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Optimization of postharvest ultrasonic treatment of kiwifruit using RSM

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ABSTRACT

This study reports the optimization of ultrasonic treatment combined with sodium hypochlorite (NaOCl) solution on kiwifruit (*Actinidia deliciosa*) to evaluate its effect on microbial population, respiration rate and its textural quality. Response surface methodology (RSM) based on four factors three level central composite design was applied to investigate the effects of process variables on ultrasonic treatment. Four independent variables include ultrasonic intensity (184–368 W/cm^2), temperature (25–40 °C), treatment time (8–15 min) and concentration of the solvent (30–60 ppm) were considered for this study. According to RSM analysis, the optimal treatment parameters obtained were ultrasonic intensity (368 W/cm^2), temperature (25 °C), treatment time (8 min) and concentration of the solvent (30 ppm). Microbial population, respiration rate and some quality parameters were compared with NaOCl treated kiwifruits. An ultrasound combined with NaOCl was found to be the most effective treatment in inhibiting the microbial growth (bacteria, yeast and mold) and preserving the quality of kiwifruits, and these results suggest that the ultrasound treatment may provide an alternative for extending the shelf life of whole kiwifruit, maintains the quality of fresh cut kiwifruits and further increases the shelf life of chitosan coated fresh cut kiwifruit.

1. Introduction

Kiwifruit (*Actinidia deliciosa*) originated from China [1]. It is also called as China's miracle fruit and the horticultural wonder of New Zealand. It is mostly grown in New Zealand, Chile and Italy and to a minor expansion in France, Greece, Iran, Japan, Turkey, Portugal and the United States [2,3] apart from these places kiwifruit also grown in India. It is considered as one of the most important horticultural crops in the world because of high medicinal and nutritional value. These fruits are rich in bioactive compounds such as ascorbic acid, polyphenols and flavonoids which have major beneficial health effects i.e. mainly due to their antioxidant properties [4].

Kiwifruit is a perishable fruit with short shelf life of 1–2 weeks depends on total soluble solids at which it was harvested, stored and transported. During storage undesirable changes and quality loss may take place which includes toughening, loss of water, changes in carbohydrates, proteins, chlorophyll degradation and amino acids. Senescence and decay are considered to be the most important factors that decrease the storage life of kiwifruits after harvest, which leads to a significant economic loss [5,6]. Postharvest decay of kiwifruits can be controlled by using fungicides, however because of the adverse effects of fungicides on environment, human health and the development of fungicide resistant pathogens, application of fungicides over fruits were nullified. Thus, maintaining the freshness and quality of kiwifruit is a pressing problem and there is an urgent need to seek alternative treatments for achieving longer shelf life.

Many studies had reported the effect of ultrasonic treatments in food processing and preservation i.e. inactivation of microorganisms and enzymes; extraction of antioxidant compounds and acceleration of heat transfer [7–14]. Ultrasound in food industry is considered to be an innovative and attractive technology because it has a unique advantage over other technologies. Ultrasound produces acoustic waves which are considered safe, nontoxic and environmental friendly [15]. Ultrasonic treatments combined with the aqueous chemicals like chlorine dioxide, ascorbic acid, sodium hypochlorite (NaOCl), peracetic acid were more effective in reducing the microbial load, decay and retaining the sensory quality of many fruits when compared with the individual treatments and untreated samples [14–16]. The combined ultrasonic treatment with the ascorbic acid was also found to have a synergistic inhibitory effect on most of the enzymes related to enzymatic browning of apple [17]. Similarly, Meng et al. [14] had showed ultrasonic treatment combined with NaOCl could increase the shelf life of the fresh cut kiwifruits. Limited information is available on the influence of ultrasonic treatments combined with

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aqueous chemicals on microbial population and quality maintenance in fruit and vegetables after harvest. Several factors affect the efficacy of the ultrasonic treatment includes ultrasonic intensities, treatment time, temperature [18–20]. A process involved with all these variables required a powerful tool (response surface methodology – RSM) for determining the effects of operational factors and its interactions [21]. RSM is the most widely used techniques for optimizing the food preservation operations and minimally processed fruits and vegetables i.e. fresh cut lettuce, pear [22–25].

However, no optimization has been carried out for the ultrasonic treatment of postharvest kiwifruit. Therefore, our objective of this study was to optimize the ultrasonic treatment of kiwifruit in terms of ultrasonic intensity, temperature, treatment time and concentration of the solvent and evaluate its effect on microbial population, respiration rate and some selected quality parameters under optimum conditions at ambient temperature.

2. Materials and methods

2.1. Materials

Hayward kiwifruits were hand harvested at a commercial farm in Dirang valley (Arunachal Pradesh, India) in the month of late November, 2015 and transported within 12 h to the laboratory. These fruits had an initial total soluble solids of 9 ± 1% (w/w) and moisture content of 82.6 ± 0.80% w.b. (wet basis). Kiwifruits were carefully selected for uniformity size, absence of visual wounds and defects for experiment.

2.2. Optimization of ultrasonic treatment

Whole kiwifruits (unpeeled) were subjected to different power/time treatments according to the combinations generated by experimental design at a constant frequency of 30 kHz. Ultrasound was applied using a probe type ultrasonicator (Sartorius, Labsonic M, Germany), which has a maximum output power density of 460 W/cm^2 with 3-mm titanium probe (maximum amplitude: 180 μm) immersed 20 mm into solvent. 0.6 s cut-off was fixed for all the experiments because cycle cut-off longer than 0.6 s for 18.5 min could rupture the fruit tissues and can increase the temperature of the solvent. The constant temperature of the solvent depends on experiment fit was controlled with the thermal cut-off switch. Kiwifruits treated with NaOCl solution were used as a control. All experiments were performed on whole kiwifruit (unpeeled) dipped in 450 mL of solvent (ratio 1:5). After treatment the fruits were removed and immediately taken for further analyses. All experiments were conducted twice with four replications of each treatment per experiment.

2.3. Experimental design

RSM was used to optimize the levels of ultrasonic intensity, treatment time, temperature and concentration of the solvent with respect to microbial population and quality parameters like fruit firmness and respiration rate. Data analysis and model construction were carried out using software design expert (version 6.0.8, Stat-Ease Inc., Minneapolis, MN). The range and center point values of four independent variables i.e. ultrasonic intensity, temperature, treatment time and concentration of the solvent were given in the Table 1. A three-level-four-factor, central composite design consisting of 30 experimental runs was employed including six replicates at the center point. Second order polynomial equation was used to express the dependent variables as a function of independent variables as follows:

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

Independent variables	Level		
	−1	0	+1
Ultrasonic intensity (X_1)	184	276	368
Temperature (X_2)	25	32.5	40
Treatment time (X_3)	8	11.5	15
Concentration of the solvent (X_4)	30	45	60

$$Y = \beta_0 + \sum_{j=1}^{k} \beta_i X_j + \sum_{j=1}^{k} \beta_{jj} X_j^2 + \sum \sum_{i<j} \beta_{ij} X_i X_j$$

where Y is the predicted response, β_0, β_j, β_{ij} and β_{jj} are the regression coefficients for the intercept, interaction and square respectively, while X_i and X_j are the independent coded variables. Coefficients were interpreted using the F test. Analysis of variance (ANOVA), regression analysis and surface plotting (Figs. 1–4) were performed to establish optimum condition for ultrasonic treatment on kiwi fruits.

2.4. Analogy experiment

Kiwifruits were subjected to ultrasonic treatment under optimal conditions (ultrasonic intensity: 368 W/cm^2, temperature: 25 °C, treatment time: 8 min and concentration of the solvent: 30 ppm). Fruits treated with NaOCl solution were used as the control. After treatment the fruits were immediately taken for microbial and other analyses. There were three replicates of 85 ± 5 g of fruit each per treatment, and the experiment was conducted thrice.

2.5. Microbial analysis

Total bacteria, yeast and mold were examined according to the methods described by [26] with minor modification. Each treated sample was put into 100 ml of previously sterilized sample bags with 0.1% peptone water. Each sample bags were kept inside another sample bags and kept on a reciprocal shaker (120 oscillations/min; 5 ± 1 °C) for 2 h. The wash solutions were evaluated for the microbial levels. Appropriate dilutions (1:10) were made with 0.1% peptone of each wash solution was surface plated on plate count agar (PCA), Potato dextrose agar (PDA) and incubated for 48 h at 35 °C for total bacterial count and 120 h at 25 °C for yeast and mold count. The results were expressed in colony forming unit per square centimeter (CFU/cm^2).

2.6. Respiration rate

Respiration rate was measured in accordance with Wang et al. [27] with minor modifications. Respiration rate was performed by sealing four replicates of about 80 ± 5 g fruits into airtight glass container (total volume of 900 ml) with rubber septum and held at 20 ± 1 °C for 1 h. Samples were then taken for respiration rate before and after the ultrasound treatment. Then 3 ml head space gas was taken by the O_2 and CO_2 meter (checkmate 3, PBI, dansensor, Ringsted, Denmark). The results were expressed in mg CO_2 - kg^{-1} h^{-1} fresh weight (FW).

2.7. Determination of the quality parameters

Firmness of the treated whole kiwifruits (unpeeled) were measured according to Meng et al. [14] with minor modifications. Puncture test was performed with a texture analyzer (TA-HD-plus, Stable Micro Systems, UK) by fitted with a 5 mm diameter stainless steel probe, at a constant speed of 5 mm s^{-1} to a depth

of 10 mm. The operating conditions maintained during analyses were pre-test speed: 1.5 mm s^{-1}, post-test speed: 10.0 mm s^{-1} and trigger force: 0.1 N. The peak puncture force (in Newton) was considered as firmness in unpeeled kiwifruit [14,28,29]. Ten grams of frozen fruit tissue were homogenized in pre chilled 40 ml of distilled water. The homogenate was centrifuged at 10,000*g* for 15 min at 4 °C. The total soluble solid was measured by adding four drops of clarified extract onto a digital refractometer (Atago, 4406 PAL-06S1) calibrated in Brix (gram of sucrose equivalent per 100 g of juice), and expressed as a percentage [1]. Titrable acidity and pH were determined using an automated titrimeter. Ten millimeter of clarified kiwifruit extracts were placed into a sample cup and titrated to the endpoint of pH 8.1 using 0.1 N sodium hydroxide. The results were expressed as % citric acid equivalent. Vitamin C content was assayed by the 2,6-dichlorophenolindophenol titration method and the results expressed as mg/100 g of FW [1].

2.8. Statistical analysis

The root mean square error (RMSE) and mean absolute error (MAE) was calculated to measure the difference between observed and the predicted data for describing the model performance using following Eqs. (1) and (2). The experimental results were analyzed statistically using an independent sample t-test (paired) by SPSS v16 for investigating the potential significant differences in the mean responses for ultrasound treated samples and control samples.

$$\mathrm{RMSE} = \left[\frac{\sum_{i=1}^{N}(R_p - R_{obs})^2}{N}\right]^{1/2} \quad (1)$$

$$\mathrm{MAE} = \frac{1}{N}\sum_{i=1}^{N}|R_{obs} - R_p| \quad (2)$$

where N is the number of points, R_p is the predicted value, R_{obs} is the observed value [36].

3. Results and discussion

3.1. Model fitting

Mean values of all dependent variables to the ultrasonic treatments influenced by ultrasonic intensity, temperature, treatment time and concentration of the solvent were shown in Table 2. Coefficients of second order polynomial equation were derived from the experimental data to obtain significance of coefficients of the models. The resultant polynomial models of the experimental data from the ANOVA with coefficients of multiple determinations (R^2), coefficient of variance (CV) for total bacteria count (TBC), yeast and mold count (Y&MC), firmness (F) and respiration rate (RR), respectively were shown in Table 3. The results suggested that the regression model could fit the dependent variable values well and the error analysis indicated that the lack of fit was insignificant for all the dependent variables. The examination of the coefficients of microbial population (TBC and Y&MC), firmness and respiration rate from ANOVA showed that the linear terms of independent variables were found to be significant ($p < 0.05$). Some quadratic and interactive terms of independent variables for all the dependent variables were found to be significant ($p < 0.05$) (Table 3). Similar kind of results was shown by Cao et al. [30] on strawberry fruit. The main advantage of RSM is to abate the number of experimental trials needed to appraise the multiple parameters and their interactions [31,32]. The performance of the constructed RSM models were also statistically measured because the use of RMSE and MAE was more appropriate to describe the deviation. Apart from R^2, RMSE and MAE as defined by Eqs. (1) and (2) were chosen as ancillary statistical indicators to measure the model performance.

3.2. RSM analysis

3.2.1. Effect of ultrasonic treatment on microbial population in kiwi fruit

The independent variables (ultrasonic intensity, treatment time and concentration of the solvent) had showed negative effect on total bacteria count i.e. The total bacteria count decreases with increase in ultrasonic intensity, treatment time and concentration of the solvent. These variables had showed significant ($p < 0.05$) effects on total bacteria count (R^2 of 0.904, CV of 1.86, RMSE of 0.046 and MAE of 0.038) which indicates the model fits extremely well (Table 3). The interaction terms between ultrasonic intensity and concentration of the solvent had showed significant ($p < 0.05$) effects on total bacteria count. Maximum bacterial load (3.88 CFU/cm^2) was observed at 184 W/cm^2, 40 °C, 8 min and 30 ppm and minimum bacterial load (3.10 CFU/cm^2) was observed at 460 W/cm^2, 32.5 °C, 11.5 min and 45 ppm. Total bacteria count decreases with increase in concentration of the solvent till 60 ppm. Any further increases in concentration of the solvent did not decreases the total bacteria count. The plot of total bacteria count as affected by ultrasonic intensity and concentration of the solvent demonstrates a marked decrease in total bacterial count with increase in ultrasonic intensity and concentration of the solvent (Fig. 1). This attributed bacteria death may be due to cavitation bubbles occur in solvent during ultra-sonication which lead to increase in localized temperature and pressure. Cao et al. [30] had reported the total bacteria numbers increases with respect to treatment power. The effect of ultrasonic intensity on yeast and mold had also showed similar type of results as total bacteria. All the independent variables had showed negative effect on total yeast and mold count i.e. the total yeast and mold count decreases with increase in ultrasonic intensity, temperature, treatment time and concentration of solvent. These variables had showed significant ($p < 0.05$) effects on total yeast and mold count (R^2 of 0.941, CV of 2.76, RMSE of 0.046 and MAE of 0.037) which indicates the model fits extremely well (Table 3). The interaction terms between ultrasonic intensity and concentration of the solvent had showed significant ($p < 0.05$) effects on total bacteria count Maximum yeast and mold load (2.60 CFU/cm^2) was observed at 184 W/cm^2, 25 °C, 8 min and 60 ppm and minimum yeast and mold load (1.78 CFU/cm^2) was observed at 460 W/cm^2, 32.5 °C, 11.5 min and 45 ppm. Total yeast and mold count decreases with the increase temperature and concentration of the solvent till 40 °C and 45 ppm respectively. Any further increase in temperature and concentration of the solvent did not decreases the yeast and mold count. The plot of total yeast and mold count as affected by ultrasonic intensity and concentration of the solvent demonstrates a marked decrease in total yeast and mold count with increase in ultrasonic intensity and concentration of the solvent (Fig. 2). This attributed yeast and mold death may be due to cavitation bubbles occur in solvent during ultra-sonication which lead to increase in localized temperature and pressure. Piyasena et al. [33] studied the effect of ultrasound and compared the reduction of microorganisms with the traditional sterilization methods, pasteurization of milk, and stabilization of wine.

3.2.2. Effect of ultrasonic treatment on quality parameter – firmness and respiration rate of kiwifruit

Firmness is one of the important quality parameter of kiwifruit which decides the eating quality and is the critical attribute of texture. Firmness closely related with the storability of the fruits

Table 2
Central composite design matrix and response values.

Experimental No.	X_1	X_2	X_3	X_4	Total bacteria count (log CFU/cm^2)	Total Yeast & Mold count (log CFU/cm^2)	Firmness (N)	Respiration rate (mg Co_2 kg^{-1} h^{-1} FW)
1	+1	+1	+1	+1	3.25	1.92	56.33	35.73
2	0	0	0	0	3.47	2.25	57.33	31.32
3	0	+2	0	0	3.45	2.30	58.33	36.34
4	−1	−1	−1	+1	3.67	2.60	61.22	27.63
5	+1	−1	−1	−1	3.55	2.30	55.44	29.07
6	−2	0	0	0	3.47	2.54	61.00	32.32
7	−1	−1	+1	+1	3.45	2.56	60.89	35.30
8	+1	−1	−1	+1	3.30	2.35	57.00	30.88
9	0	0	0	0	3.45	2.29	56.33	30.04
10	0	0	0	0	3.40	2.33	57.44	30.45
11	0	0	0	0	3.53	2.35	58.00	32.23
12	+1	+1	−1	−1	3.49	2.44	57.11	31.77
13	0	0	0	−2	3.55	2.46	59.44	28.59
14	+1	+1	+1	−1	3.37	2.20	55.78	33.84
15	0	−2	0	0	3.45	2.50	58.89	27.81
16	0	0	−2	0	3.66	2.37	58.89	28.20
17	+2	0	0	0	3.10	1.78	55.33	36.90
18	+1	−1	+1	−1	3.55	2.25	57.11	27.85
19	−1	+1	+1	+1	3.45	2.56	60.56	35.73
20	+1	−1	+1	+1	3.30	2.05	56.67	36.34
21	−1	−1	+1	−1	3.50	2.55	58.89	30.45
22	0	0	0	0	3.52	2.33	59.00	31.32
23	0	0	0	+2	3.57	2.35	59.22	36.34
24	+1	+1	−1	+1	3.38	2.20	56.67	36.81
25	−1	+1	−1	−1	3.88	2.56	62.89	31.32
26	−1	+1	+1	−1	3.52	2.47	58.56	34.31
27	−1	+1	−1	+1	3.77	2.53	60.89	33.84
28	−1	−1	−1	−1	3.60	2.50	62.33	26.70
29	0	0	+2	0	3.40	2.29	55.89	35.81
30	0	0	0	0	3.50	2.30	58.00	31.32

Table 3
Regression coefficient for the responses.

Coefficients	Total bacteria count	Total yeast and mold count	Firmness	Respiration rate
$X_1(\beta_1)$	−0.100***	−0.173***	−1.894***	0.674***
$X_2(\beta_2)$	0.008	−0.028**	−0.078	1.925***
$X_3(\beta_3)$	−0.074***	−0.045**	−0.616**	1.531***
$X_4(\beta_4)$	−0.035**	−0.030**	0.070	1.769***
$X_1^2(\beta_5)$	−0.041**	−0.028**	0.165	0.801**
$X_2^2(\beta_6)$	0.001	0.032**	0.277*	0.167
$X_3^2(\beta_7)$	0.021	0.014	−0.029	0.149
$X_4^2(\beta_8)$	0.028**	0.033**	0.457**	0.264
$X_1X_2(\beta_9)$	−0.038**	−0.006	0.007	−0.069
$X_1X_3(\beta_{10})$	0.047**	−0.051**	0.507**	−0.692**
$X_1X_4(\beta_{11})$	−0.036**	−0.053**	0.021	0.469
$X_2X_3(\beta_{12})$	−0.038**	−0.015	−0.243	−0.612*
$X_2X_4(\beta_{13})$	0.004	−0.026	−0.118	−0.326
$X_3X_4(\beta_{14})$	−0.006	−0.016	0.382	0.397
R^2	0.904	0.941	0.894	0.915
Adj R^2	0.815	0.885	0.795	0.835
Pred R^2	0.527	0.684	0.526	0.552
Adeq Precision	14.955	16.491	11.519	12.855
Std dev	0.065	0.065	0.940	1.300
C.V	1.860	2.760	1.600	4.030
RMSE	0.046	0.046	0.662	0.918
MAE	0.038	0.037	0.519	0.773
Lack of fit	NS			

* Significant at $p < 0.1$.
** significant at $p < 0.05$.
*** significant at $p < 0.001$.

because characterizes the change in textural quality [30]. The independent variables (ultrasonic intensity, temperature and treatment time) had showed negative effect on firmness i.e. firmness decreases with increase in ultrasonic intensity, treatment time, temperature and concentration of solvent. Ultrasonic intensity and treatment time had showed significant ($p < 0.05$) effect on the fruit firmness (R^2 of 0.894, CV of 1.60, RMSE of 0.662 and MAE of 0.519) which indicates the model fits well (Table 3). The interaction terms between ultrasonic intensity and treatment time had showed significant ($p < 0.05$) effects on firmness. Maximum firmness (62.89 N) was observed at 184 W/cm^2, 40 °C, 8 min and 30 ppm and minimum firmness (55.33) was observed at 460 W/

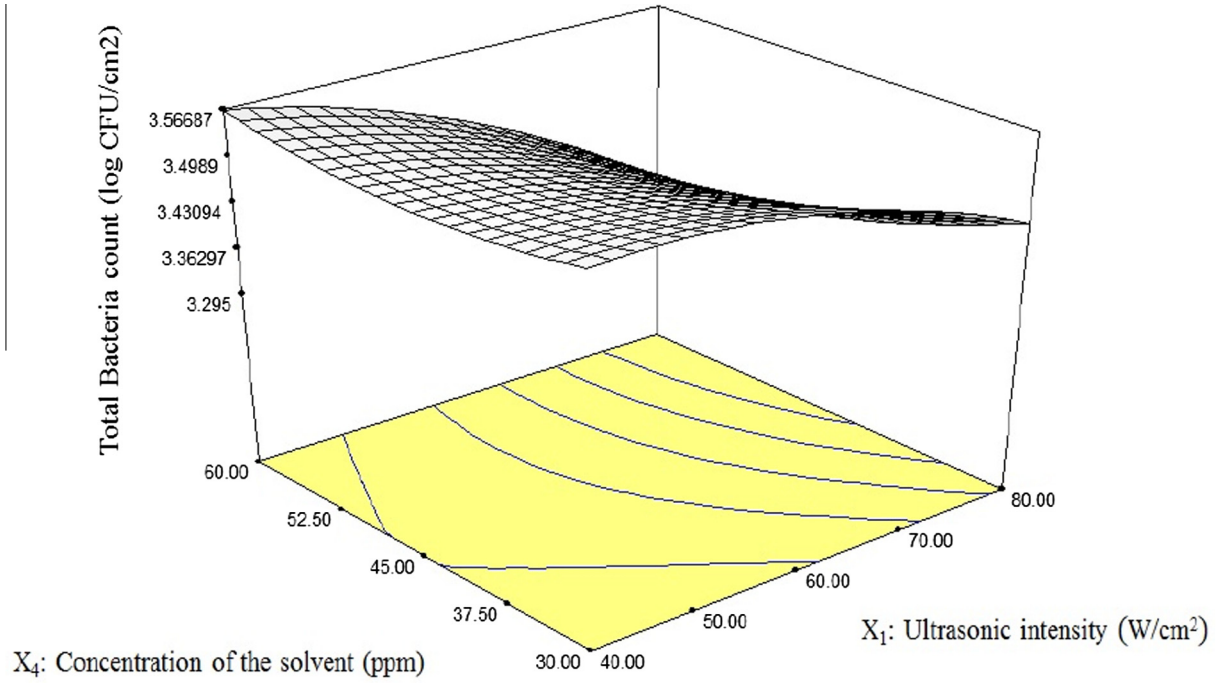

Fig. 1. Effect of ultrasonic intensity and concentration of the solvent on bacteria.

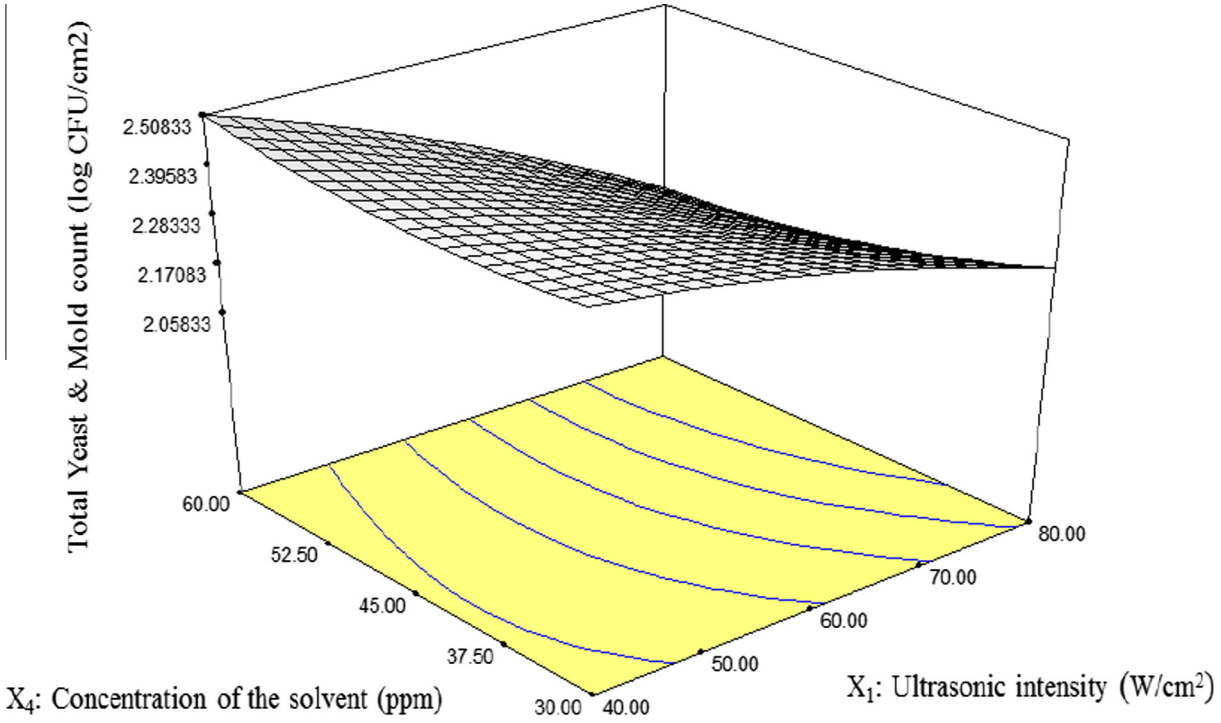

Fig. 2. Effect of ultrasonic intensity and concentration of the solvent on yeast and mold.

cm^2, 32.5 °C, 11.5 min and 45 ppm. Firmness decreases with increase in temperature till 32.5 °C. The plot of firmness as affected by ultrasonic intensity and treatment time demonstrates a marked decrease in firmness with increase in ultrasonic intensity and treatment time (Fig. 3). This may be due to the mechanical effects of ultrasound which leads to fruit softening. Samples of similar mass i.e. 80 ± 5 g was taken for observing the effect of ultrasonic treatment on respiration rate of kiwifruits. All the independent variables had showed positive effect on respiration rate i.e. respiration rate increase with increase in ultrasonic intensity, treatment time, temperature and concentration of solvent. These variables had showed significant ($p < 0.001$) effects on the fruit respiration rate (R^2 of 0.915, CV of 4.03, RMSE of 0.918 and MAE of 0.773) which indicates the model fits extremely well (Table 3). The interaction terms between ultrasonic intensity and treatment time had showed significant ($p < 0.05$) effects on respiration rate. Maximum

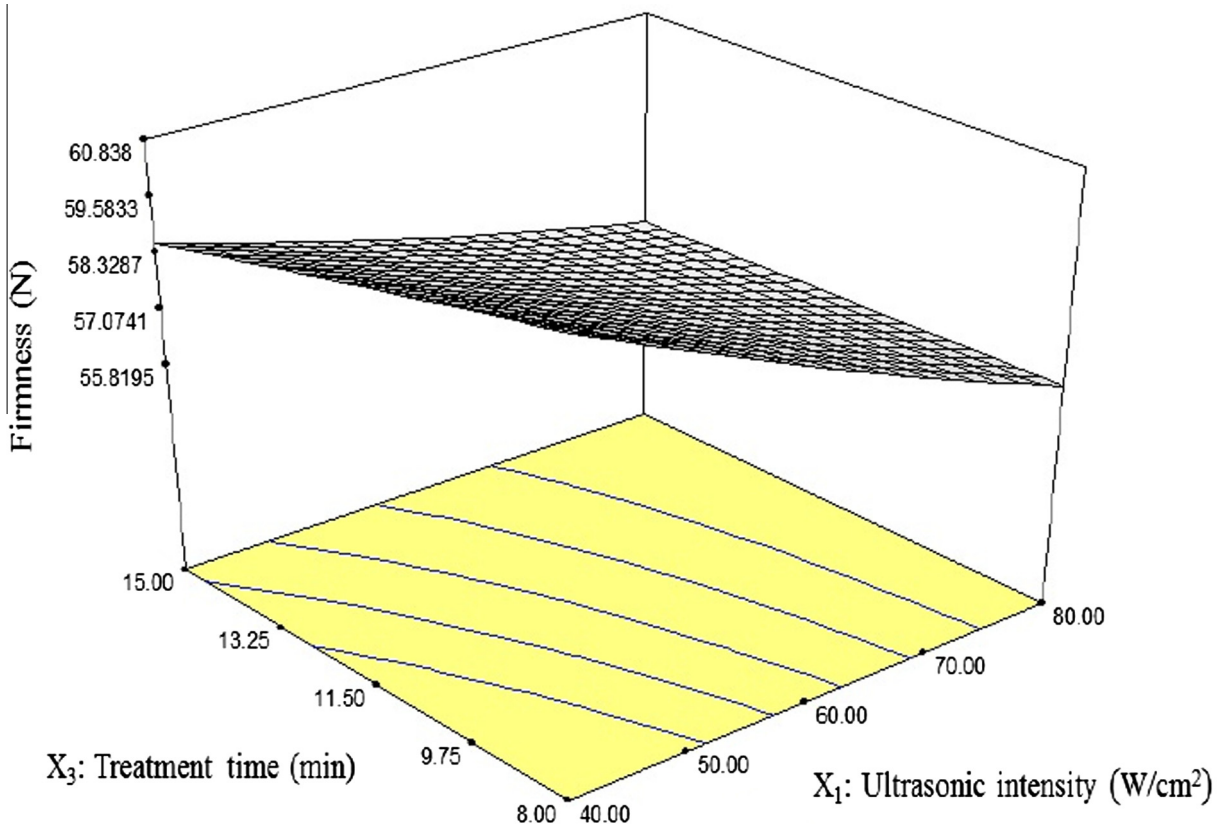

Fig. 3. Effect of ultrasonic intensity and treatment time on firmness.

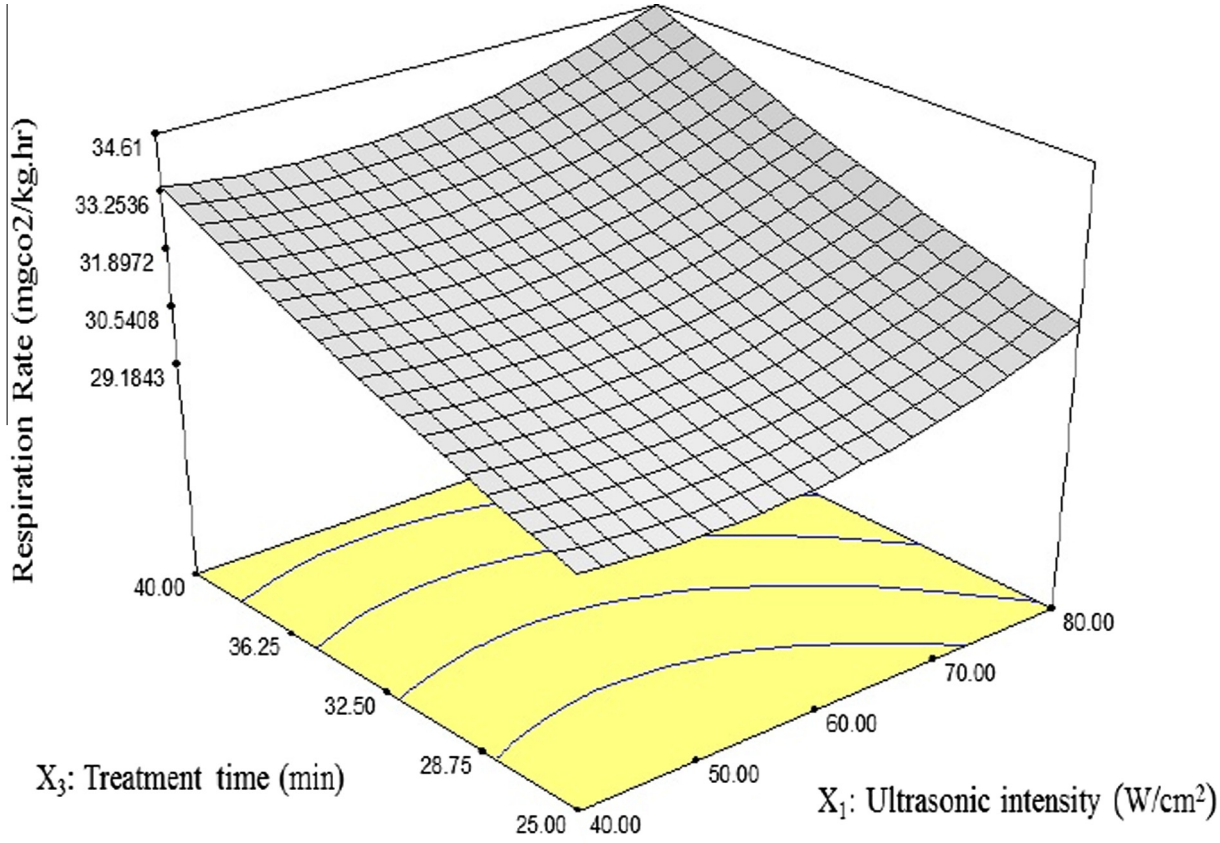

Fig. 4. Effect of ultrasonic intensity and treatment time on respiration rate.

Table 4
Responses and limits of optimizer for optimization using numerical optimization in design expert.

Constraints		Lower	Upper	Lower	Upper	
Name	Goal	Limit	Limit	Weight	Weight	Importance
Total bacterial count	Minimize	3.10	3.88	1	1	3
Total yeast and mold count	Minimize	1.78	2.60	1	1	3
Firmness	Maximize	55.33	62.89	1	1	3
Respiration rate	Minimize	26.70	36.90	1	1	3

Table 5
Optimized solution – response optimizer in design expert.

Variables	Optimized condition
Ultrasonic Intensity (W/cm^2)	368
Temperature (°C)	25
Treatment time (min)	8
Concentration of the solvent (ppm)	30
Total bacterial count (log CFU/cm^2)	3.48
Total yeast and mold count (log CFU/cm^2)	2.34
Firmness (N)	56.77
Respiration rate (mg CO_2 kg^{-1} h^{-1} FW)	27.71

respiration rate (36.90 mgCO_2/kg h) was observed at 460 W/cm^2, 32.5 °C, 11.5 min and 45 ppm and minimum respiration rate (26.70 mg CO_2/kg h) was observed at 184 W/cm^2, 25 °C, 8 min and 30 ppm. Respiration rate of kiwifruit decreased as the ultrasonic intensity increased and reached a minimum at 276 W/cm^2. Any further increase in ultrasonic intensity increases the respiration rate of kiwifruit. This may be due to the cavitation bubbles which generates the energy for chemical and mechanical effects leads to fruit softening (tissue rupture). The plot of respiration rate as affected by ultrasonic intensity and treatment time demonstrates a marked increase in respiration rate with increase in ultrasonic intensity and treatment time (Fig. 4). Ketsa et al. [34] had showed the higher respiration resulted in mango when subjected to a heat treatment. While Serrano et al. [35] had reported that the plums subjected to mechanical damage evidenced lower respiration after being heat treated (45 °C/10 min).

3.3. Optimization of ultrasonic treatment conditions

Numerical optimization was used in design of expert '6.0.8', which gives a desirability function of 0.82. Equal importance of '5' was given to all the independent variables (ultrasonic intensity, temperature, treatment time and concentration of solvent). Based on the relative contribution to quality of final product importance of '3' was given to total bacterial count, yeast & mold, firmness and respiration rate (Table 4). The computer program predicts the optimum ultrasonic treatment conditions for the kiwi fruit were an ultrasonic power, treatment time, temperature and concentration of the solvent as shown in Table 6.

3.4. Effect of ultrasonic treatment under optimum condition on microbial population and quality of kiwi fruit

The optimum conditions (ultrasonic intensity (368 W/cm^2); temperature (30 °C); treatment time (8 min) and concentration of the solvent (30 ppm)) were taken for evaluating the microbial population and quality for fifteen kiwifruit samples in order to verify and compare the effects of ultrasonic treatment with the control (Table 5). The mean values of total bacteria count, yeast and mold count, firmness, respiration rate, total soluble solids, titrable acidity, vitamin C for ultrasound treated samples and control were shown in Table 6. Ultrasonic treatment significantly ($p < 0.05$) inhibited the increase in microbial populations (total bacterial count, yeast & mold count) and showed significant ($p < 0.05$) difference for firmness and respiration rate when compared with the control (Table 6). Insignificant ($p > 0.05$) difference was observed between total soluble solids, pH, titrable acidity and vitamin C for both the ultrasound treated samples and control. The respiration rate and titrable acidity for the ultrasound treated samples were slightly higher (22.1% and 2.3%) when compared with control. While the vitamin C for the ultrasound treated sample were slightly lower (1.1%) than the control. These results showed that there was no significant ($p > 0.05$) difference between predicted and the experimental values at 95% confidence interval, which confirmed the validity and adequacy of the predicted models. Similar kind of results was shown by Cao [30]. Firmness of kiwi fruits were resulted 5.42% less for ultrasound treated samples when compared with the control, while the TSS of the ultrasound treated samples were unaffected.

4. Conclusion

In this study ultrasonic technology was used for reducing the microbial load on kiwifruit. RSM was used to optimize the experimental variables (ultrasonic intensity, temperature, treatment time and concentration of solvent). The optimal solution to obtain reduction of microbial population (bacteria, yeast and mold) by 1.5–3 log cycle, improving the quality of kiwi fruit for further edible chitosan coating to enhance the shelf life of the fresh cut kiwi fruit at 5 °C for 10 days were determined as follows: 368 W/cm^2 of ultrasonic intensity, 8 min of treatment time, 25 °C temperature and 30 ppm of concentration of the solvent. Ultrasonic treatment

Table 6
Effect of ultrasonic treatment under the optimized conditions on responses.

Responses	Ultrasound treated sample (optimized condition)	Control sample (NaOCl treated for 8 min)	p-Value
Total bacterial count (log CFU/cm^2)	3.48 ± 0.06	5.83 ± 0.04	0.000*
Total yeast and mold (log CFU/cm^2)	2.32 ± 0.02	3.68 ± 0.08	0.001*
Firmness (N)	56.61 ± 0.46	61.23 ± 0.90	0.001*
Respiration rate (mg CO_2/kg h)	27.80 ± 0.62	21.65 ± 1.60	0.000*
Total soluble solids (%)	9.67 ± 0.48	9.71 ± 46	0.790
Titrable acidity (%)	0.43 ± 0.18	0.42 ± 0.15	0.076
Vitamin C (mg/100 g of FW)	95.43 ± 3.60	96.48 ± 1.83	0.187

p value corresponds to Student's t-test to related samples (paired).
* Significant at $p < 0.1$.

increases the respiration rate (26.7–36.90 mgCO_2/kg h) of the kiwi fruit with reduced firmness (62.89–55.33 N).

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