

Study on Power Ultrasound Optimization and Its Comparison with Conventional Thermal Processing for Treatment of Raw Honey

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Summary

The present study was done to optimize the power ultrasound processing for maximizing diastase activity and minimizing hydroxymethylfurfural (HMF) content in honey using response surface methodology. Experimental design with treatment time (1-15 min), amplitude (20-100 %) and volume (40-80 mL) as independent variables under controlled temperature conditions was studied and it was concluded that treatment time of 8 min, amplitude of 60 % and volume of 60 mL give optimal diastase activity and HMF content, *i.e.* 32.07 Schade units and 30.14 mg/kg, respectively. Further thermal profile analyses were done with initial heating temperatures of 65, 75, 85 and 95 °C until temperature of honey reached up to 65 °C followed by holding time of 25 min at 65 °C, and the results were compared with thermal profile of honey treated with optimized power ultrasound. The quality characteristics like moisture, pH, diastase activity, HMF content, colour parameters and total colour difference were least affected by optimized power ultrasound treatment. Microbiological analysis also showed lower counts of aerobic mesophilic bacteria and in ultrasonically treated honey than in thermally processed honey samples complete destruction of coliforms, yeasts and moulds. Thus, it was concluded that power ultrasound under suggested operating conditions is an alternative nonthermal processing technique for honey.

Key words: power ultrasound, amplitude, diastase activity, HMF, thermal processing

Introduction

Honey is a supersaturated sugar solution produced by honeybees from nectar of flowers, which they collect, transform and combine with some enzymes of their own, store and leave in comb to ripen and mature (1). Processing of raw honey after harvesting is important from the shelf-life point of view because with the passage of time glucose crystallizes into D-glucose monohydrate crystals and leads to phase separation (2). The upper liquid phase with reduced sugar concentration becomes more susceptible to yeast fermentation and ultimately results in off-flavour or spoilage. The only commercial method that

industries follow for delaying crystallization and destruction of existing microorganisms is thermal pasteurization (3). However, heating above 40 °C affects the quality and causes protein denaturation and deactivation of several enzymes which are mainly responsible for its functional behaviour. Another negative aspect of thermal processing is darkening of honey due to hydroxymethylfurfural (HMF) formation, which is the breakdown product of fructose in acidic conditions because of nonenzymatic browning or Maillard reaction (4). Light coloured honey usually has milder flavour and higher commercial value than dark coloured honey (5).

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Because of high sugar concentration and acidic pH, honey does not allow the growth of most of the microorganisms. Therefore, only those who withstand such a high carbohydrate concentration and low pH can be expected to survive in it (6). The major sources of microbial contamination of honey include nectar, pollen, dust, air, honeybee and postharvest handling. Contamination through postharvest handling can be prevented by good hygienic practices, but the rest is not at all possible to control and may lead to human illness under specific conditions. The microbes mainly present in honey include yeast, fungi, coliforms and some aerobic mesophilic bacteria (7). Some researchers reported the presence of several *Bacillus* species in commercial honey samples in Nigeria (8).

Nowadays consumers are more health conscious and concerned about their diet and food nutritional aspects. This has resulted in research focusing on advanced processing and preservative techniques with reduced impact on the nutrients and overall quality of the food (9-11). That is why nonthermal or minimal processing techniques like power ultrasound are gaining more importance (12). Moreover, ultrasound processing has been identified as a green technology in food industry with varied functions such as effective mixing, faster energy and mass transfer, reduced thermal and concentration gradients, reduced temperature, selective extraction, reduced equipment size, faster response to process extraction control, faster start-up, increased production, elimination of process steps, lower usage of energy, improved extraction using food-grade solvents, and lower production of waste (13,14). Exposing honey to ultrasound at a particular frequency for specific period of time keeps it longer in liquid state by dissolving the existing crystals and delaying further crystallization, extending the shelf life by destroying most of the yeast cells, while the remaining cells lose the ability to grow (2). Ultrasonic treatment at 40 % amplitude and 20 kHz for 30 min minimally altered the colour, pH, HMF content and enzymatic activity, and noticeably improved the mass fractions of phenols, flavonoids in and antioxidant activity of longan, lychee and wildflower honey when compared to the conventional thermal treatment (15).

Response surface methodology (RSM) is a combination of various techniques including statistical and mathematical analyses of one or more independent and dependent variables, which are also known as variables and responses respectively (16). This tool has been successfully used to design and optimize different biotechnological and biochemical processes in relation to food (17). It is very efficient in determining optimum processing conditions and ingredient level, reaching desired minimum and maximum output values and reducing number of experiments, which ultimately leads to time, cost and energy savings (18). Different researchers worked on various food products like snacks (19), biscuit dough (20) and cake (21) using RSM.

Until today no work related to optimization of power ultrasound for honey processing and its comparison with industrial thermal processing of honey has been reported. Thus, the current study was carried out to develop an optimum power ultrasound processing technique for honey, which minimally alters the nutritional and physicochemi-

cal properties, by using response surface methodology, and further compared its effects with commercial heat processing protocol for honey.

Materials and Methods

Honey samples

The raw honey samples were procured directly from the professional beekeepers of Hisar city, Haryana, India during winter season, in January 2016. The samples of honey were unprocessed and freshly extracted (not older than one week) from the hives kept near the fields of mustard crop. After sampling in dark coloured glass jars, the honey was stored at 4 °C until analysis (completed within 20 days). Further, all the parameters of honey, *i.e.* diastase activity, hydroxymethylfurfural (HMF), content, colour changes and microbiological analysis were done on the same day following thermal and ultrasonic treatments.

Thermal treatment of honey

The raw honey samples were thermally treated as per the conventional method widely applied in food industries, *i.e.* for 25 min at 65 °C (22). The honey samples (50 g) were weighed in 50-mL beakers covered with aluminium foil and immersed in thermostatic water bath (Industrial and Laboratory Tools Corporation, Chennai, India) at 65, 75, 85 and 95 °C with continuous stirring until the temperature of all samples reached 65 °C. Then the temperature of honey was maintained at 65 °C for 25 min, after which the samples were drawn and cooled at room temperature for analysis.

Ultrasonic treatment of honey

Raw honey sample was put in a 100-mL glass beaker and placed into treatment chamber. Ultrasound system (VCX 750 ultrasonic processor; Sonics and Materials, Inc., Newtown, CT, USA) with constant frequency of 20 kHz and 19-mm diameter probe attached with the transducer at fixed pulsation (100 %) was used to treat the sample. The ultrasound treatment of raw honey sample was carried out at different treatment times, amplitude (%) and volumes for optimization. Temperature of the samples during ultrasound treatment was kept below 40 °C. Overheating was prevented by circulating chilled water around the beaker during treatment inside the chamber. Each experiment was conducted in triplicate to minimize the error and ensure accuracy.

The energy consumption during thermal and ultrasound treatments was calculated using calorimetric method (10) given by the equation:

$$P = m \cdot C_p \cdot dT/dt \quad /1/$$

where P is the used power (W), m is the mass (g), dT/dt is the change in temperature over time (°C/s) and C_p is the specific heat of honey (0.60 cal/(g·°C)).

Moisture analysis of honey

The refractive index of honey sample was measured at 20 °C with RX7000α refractometer (ATAGO, Tokyo, Japan) and moisture content was determined using stand-

ard reference table of honey refractive indices and water content, according to the Harmonized Methods of the International Honey Commission (23). The reading of each sample was done in triplicate.

pH value

Acidity of honey was checked as per the protocol given in Harmonized Methods of the International Honey Commission (23) using calibrated (at pH=4.0 and 7.0) digital pH meter (pH Tutor; Eutech Instruments, Thermo Fisher Scientific, Singapore). The reading of each sample was done in triplicate.

Colour measurement and total colour difference calculation

HunterLab ColourFlex EZ, 45°/0° colour spectrophotometer (Hunter Associates Laboratory, Inc., Reston, VA, USA) was used to measure colour parameters of honey, under the following conditions: illuminant D65, observer 10° and reflectance mode. The three-dimensional colour space is perceived in L^* , a^* and b^* , where L^* (luminance) from vertical axis expresses brightness, between complete black to complete white (*i.e.* 100 % black to 100 % white), and a^* and b^* axes range from greenness ($-a^*$) to redness ($+a^*$) and blueness ($-b^*$) to yellowness ($+b^*$) respectively (24,25). The reading of each sample was done in triplicate.

For calculating total colour difference (ΔE^*) the values of L^* , a^* and b^* were used according to the following formula (24,25):

$$\Delta E = [(L_0^* - L^*)^2 + (a_0^* - a^*)^2 + (b_0^* - b^*)^2]^{1/2} \quad /2/$$

Diastase activity

Diastase activity was calculated in Schade units (SU) on the basis of 1 % starch hydrolyzed by α -amylase enzyme in 1 g of honey for 1 h at a temperature below 45 °C (23,26). Decomposition of starch results in the discolouration of the solution from blue to violet/pink/no colour, which is directly proportional to the amount of decomposed starch. The degree of decomposition mainly depends on the intensity of enzymatic activity. This colour change was observed by UV-Vis- spectrophotometer (UV-1800; Shimadzu, Tokyo, Japan) at a wavelength of 660 nm against distilled water as blank. The reading of each sample was done in triplicate.

HMF analysis

HMF content (in mg/kg) in honey samples was measured using a spectrophotometric direct absorption method (27), where honey solution was filtered after dissolving in Carrez solution (Merck Millipore, Darmstadt, Germany) and then absorption was measured at 284 and 336 nm (UV-1800; Shimadzu) against the reference solution of honey and 0.2 % sodium bisulphate (Merck Millipore). The final value (in triplicate for each sample) was obtained using the following equation:

$$\text{HMF} = (A_{284 \text{ nm}} - A_{336 \text{ nm}}) \cdot 149.7 \cdot \frac{D}{m} \quad /3/$$

where $A_{284 \text{ nm}}$ and $A_{336 \text{ nm}}$ are the absorbance of sample solution at 284, and 336 nm respectively, 149.7 is the constant,

5 is the initial mass of honey sample (5 g), D is the dilution factor (in case dilution is necessary) and m is the mass of honey sample (in g).

Microbiological analysis

For determination of microbial flora in the honey, the methodology suggested by Omafuvbe and Akanbi was followed (7). Accordingly, 10 g of honey sample were mixed thoroughly in 90-mL pre-sterilized maximum recovery diluent (MRD-7658; HiMedia Laboratories Pvt. Ltd., Mumbai, India) under sterile conditions. After appropriate dilutions with MRD, the sample (100 μ L) was cultured in triplicate using spread plate method. Total aerobic mesophilic bacteria were counted on plate count agar (PCA; HiMedia Laboratories Pvt. Ltd.) after incubation for 48 h at (30 \pm 2) °C. Total coliform count was enumerated on violet red bile glucose (VRBG) agar (HiMedia Laboratories Pvt. Ltd.), incubated for 24-48 h at (35 \pm 2) °C. Yeast and fungi were counted on Sabouraud dextrose agar (SDA) supplemented with chloramphenicol (100 mg/L; HiMedia Laboratories Pvt. Ltd.) after incubation period of 3-5 days at (25 \pm 2) °C. After the completion of incubation period, all colonies that appeared were counted using digital colony counter (JAINCO 1037; HiMedia Laboratories Pvt. Ltd.) and average number of colonies, multiplied by the dilution factor, was considered for counting. Results were expressed in colony forming unit per g of honey (CFU/g).

Statistical analysis

The experimental results were analyzed by response surface methodology (RSM) using commercial statistical package, Design Expert v. 10.0.6.0 (Stat-Ease Inc., Minneapolis, MN, USA). A three-factor and three-level central composite design (CCD) with face-centred model was chosen (28) to determine optimum levels of three independent variables, *i.e.* treatment time (min), amplitude (%) and volume (mL) against two dependent variables: diastase activity (SU) and HMF content (mg/kg). On the basis of three independent variables, 20 experimental trials were designed including six replicates at centre point. The order of experiments was fully randomized and data were analyzed by multiple linear regression model. A second order polynomial equation was used to express the responses as a function of the independent variables:

$$Y_k = \beta_0 + \sum_{i=1}^n \beta_{ki} X_i + \sum_{i=1}^n \beta_{kii} X_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^n \beta_{kij} X_i X_j \quad /4/$$

where Y_k is the response variable (Y_1 is the diastase activity, Y_2 is the HMF), β_0 is the constant, β_{ki} is the linear coefficient, β_{kii} is the quadratic coefficient, β_{kij} is the cross product coefficient, X_i represents coded independent variables (X_1 is treatment time, X_2 is amplitude and X_3 is volume). Statistical significance test was performed using total error criteria with the confidence level of 95 %. The significant terms in the model were found by analysis of variance (ANOVA) for all responses and the homogeneity of the variance was checked using Tukey's test. The adequacy was checked on the basis of coefficient of multiple determination (R^2) and adjusted coefficient of determination (R^2_{adj}). The numerical optimization of the design

was used for the simultaneous optimization of responses. The designed goals of each variable and response were selected individually. Treatment time and amplitude range were kept at minimum, and volume was kept at maximum, so that maximum amount of honey can be treated in minimum time with minimum energy, whereas in the case of responses diastase activity was kept at maximum and HMF content at minimum.

Results and Discussion

Power ultrasound process optimization

In different ultrasound treatments, the combined effect of time (X_1), amplitude (X_2) and volume (X_3) was studied using RSM and calculations were done at 95 % confidence level. Central composite design with face centred model consisting of 20 experimental trials was designed. The responses of diastase activity and HMF mass fractions obtained after power ultrasound treatment at different independent variable combinations are listed in Table 1. The experimental data were used to calculate various factors of the second order polynomial equation. For any of the terms in the model, large regression coefficient and small p-value indicate a more significant effect on the respective dependent variables. ANOVA for diastase activity and HMF content showed that the resulting quadratic model adequately represented the experimental data, with coefficients of multiple determinations (R^2) of 0.9601 (Table 2) for diastase activity and 0.9551 (Table 2) for HMF content. The results obtained with the used model indicate that the current study was able to identify optimum processing conditions for freshly harvested raw honey.

Changes in diastase activity

Diastase activity of honey has been reported to range from 12 to 58 SU with an average value of 38 SU. The analysis of variance (ANOVA) confirmed that the diastase activity of honey had highly significant ($p < 0.001$) negative linear response on treatment time (X_1) and amplitude (X_2). The effect of various ultrasonic processing conditions on diastase activity is shown in Figs. 1a-c. It was observed that the increase in the amplitude with time led to decrease in the diastase activity (Fig. 1a). This phenomenon may be due to high energy input; at 100 % amplitude and 8-minute treatment acoustic energy was 39 W, at 60 % amplitude and 8-minute treatment acoustic energy was 8 W, while in thermal treatment it was 4, 14, 18 and 26 W respectively at 65, 75, 85 and 95 °C in the $V=60$ mL. The enzyme deterioration was higher and the effect was more intense when treatment time increased with amplitude. Other researchers reported similar findings and concluded that with the increase of amplitude (40-80 %) during high power ultrasound treatment the diastase activity was significantly reduced in longan, lychee and wildflower honey samples (15,29). Some researchers (30,31) have reported that the ultrasound increases degradation of natural products during food processing, however, degradation of the metabolites depends on the dosage or energy input; hence a lower input energy may just be sufficient to retain the natural products.

The predicted model for calculating diastase activity of honey was described by polynomial equation:

Table 1. Central composite design showing the effect of treatment time, amplitude and volume on diastase activity of and hydroxymethylfurfural (HMF) content in raw honey samples

Sample	Independent variable			Dependent variable	
	t/min (X_1)	Amplitude/% (X_2)	V/mL (X_3)	Diastase activity/SU	w(HMF)/(mg/kg)
1	15.0	60.0	60.0	28.0±0.5	40.1±1.0
2	1.0	20.0	40.0	57.0±0.2	13.4±0.2
3	8.0	60.0	60.0	35.0±0.3	34.4±0.8
4	8.0	60.0	40.0	32.0±0.3	36.0±0.9
5	8.0	60.0	60.0	30.0±0.2	29.2±0.3
6	15.0	100.0	80.0	32.0±1.2	34.4±0.8
7	8.0	20.0	60.0	55.0±0.7	15.7±0.5
8	8.0	60.0	60.0	30.0±0.2	29.6±0.4
9	1.0	100.0	80.0	43.0±0.9	22.4±0.5
10	8.0	100.0	60.0	20.0±0.3	39.3±0.8
11	8.0	60.0	60.0	30.0±0.2	29.6±1.6
12	8.0	60.0	60.0	30.0±0.4	29.0±0.3
13	15.0	20.0	40.0	50.0±0.2	18.7±0.4
14	15.0	20.0	80.0	55.0±0.6	14.2±0.8
15	8.0	60.0	80.0	38.0±0.4	26.9±0.6
16	15.0	100.0	40.0	12.0±0.8	48.7±1.8
17	8.0	60.0	60.0	30.0±0.5	29.3±0.4
18	1.0	20.0	80.0	58.0±0.6	12.7±0.3
19	1.0	60.0	60.0	45.0±0.1	19.2±1.4
20	1.0	100.0	40.0	42.0±0.8	23.9±0.5

Values are mean of triplicate determinations

$$Y_1 = 31.37 - 6.80A - 12.60B + 3.30C + 4.32A^2 + 5.32B^2 + 2.82C^2 - 3.87AB + 2.88AC + 1.88BC \quad /5/$$

where Y_1 is diastase activity and A, B and C are the coded values of treatment time (min), amplitude (%) and volume (mL), respectively.

Changes in HMF content

The maximum HMF content (48.7 mg/kg) of honey was about 3.8 times higher than the minimum HMF content (12.7 mg/kg) and the average value was 27.3 mg/kg. The analysis of variance (ANOVA) of the fitted model showed that HMF content of honey had highly significant ($p < 0.001$) positive linear response on treatment time (X_1) and amplitude (X_2) during power ultrasound processing and significant ($p < 0.05$) negative linear response on volume (X_3). This indicates that increase in treatment time and amplitude leads to the increase of the HMF content in honey. The 3D response surface plots in Figs. 1d-f, show the effect of ultrasonic processing conditions on HMF content of honey. Ultrasound treatment has been reported to form free radicals in the medium (32). Hence, the stronger the ultrasound treatment, the more free radicals are formed, which in turn may lead to the formation of HMF. Moreover, HMF formation is accelerated when the amplitude is increased from 40 to 80 % (10). Increased amplitude leads to increase in the temperature of honey

Table 2. Regression coefficients (R^2) of second order polynomial and their significance for diastase activity and hydroxymethylfurfural (HMF) content after ultrasonic treatment

Source	Diastase activity			HMF content		
	Coeff. value	F-ratio	p-value	Coeff. value	F-ratio	p-value
X_0	31.37 ^{***}	26.76	<0.0001	30.86 ^{***}	19.14	<0.0001
X_1	-6.80 ^{***}	37.77	0.0001	6.45 ^{***}	40.97	<0.0001
X_2	-12.60 ^{***}	129.67	<0.0001	9.40 ^{***}	86.99	<0.0001
X_3	3.30 ^{**}	8.89	0.0138	-3.01 ^{**}	8.92	0.0137
X_{12}	-3.87 ^{**}	9.81	0.0106	3.75 ^{**}	11.08	0.0076
X_{13}	2.88 ^{**}	5.40	0.0425	-2.07 [*]	3.39	0.0954
X_{23}	1.88 ^{ns}	2.30	0.1606	-1.33 ^{ns}	1.38	0.2669
X_1^2	4.32 [*]	4.19	0.0679	-2.26 ^{ns}	1.38	0.2671
X_2^2	5.32 ^{**}	6.35	0.0304	-4.37 ^{**}	5.18	0.0461
X_3^2	2.82 ^{ns}	1.78	0.2113	-0.42 ^{ns}	0.049	0.8300
R^2		0.9601			0.9551	
R^2_{adj}		0.9242			0.9007	
Adeq. precision		19.521			17.085	
Lack-of-fit		4.88 ^{ns}			3.70 ^{ns}	

*significant at $p < 0.1$, **significant at $p < 0.05$, ***significant at $p < 0.001$, ns=not significant

and finally enhances the HMF level. Likewise, significant HMF accumulation was observed when temperature and processing time were increased (33). Similar findings were obtained by others with honeydew honey and manuka honey (34,35).

The predicted model for HMF formation in honey was described by polynomial equation:

$$Y_2 = 30.86 + 6.45A + 9.40B - 3.01C - 2.26A^2 - 4.37B^2 - 0.42C^2 + 3.75AB - 2.07AC - 1.33BC \quad /6/$$

where Y_2 is HMF content and A, B and C are the coded values of treatment time (min), amplitude (%) and volume (mL), respectively.

Ultrasound processing optimization

The optimum conditions for power ultrasound processing of honey were determined to retain maximum diastase activity with minimal HMF accumulation during processing. Second order polynomial models obtained in the current study were used for both responses, *i.e.* diastase activity and HMF formation in identifying the optimum processing conditions. In this study, time, amplitude and volume were optimized to 1-15 min, 20-100 % and 40-80 mL, respectively. By applying point prediction method, it was determined that the optimum power ultrasound processing conditions are treatment time of 8 min, 60 % amplitude and volume of 60 mL, which corresponds to 8 W of acoustic energy given to the system. At this point, predicted diastase activity and HMF content were 31.37 SU and 30.86 mg/kg, respectively. Validation of the model was conducted by using the optimized treatment conditions and experimentally obtaining the value of the responses. The predicted (31.37 SU and 30.86 mg/kg) and experimental values (32.07 SU and 30.14 mg/kg) were compared and found to be close, with low error. There-

fore, the model obtained in this study could be used for optimization of power ultrasound process for raw honey treatment.

Effects of power ultrasound and thermal treatment on physicochemical parameters

In the current investigation the comparison between commercial heat processing of honey (*i.e.* with initial heating at different temperatures and holding time of 25 min at 65 °C) and optimized power ultrasound conditions (treatment time 8 min, amplitude 60 % and volume 60 mL) was done and their effects on different quality parameters of honey are described below.

The temperature profile study of thermally treated honey shown in Fig. 2 clearly indicates that speed of temperature increase of honey varies drastically when exposed to different temperature conditions, *i.e.* heating in water bath at 65 °C takes more than 21 min to reach the honey temperature of 65 °C, whereas heating at 75, 85 and 95 °C takes less than 8, 7 and 5 min respectively. Achieving honey temperature of 65 °C and holding time of 25 min at the same temperature are recommended for complete decrystallization and microbial growth inhibition (23). With longer exposure time qualitative damage is greater. Similar findings were documented stating that heating results in degradation of honey quality, and to calculate the intensity of heat to which honey has been subjected, diastase activity and HMF content are used together as quality parameters (4).

Moisture is the second largest component in honey after sugar and it is one of the deciding factors for shelf life of honey. During processing, some water loss is observed because of high temperature, which is minimal in the case of ultrasonically treated honey because of controlled temperature treatment, but the highest loss, *i.e.* 0.67 %, was ob-

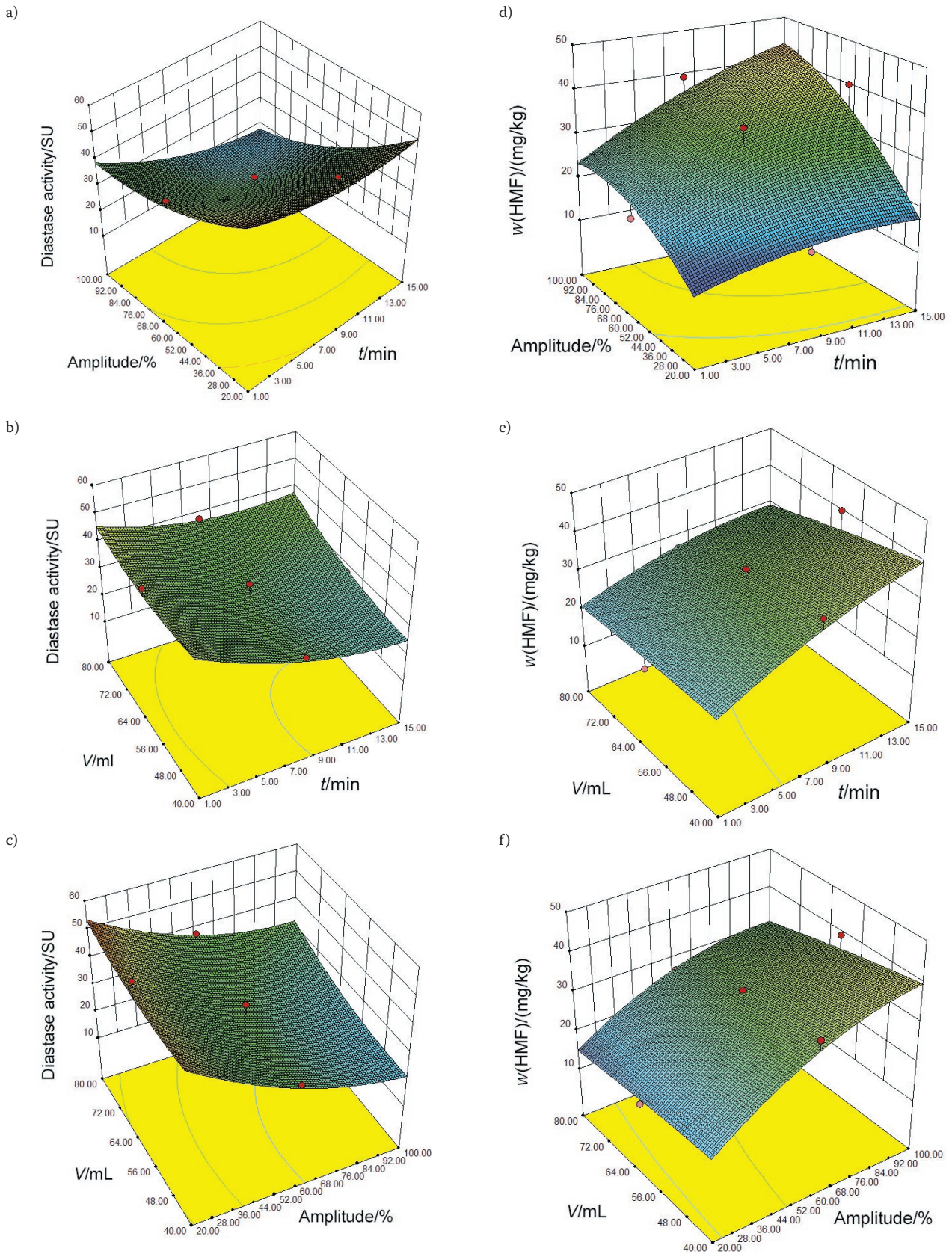


Fig. 1. Response surface plot for: a) diastase activity as a function of treatment time and amplitude at fixed $V=60$ mL, b) diastase activity as a function of treatment time and volume at fixed amplitude of 60 %, c) diastase activity as a function of amplitude and volume at fixed treatment time $t=8$ min, d) hydroxymethyl furfural (HMF) content as a function of treatment time and amplitude at fixed $V=60$ mL, e) HMF content as a function of treatment time and volume at fixed amplitude of 60 %, f) HMF content as a function of amplitude and volume at fixed treatment time $t=8$ min. SU=Schade unit

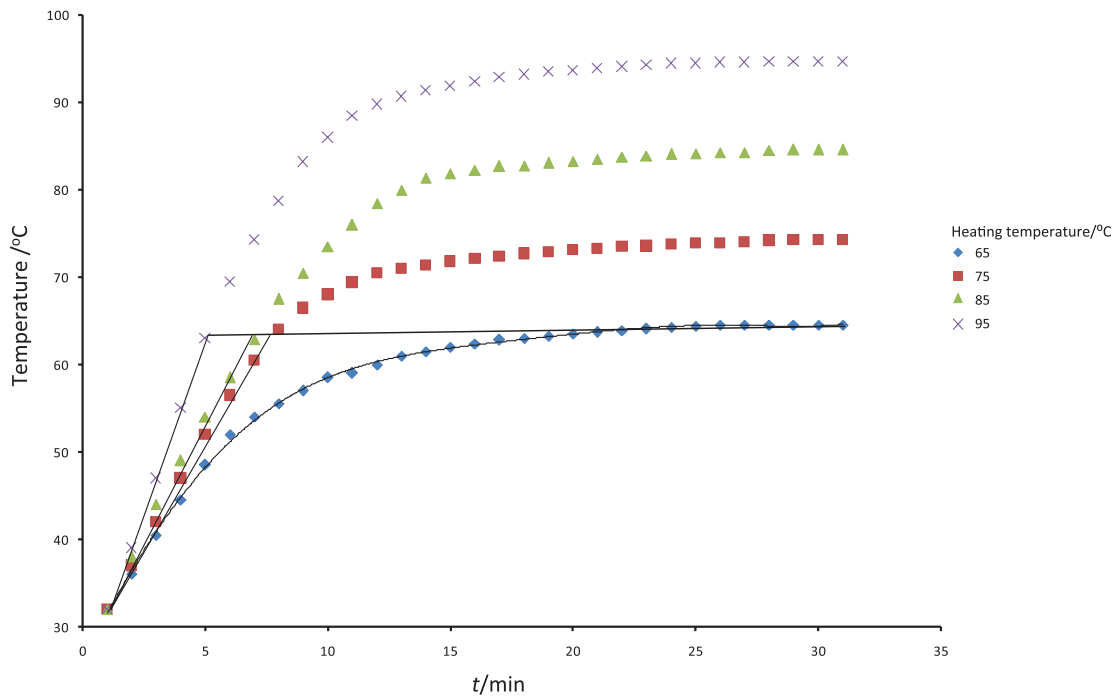


Fig. 2. Temperature profile of thermally treated honey sample from 65 to 95 °C. The line represents the time taken to achieve the temperature of honey of 65 °C after initial heating at temperatures of 65, 75, 85 and 95 °C

served in honey heated at 65 °C. Initial moisture content of raw honey was 16.5 %, which was reduced up to 15.8, 16.01, 16.2 and 16.3 % when initial heating temperature was 65, 75, 85 and 95 °C respectively (Table 3). Moisture loss was higher at longer heat exposure time, and it was the highest at 65 °C (46-minute treatment, *i.e.* 21 min arrival time and 25 min holding time) and least at 95 °C (30-minute treatment, *i.e.* 5 min to achieve 65 °C and 25 min holding time).

Only power ultrasound treatment of honey did not have a significant ($p>0.05$) effect on pH when compared to unprocessed honey, whereas pH of all thermally treated honey samples significantly decreased. More specifically, greater pH decrease ($p\leq 0.05$) was observed in the samples exposed to heat for a longer period of time. Initial heating at 65 °C resulted in maximum pH drop, *i.e.* from 3.52 to 3.35 in raw honey, while initial heating at 75, 85 and 95 °C also

resulted in the decrease of pH in descending order: 3.40, 3.43 and 3.43 respectively when compared to unprocessed honey ($pH=3.52$), as shown in Table 3. The finding was similar to a previous study with conventionally and ultrasonically treated longan, lychee and wildflower honey (15). The decrease in pH might be because of the increase in hydrogen ion concentration due to heat treatment or because pollen releases organic acids during heating, which also affects the pH of the final sample (36).

Diastase activity in honey is extensively dependent on floral source of honey and may vary from season to season in the honey from the same flora (37). Diastase activity of unprocessed raw honey was 60 SU and it was reduced to 32.1 SU during ultrasound treatment (Table 3). But during heat processing with different initial temperatures, the loss in diastase activity is higher than in ultrasonically treated

Table 3. Effects of power ultrasound and heat treatments on physicochemical properties of raw honey

Treatment	w(moisture)/%	pH (at 25 °C)	Diastase activity/SU	w(HMF)/(mg/kg)	L^*	a^*	b^*	ΔE^*
Raw honey	16.5±0.2	3.52±0.05	60±5	11.9±0.2	29.1±0.9	2.21±0.04	18.5±0.6	
Ultrasound	16.4±0.2	3.48±0.02	32.0±0.1	30.1±1.3	28.8±0.3	2.5±0.3	20.1±0.5	1.8±0.1
Temperature/°C								
65	(15.8±0.3) ^a	(3.35±0.03) ^b	(16.5±0.3) ^d	(48.8±2.7) ^e	(23.8±1.3) ^j	(5.4±0.5) ^k	(25.7±0.4) ^l	(9.5±0.3) ⁿ
75	(16.01±0.09) ^a	(3.40±0.02) ^{bc}	(21.9±0.5) ^e	(43.6±0.4) ^b	(25.4±1.3) ^j	(4.3±0.3) ^k	(22.96±0.06) ^m	(6.2±0.5) ^o
85	(16.2±0.7) ^a	(3.43±0.03) ^c	(23.3±0.8) ^f	(39.4±0.9) ⁱ	(25.5±1.4) ^j	(4.2±0.6) ^k	(22.9±0.5) ^m	(6.1±0.2) ^o
95	(16.3±0.3) ^a	(3.43±0.01) ^c	(24.1±0.2) ^f	(38.9±1.1) ⁱ	(25.5±0.5) ^j	(4.2±1.1) ^k	(22.78±0.02) ^m	(6.0±0.2) ^o

Mean values in the column followed by different letters are significantly different according to Tukey's test ($p<0.05$). Three replicates were performed for each measurement. Ultrasonic treatment was performed at $t=8$ min, amplitude=60 % and $V=60$ mL. For each thermal treatment, when temperature of honey reached 65 °C it was maintained for 25 min and then the samples were drawn and cooled before analysis. L^* =lightness, a^* =redness, b^* =yellowness, ΔE^* =total colour difference

honey. The diastase activity decreased from 24.0 to 16.5 SU within the initial heating temperature range from 95 to 65 °C. This clearly indicates that heating for longer time depletes more enzymes in honey, which is why the highest loss is at 65 °C for 46 min, followed by 75 °C (diastase activity 21.9 SU), 85 °C (diastase activity 23.3 SU) and 95 °C (diastase activity 24.0 SU) with total exposure times of 34, 32 and 30 min, respectively. In contrast, the diastase activity of all samples after thermal treatment is higher than the level (8 SU) recommended by International Honey Commission (23), which was similar to a previous study where greater loss in diastase activity was reported during heat treatment (90 °C/5 min) than during ultrasound treatment (40-80 % amplitude/30 min) of longan, lychee and wildflower honey (15). Similar results (20-40 % amplitude/20 kHz/1 h) were obtained for sunflower honey treated with ultrasound (29).

5-Hydroxymethylfurfural (HMF) is a breakdown product of sugar degradation formed during exposure to high temperature under acidic conditions and is used as freshness or thermal damage indicator for honey (15,37). As per our observations, the initial HMF content in unprocessed fresh honey sample was 11.9 mg/kg (Table 3), which increased in all samples after treatments. However, the increase in HMF content was greater in thermally treated samples than in the ultrasonically treated sample. It increased up to 48.8 mg/kg at initial heating temperature of 65 °C due to longer heat exposure time, which is beyond the acceptable limit of 40 mg/kg (38), while in the ultrasonically treated sample the HMF increase was minimal, *i.e.* 30.1 mg/kg, which is because of the temperature control (below 40 °C) during power ultrasound treatment. The increased HMF content is directly proportional to the browning index of honey, as shown in Table 3. The accumulation of HMF is mainly dependent on treatment temperature, exposure time and storage (33). Similarly, thermal processing is more effective in producing HMF and decreasing diastase activity in honey than ultrasonication (40 % amplitude/30 min/52.63 °C and 80 % amplitude/30 min/75.09 °C) (29). Higher rate of HMF formation in honey at 100 °C than in those heated at 90 and 75 °C was also reported (34).

Colour of honey is one of the most important acceptability factors for consumers. Light coloured honey is mild in flavour with high commercial value and preferred over dark honey (5). The colour of honey depends on many factors like nectar source, climatic conditions, floral origin, processing technique, *etc.* In the current investigation, comparative analysis of changes in colour parameters (L^* , a^* , b^* and ΔE^*) of honey due to thermal and power ultrasound treatments was done (Table 3). In all honey samples significant decrease in the lightness was observed when compared to the control. The decrease in lightness was the lowest in ultrasonically treated sample compared to all other thermally processed honey samples. Higher values of redness ($+a^*$) and yellowness ($+b^*$) were observed in thermally processed honey sample followed by ultrasonically treated samples than in control. In particular, samples treated at initial temperature of 65 °C revealed maximum $+a^*$ (5.4) and $+b^*$ (25.7) values followed by samples treated at initial temperatures of 75, 85 and 95 °C. Smaller increase in $+a^*$ (2.5) and $+b^*$ (20.1) values was observed in ultrasonically treated honey samples than in control sample

($+a^*=2.21$ and $+b^*=18.5$). Similar results were reported in floral honey from Thailand, where maximum increase in $+a^*$ and $+b^*$ values was observed in thermally processed (90 °C/5 min) and the lowest in ultrasonically treated (80 and 40 % amplitude/30 min) samples (15). L^* value was decreased compared to the control, and the decrease was higher in thermally processed than in ultrasonically processed honey samples because of longer heating time, which caused the formation of more HMF. The results are further supported by significant increase in $+a^*$ and $+b^*$ parameters after ultrasonic treatment of guava juice (35 kHz/20 °C/30 min) and decreased L^* value when compared to the control sample (39).

Total colour difference (ΔE^*) data of both thermally and power ultrasound-treated honey samples in Table 3 further confirms that the colour change is minimum in ultrasonically treated honey (1.8), which is close to the control. However, ΔE^* is more than double in thermally processed honey samples. This clearly indicates that browning reaction occurred due to high temperature treatment and the effect is more severe in samples treated for a longer period of time.

Effects on microbiological qualities

The microbial counts in raw, thermally processed and power ultrasound-treated honey samples are reported in Table 4. The total aerobic mesophilic bacterial count in raw honey sample was $5.2 \cdot 10^3$ CFU/g, which is close to the value reported by other researchers (7,40). After heat treatment, total aerobic mesophilic bacterial count was reduced to $2.6 \cdot 10^2$ CFU/g and in ultrasonically treated honey reduction was $1.8 \cdot 10^2$ CFU/g, showing that both thermal and ultrasound treatments were effective in total aerobic mesophilic bacterial reduction in honey. Total coliform count (TCC) was very low, *i.e.* $0.6 \cdot 10^5$ CFU/g in raw honey sample, which was completely destroyed during heat treatment for 25 min at 65 °C and also no sign of coliforms was found in ultrasonically treated honey samples. Similarly, total fungal count and total yeast count were reduced up to half of their existing level by thermal processing, whereas in ultrasound treatment complete destruction took place.

These results clearly indicate that power ultrasound is more effective than conventional heat processing for microbial inactivation. Microbial inactivation in ultrasonic treatment is due to micromechanical shockwaves which destroy cellular components and ultimately cell lysis takes place. Similar findings were reported in research using ultrasonic treatment (60 °C/9 min/120 μ m) where complete destruction of *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* sp., *Listeria monocytogenes* and better *Bacillus cereus* inactivation (3.48 log CFU/mL) were obtained (41). Thus, the ultrasound processing of honey has significant industrial potential as it could improve the diastase retention in honey as well as decrease the formation of HMF, which in turn retains the colour of honey when compared to thermal processing with the added advantage of microbial inactivation for extended shelf life. Moreover, development in the improved ultrasound systems to date offers better control of treatment conditions which can be easily integrated in industry, and is environmentally friendly.

Table 4. Mean microbial count of raw, thermally treated and ultrasonically treated honey

Honey sample	N/(CFU/g)			
	TAMB	TCC	TFC	TYC
Raw	$(5.2 \pm 0.2) \cdot 10^3$	$(0.6 \pm 0.1) \cdot 10^5$	$(0.2 \pm 0.1) \cdot 10^2$	$(3.2 \pm 0.2) \cdot 10^2$
Thermally treated*	$(2.6 \pm 0.3) \cdot 10^2$	++	$(0.1 \pm 0.1) \cdot 10^2$	$(1.00 \pm 0.08) \cdot 10^3$
Ultrasonically treated**	$(1.8 \pm 0.1) \cdot 10^2$	++	++	++

TAMB=total aerobic mesophilic bacteria, TCC=total coliform count, TFC=total fungal count, TYC=total yeast count, ++=absence of microbes. *Treatment temperature $T=65$ °C, time $t=25$ min, **treatment time $t=8$ min, amplitude=60 % and $V=60$ mL

Conclusions

Power ultrasound processing under optimized conditions and controlled temperature could be an alternative to heat processing of honey. In the current research, optimized power ultrasound completely destroyed yeast, mould and coliform bacteria, while the total plate count results were better than in thermally processed honey. Similarly, other physicochemical properties like moisture, pH, diastase activity and HMF formation were least affected under optimum power ultrasound treatment and higher nutritional value was achieved than in thermally processed honey samples. Moreover, ultrasound treatment can lead to a microbially safe honey with minimum deviation in colour and browning index, which can have a better commercial value. However, further research is needed to study the changes during storage at various temperature conditions.

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