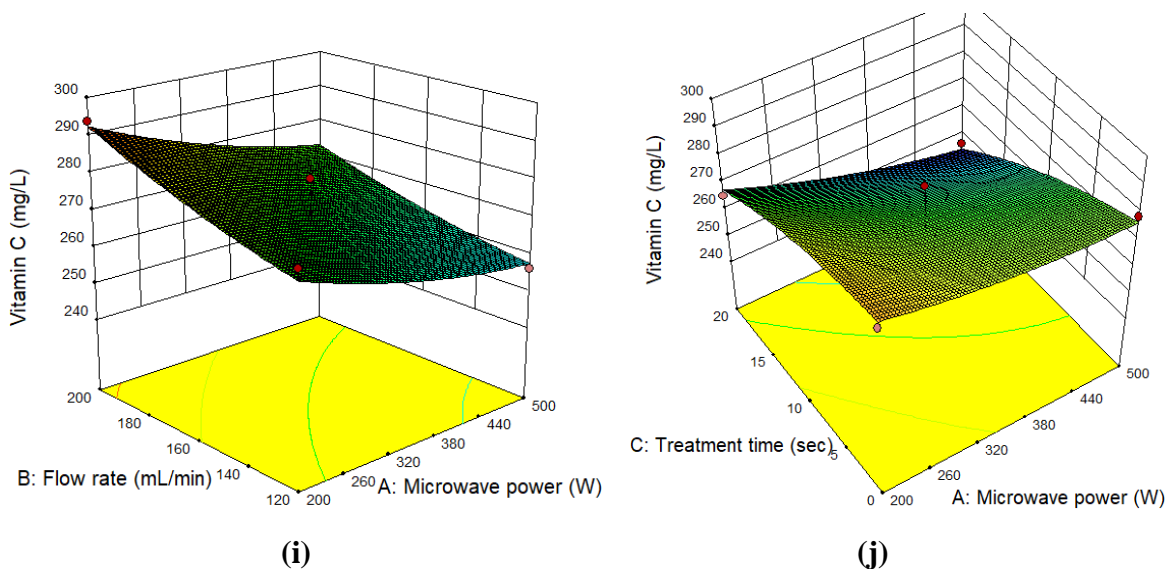


Figure 3 (i, j). Response surface plots (3D) for vitamin C as function of microwave power, flow rate and treatment time.

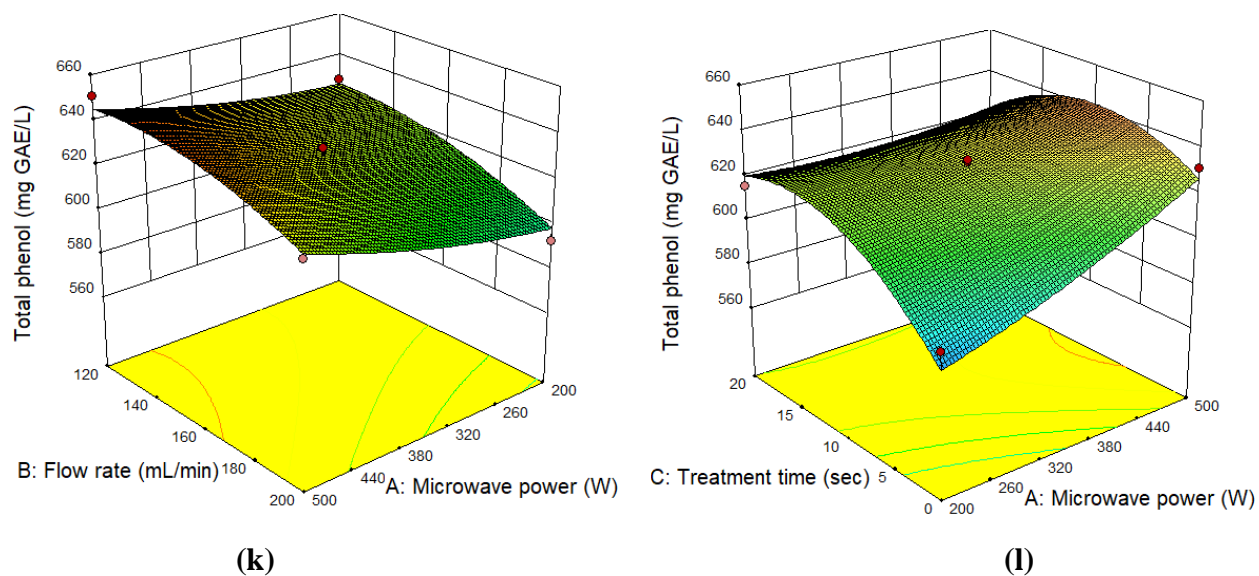


Figure 4 (k, l). Effect of microwave power, flow rate and treatment time on total phenolic content.

Table 5. Optimized values obtained from the Design expert software after optimization

Variables	MWUV treated sample (Optimized values)	Control sample (Fresh orange juice)
Microwave power (W)	500.00	-
Flow rate (mL/min)	166.00	-
Treatment time (sec)	9.51	-
Total phenols (mg GAE/L)	641.00	540.00
L*	57.63	61.82
a*	6.37	6.22
b*	53.81	54.26
Vitamin C (mg/L)	264.20	330.52
Total plate count (log CFU/mL)	1.26	5.38

3.4. Total phenols

Treatment time has a higher impact than microwave power on reducing total phenolic content when both the parameters are increased Figure 4 (k, l). With respect to the total phenolic content of the treated samples, the three independent variables: microwave assisted UV power showed a positive significant difference at $p < 0.001$; flow rate showed a negative significant difference at $p < 0.001$ on total polyphenols and treatment time showed positive significant difference at $p < 0.05$. Also the combination of microwave assisted UV power and treatment time showed significant negative difference at $p < 0.05$ on total polyphenols while the interaction effect of flow rate and treatment time showed significant positive difference at $p < 0.10$. The coefficient of determination (R^2) and adjusted coefficient of determination (Adj R^2) for the total polyphenols obtained were 90.75 and 78.85 (Table 3); hence the equation fits precisely well to the data points. Overall, there is increased in total phenolic content in the juice after the treatment with the increased in microwave power, flow rate and treatment time. Many researchers have also found the increased in phenolic content after heat or for the liquid foods like fruit and vegetable juices.

radiation treatment of plant materials (Xu et al., 2007; Jeong et al., 2004; Gulati et al., 2003).

4. Conclusions

The optimum conditions for microwave assisted UV sterilization system were calculated on the basis of microbial count (Total plate count), colour values (L*, a*, b*), total phenolic contents and vitamin C content. As per the design, the optimised independent parameters obtained were 500 W microwave power, 166 mL/min flow rate and 9.51 sec treatment time. Also, at the optimised condition Table 5, the values for the dependent parameters were total phenol: 621 mg GAE/L; L*: 57.63; a*: 6.37; b*: 53.81; vitamin C: 264.2 mg/L and total plate count: 6.46 log (CFU/mL). The system developed for the sterilization of liquid foods using microwave assisted UV treatment gave better results compared to microwave treatment alone in terms of microbial load and preservation of biochemical properties of foods with minimal treatment time. After a proper optimization of processing parameters, this system can be effectively scaled up to industrial and commercial level

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