CHAPTER 3

To study the combined effects of microwave with ultrasound treatment on the pasteurization and nutritional properties of bottle gourd (*Lagenaria siceraria*) juice

3.1. Introduction

Bottle gourd (*Lagenaria siceraria*) is a commonly grown vegetable in India which is popular due to its high nutritional value and low cost [8,9]. Bottle gourd juice has been reported to have different health beneficial properties and medicinal values against several diseases like hyperthyroidism, diabetes, flatulence and piles [53]. Bottle gourd has been reported to contain triterpenoid compounds like cucurbitacins B, D, G and H [27], which may help in regulating the blood glucose levels [80]. It also contains high choline content among other vegetables which act as neurotransmitters that reduces depression and mental disorders [54]. Consumers prefer fruit and vegetable juice with nutritious quality, natural taste and freshness, preserved without any preservatives as they are perceived as safe. However, the safety and shelf life of bottle gourd juices remains a challenge. Important factors affecting the spoilage of bottle gourd juice includes pH, water activity and its nutritional parameters for microorganism growth [3]. Bottle gourd juice is normally treated for 121, 63, 75 ºC for 7, 30 and 10 min, respectively [8]. However, thermal processing can damage the naturally occurring compounds in the food, as long heating process have negative effects on the nutritional qualities of the product [13, 41, 48, 68]. HTST (High temperature and short times) which was considered as a mild heat treatment also showed decrease in the nutritional value by bringing chemical changes in the product [33, 46, 68]. Consumer demand for nutritious foods, which are minimally and naturally processed, has led to processor interest in non-thermal or mild thermal technologies. Microwave heating as a promising alternative to conventional pasteurization has been reported in different studies [17, 45, 50]. Microwaves have the capacity to penetrate and dissipate energy into the food with the interaction of water molecules inside the food, and this property of microwave helps in heating the food rapidly. Many researchers [10, 12, 15, 23, 35, 33, 46, 60, 65, 69] have also investigated and explained the lethality of microwaves for microorganisms. Many studies also reported ultrasound as an alternative to conventional thermal processing to decrease microbial population [1] with minimal changes in the available bioactive compounds [51, 71]. Ultrasound has been reported to be useful in sterilization and extraction in reduced processing times and with high efficiency [44, 62, 70]. The lethality of ultrasound can be increased if it is combined with different other techniques like the use of heat, microwave, UV and high pressure.

L. monocytogenes is a gram positive, nonsporing, heat resistant causative organism in several outbreaks of foodborne disease [22, 24] and occurrence of *L. monocytogenes* in vegetables have been reported in many studies [6, 18, 32]. *L. monocytogenes* is known to survive mild heat treatments, low pH, high NaCl and in refrigeration conditions [47]. The present study was conducted to optimize a combined microwave and ultrasound process using reponse surface methodology for the pasteurization of bottle gourd juice spiked with *L. monocytogenes* while keeping the nutritional value intact. The process includes a short rapid microwave treatment followed by a cold ultrasound treatment. To the best of our knowledge this is the first report using *L. monocytogenes* as a marker organism on combined effect of microwave and ultrasound (MW-US) in bottle gourd juice without affecting its nutritional parameters.

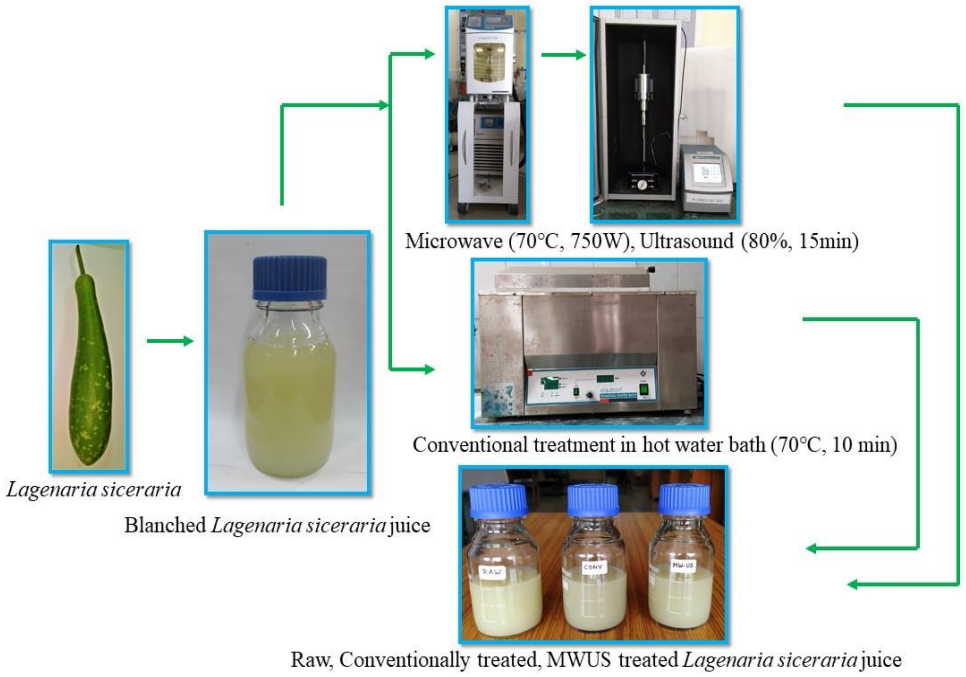


Figure 3.1: Microwave and ultrasound processing of *Lagenaria siceraria* juice

3.2 . Materials and Methods

3.2.1. Materials

The bottle gourd was collected from homestead garden of Napaam, Tezpur, Assam, India (latitude 26.651218, longitude 92.783813). The herbarium of the sample was identified by The Department of Botany, Gauhati University, Guwahati, Assam, India (*Lagenaria siceraria*, Accession No: GUBH18494). *Listeria monocytogenes* (ATCC 13932) was purchased from HiMedia Laboratories Pvt. Ltd. Chemicals and media for *L. monocytogenes* were purchased from HiMedia (M1865), HPLC phenolic standards were purchased from Sigma Aldrich (USA). All other reagents were of analytical grade and purchased from Sigma-Aldrich (USA).

3.2.2. *L. monocytogenes* spiked to the bottle gourd juice (BGJ)

Good quality bottle gourds were washed, cleaned and wiped with an absorbent paper, peeled and cut into uniform cubes ($2 \times 2 \times 2$ cm^3). Blanching was carried out in distilled water following the protocol described by [8] with minimal modification (85 ºC for 5 min). Blanched samples were allowed to cool immediately with the help of chilled water. BGJ was extracted using a commercial grinder (Bajaj Majesty 1.5 L JX4 Juicer Mixer Grinder). The juice was centrifuged (Eppendorf Centrifuge 5430 R, Germany) at 5,000 rpm for 15 min at room temperature (23 ± 2ºC) and filtered through Whatman No. 1 filter paper (0.45 µm), to remove debris and suspended particles.

Strain of *L. monocytogenes* was cultured on *Listeria* Selective Enrichment Broth (HiMedia, M1865) for 24 h at 37ºC. After 24 h growth, the culture containing the cells were centrifuged for 5 minutes at 8,000 rpm. The bacterial pellet was suspended in sterile BGJ making a population of log 6 c.f.u/ml in the sample. The task was performed aseptically in a laminar airflow (MICRO-FILT, INDIA). The spiked juice was kept in an incubator for half an hour at 37 ºC before the pasteurization treatments for adaptation purpose.

To determine the *L. monocytogenes* survival count, the processed and non-processed samples were serial diluted using saline solution (0.86 % Nacl) and they were superficially cultivated on *Listeria* selective media agar plates. *L. monocytogenes* plates

were placed inside an incubator (New Brunswick, EXCELLA E24 R) at 37ºC.The emerged colonies in the plates were counted after 48 hours [40]. Each test was performed in triplicate and results were expressed as log colony-forming units (c.f.u) per ml.

3.2.3. Microwave and ultrasound processing and experimental design (MW-US)

The effect of combined microwave-ultrasound pasteurization of BGJ was studied (Figure. 1). The destruction level of *L. monocytogenes* cell load was considered as an indicator for the effectiveness of the process on microorganisms. For the simultaneous microwave followed by ultrasound (MW-US) treatment, a response surface methodology face centred composite design (FCCD) was employed with microwave power (MP) (250-750W), microwave induced temperature (MT) (30-70ºC), ultrasound amplitude (UA) (20-80%) and ultrasound exposure time (UT) (5-15min) as factors. Moreover, total phenolic contents, total terpenoides and antioxidant activities were taken as positive response while survivability of the microbial culture was taken as the negative response of the design. BGJ (100 ml) was first put into a beaker inside the microwave system (Neos GR, Milestone Technologies) with sample temperature sensor. The temperature of the sample was brought to the desired temperature according to Table 3.1. The microwave system was set to immediately stop at the desired temperature. The time of microwave exposure was 15-30 sec. The sample was further transferred to ultrasound chamber aseptically. Samples were cooled and brought down to 20ºC in the ultrasound chamber with circulating cold water from a condenser [51]. The ultrasound process was performed later according to Table 3.1. Sample temperature during the ultrasound process was maintained below 30 ºC.

Table 3.1: Experimental design and data for response surface analysis.

No	Independent variables				Dependent variables			
	Microwave power (W)	Microwave temperature (ºC)	Ultrasound amplitude percentage (%)	Ultrasound exposure time (min)	Cell viability (Log c.f.u/ml)	TPC (µg/ml)	Terpenoids (µg / ml)	DPPH (%)
1	250	30	20	5	6	280.11	460.00	20.01
2	750	30	20	5	6	276.41	488.75	19.02
3	250	70	20	5	4	280.49	426.25	37.98
4	750	70	20	5	4	272.37	532.50	35.53

5	250	30	80	5	6	287.61	480.00	34.97
6	750	30	80	5	6	290.29	508.75	34.86
7	250	70	80	5	4	282.59	448.75	28.27
8	750	70	80	5	4	280.85	553.75	30.37
9	250	30	20	15	6	279.65	416.25	21.37
10	750	30	20	15	6	280.06	527.50	31.73
11	250	70	20	15	4	287.79	466.25	34.58
12	750	70	20	15	4	283.77	653.75	41.87
13	250	30	80	15	4	286.48	431.25	38.31
14	750	30	80	15	4	293.28	542.50	41.93
15	250	70	80	15	1	289.22	483.75	42.57
16	750	70	80	15	1	291.59	670.00	42.63
17	250	50	50	10	5	285.34	420.00	23.16
18	750	50	50	10	5	284.34	607.50	26.59
19	500	30	50	10	5	276.74	505.00	23.17
20	500	70	50	10	4	275.76	577.50	29.60
21	500	50	20	10	6	274.87	482.50	27.12
22	500	50	80	10	4	286.35	512.50	39.56
23	500	50	50	5	6	284.94	500.00	23.21
24	500	50	50	15	4	292.65	512.50	34.67
25	500	50	50	10	5	279.03	487.50	24.63
26	500	50	50	10	5	282.56	525.00	28.11
27	500	50	50	10	4	282.50	507.50	23.32
28	500	50	50	10	6	280.27	518.75	26.89
29	500	50	50	10	5	285.90	490.00	24.13
30	500	50	50	10	5	277.55	528.75	29.78

3.2.3.1. Death and reaction order kinetics

For the investigation of reaction order and microbial death kinetics of microwave-ultrasound based pasteurization process, the equation 3.1 was applied

$$Y = Y_0 exp(kt) \quad (3.1)$$

Where, *Y* stands for various responses (TPC, terpenoids and DPPH), Y_0 is value of the response at *t=0*, and *t* refers to ultrasound exposure time and *k* is reaction order rate. The values of *t* and *D* were obtained by equation 3.2 and 3.3, respectively

$$t = Dlog\left(\frac{N_0}{N}\right) \quad (3.2)$$

$$D = \frac{2.303}{k} \quad (3.3)$$

Where, *t* is ultrasound exposure time, *D* is decimal reduction time, N_0 is initial population of the microorganism, *N* is final population microorganism and *k* refers to reaction order rate.

3.2.4. Conventional processing of BGJ

The extracted bottle gourd juice was conventionally pasteurized at 75 °C for 10 min [8] in a hot water bath (Equitron, India). The temperature of the sample was monitored with a laser point infrared digital thermometer (Bexco, South Korea).

3.2.5. Determination of total phenolic contents (TPC)

The content of total phenolics was determined using Folin-ciocalteu reagent (FCR) [66] with minor modification. The absorbance was measured in a spectrophotometer at 725 nm (Eppendorf BioSpectrometer, Germany). The phenolic content was calculated from a gallic acid standard curve and finally expressed as mg/gm gallic acid equivalent (GAE).

3.2.6. Determination of total terpenoids

The content of total terpenoids was determined using linalool 97% (L2602-100G, Sigma-Aldrich) following standard protocol with minor modification [26]. Briefly 10 ml of juice was taken, and 3.5 ml of ice cold (95% v/v) and methanol was added. The mixture was centrifuged at 4000g for 15 min. The supernatant was collected, and another 1.5 ml of chloroform was added to 200 µl of supernatant. The mixture was vortexed and kept for 3 min to rest. Further 100 µl of concentrated H_2SO_4 was added to each tube in ice cold condition. The mixture was kept in the dark for 1.5 h, after which a reddish precipitation occurs and the supernatants were removed. Another 1.5 ml of methanol was added till the precipitation was completely dissolved. Absorbance was taken in a spectrophotometer (Eppendorf BioSpectrometer Germany) at 538 nm taking methanol as a blank.

3.2.7. Determination of antioxidant activity by DPPH free radical scavenging assay

DPPH radical scavenging activity of the BGJ was determined according to the previously described method [38] with minor modification. Briefly, DPPH solution of 0.135 mM was prepared in methanol. The DPPH solution was then mixed with BGJ (1.0 mg/ml) and standard (BHA) in methanol in a total volume of 1.0 ml and vortexed thoroughly. The test tubes were then incubated in the dark for 20 minutes at room temperature. The decrease in absorbance was measured at 517 nm using a UV-VIS spectrophotometer. The reaction mixtures were measured in a spectrophotometer (Eppendorf BioSpectrometer Germany) at 517 nm after 30 minutes of incubation in dark. The scavenging activity was calculated using the equation 3.4.

$$\%\ \text{DPPH radical scavenging activity} = \frac{\text{Abs control} - \text{Abs test sample}}{\text{Abs control}} \text{X } 100 \quad (3.4)$$

3.2.8. Comparison between MW-US pasteurized juice with raw BGJ and conventionally pasteurized juice

3.2.8.1. Determination of total solids (TS) and total soluble solids (TSS)

TS were determined by standard method [56]. The BGJ juice (10 ml) was weighed in a petri-dish and kept at 50 ºC in an oven. The sample was cooled in a desiccator to be weighed again. The procedure was repeatedly carried out until the weight was found to be constant. TSS was determined by a handheld refractrometer (Mettler Toledo, Germany).

3.2.8.2. Determination of titratable acidity (TA) and pH

Titratable acidity (TA) was determined by using the standard protocol [56]. TA was calculated in terms of citric acid by using the equation 3.5.

$$\%\ \text{TA} = \frac{\text{Titre} \times \text{Normality of alkali} \times \text{volume made up} \times \text{Eq wt of citric acid} \times 100}{\text{Volume of sample taken for estimation} \times 1000} \quad (3.5)$$

pH was measured using a digital pH meter (Cyber scan, EUTECH Instruments). pH meter was calibrated by buffer solutions of pH 4 and 7. Each sample was measured in triplicate.

3.2.8.3. Polyphenol profiling by HPLC method.

The content of total phenolics was determined as explained in section 3.2.6. Polyphenols profiling of BGJ was carried out using RP-HPLC (Waters, United States) gradient elution method [63]. Symmetry 300^{TM} C_{18} (5 µm, 4.6 x 250 mm) column with a binary pump (Waters, 1525) and UV-Vis detector (Waters, 2489) were used. 100 ml juice was concentrated to 10 ml in a rotary evaporator (IKA 10) under reduced pressure at 40ºC. The sample (20 µl) was then filtered using 0.22 µm syringe filter. The mobile phase used was acidified ultrapure water (0.1% acetic acid, pH 3.2, mobile phase A) and methanol (mobile phase B). The gradient method used was: 80% A (0-8 min), 65% A (9-12 min), 45% A (13-16 min), 30% A (17-20 min), 20% A (21-30 min), 10% A (31-34 min). Washing of the column was done with 65% A (35-39 min), In the end 80% A (42-45 min) was used with a flow rate of 0.8 ml/min throughout the analysis. The detection was measured at 254 and 325 nm. The standards used for comparison and identification were caffeic acid, catechin, coumaric acid, ferulic acid, gallic acid, quercetin, rutin, and sinapic acid.

3.2.8.4. Protein content and amino acid compositional analysis

The protein content was estimated by Bradford's method using the manufacturer's protocol (Sigma Aldrich, USA). The described protocol was also followed for amino acid compositional analysis [30, 42]. Raw, conventional and microwave treated BGJ protein (500 µg) were hydrolyzed at 110 ºC for 24 h under vacuum using 6 N HCl. Then, 25 µl hydrosylate was mixed with ortho-pthaldialdehyde (Sigma), 3-merceptopropionic acid and 9-fluorenylmethyl chloroformate (Sigma) reagents in borate buffer 1:1. Then 8 µl of 1 M acetic acid was added. After that 3 µl of the resulting mixture was injected in Acclaim^{TM} RSLC 120 C_{18} RP-UPLC column (2.1 mm x 100 mm, 2.2 µm particle size, 120 Å pore size) coupled to ultimate 3000RSLC HPLC system (Dionex, Dreieich, Germany UHPLC NaN_3), the amino acid derivatives were separated by $CH_3CN/MeOH/H_20$ solvent system (45:45:10) at a flow rate of 0.722 ml/min. The elution was monitored initially at 338 nm for 0 -7.2 min and then at 262 nm for the next 7.2-10.5

min [31, 59]. A calibration curve of standard amino acid derivatives was run in the same RP-UHPLC column under identical conditions and the concentration of individual amino acid was determined.

3.2.8.5. Estimation of vitamin content in the juice using HPLC method

Water soluble vitamins and fat-soluble vitamins were quantified using AOAC/relevant official methods after duly validated with Certified Reference Materials / Standard Reference Materials and as explained by [16].

Vitamin C extraction from the sample was performed using metaphosphoric acid, acetic acid and tris-2-carboxy ethyl phosphine hydrochloride [52]. The extracts were filtered and run into the HPLC through a C_{18} column (Thermo BDS Hypersil C_{18} column, 250 x 4.6 mm, 5µm) using acetonitrile and potassium phosphate buffer as mobile phase.

Riboflavin was determined after acid hydrolyzing the sample using 0.1M HCl /5%metaphosphoric acid according to the protocol described by [4]. The separation was carried out using thermo BDS HYPERSIL C_{18} (250x4.6 mm, 5µm) column at 40^{o}C in the reversed-phase UHPLC (Thermo Fisher SCIENTIFIC Ultimate 3000) system. Eluted compounds were detected with fluorescence detector at Ex: 445nm, Em; 522nm.

Sample was extracted using 3% (v/v) acetic acid solution for B_5 analysis [4]. The extract was then analyzed by HPLC through a C_{18} column (µm Bondapak C_{18} column, 300 x 3.9 mm, 10 µ) using phosphate buffer (0.1M, pH-2.25) as mobile phase with a Diode Array Detector (DAD) detector.

Vitamin B_6 was extracted using metaphosphoric acid following analysis in the U-HPLC using a C_{18} column (Thermo, Hypersil BDS 250 x 4.6mm, 5µm). Phosphate and acetonitrile were used as mobile phase [75]. Dipotassium hydrogenphosphate, ascorbic acid, sodium azide and 2-mercapto ethanol at pH 7.2 was used for Vitamin B_9 extraction, followed by tri-enzyme (α-amylase, protease and deconjugase) treatment to convert folates into its different forms [21]. Extract was purified through a strong anion exchange cartridge (SEP-PAK cartridge) and analyzed by U-HPLC (Dionex ultimate 3000 RSLC).

Alkaline saponification using n-hexane was performed for tocopherols and tocotrienols extraction and separated by normal phase U-HPLC (Dionex ultimate 3000 RSLC) through a silica column (Waters, Superisorb silica 100 x 4.6 mm I.D., 3 µm) [11].

Vitamin K was extracted using methanol and dichloromethane (1:2) followed by n-hexane [36]. The extract solution was analyzed by RP-HPLC (Dionex ultimate 3000 RSLC) using C_{18} column.

Carotenoids and xanthophylls were extracted after saponifying the sample with petroleum ether in the presence of 12% (w/v) alcoholic potassium hydroxide [58]. The total carotenoids were determined spectrophotometrically at 450 nm.

3.2.8.6. Determination of total color change

The change in color was checked using Hunter Lab colorimeter (Ultrascan Vis, HunterLab, USA). Hunter scales L^*, a^*, b^* were used [19, 55]. The change in color was determined using the equation 3.6.

$$\Delta E = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2} \quad (3.6)$$

3.2.8.7. Determination of antioxidant activity

The anti-oxidant activity of the three juice samples were determined using four different methods namely DPPH free radical scavenging assay (explained on section 2.8), nitric oxide (NO) assay, superoxide radical scavenging (SOD) assay and reducing power (RP) assay.

3.2.8.7.1. Nitric oxide (NO) assay

NO assay was determined using Griess reagent [43]. 2.0 ml of 10 mM sodium nitroprusside dissolved in 0.5 ml phosphate buffer saline (pH 7.4) was mixed with 0.5 ml of sample at a concentration (1.0 mg/ml) and incubated at 25 ºC. for 150 min. Incubated solution was mixed with of Griess reagent [(1.0 ml sulfanilic acid reagent (0.33% in 20% glacial acetic acid at room temperature for 5 min with 1.0 ml of naphthylethylenediamine dichloride (0.1% w/v)] at 1:1 ratio and incubated at room temperature for 30 min and OD was measured in a spectrophotometer (Eppendorf

BioSpectrometer Germany) at 546 nm. The amount of nitric oxide radical inhibition was calculated following the equation 3.7.

$$\% \text{ Inhibition of nitric oxide} = \frac{Ao - Ai}{Ao} X\ 100 \quad (3.7)$$

Where, Ao is the absorbance before reaction and

Ai is the absorbance after reaction has taken place with Griess reagent.

3.2.8.7.2. Superoxide radical scavenging (SOD) assay

SOD activity was measured following standard protocol [57]. The superoxide radicals were generated in 3.0 ml of Tris–HCl buffer (16 mM, pH 8.0), containing 0.5 ml of nitrobluetetrazolium (NBT) (0.3 mM), 0.5 ml NADH (0.936 mM) solution, 1.0 ml extract (1.0 mg/ml) and 0.5 ml Tris–HCl buffer (16 mM, pH 8.0). The reaction was initiated by adding 0.5 ml of 0.12 mM phenazinemethosulfate (PMS) solution to the mixture, followed by incubation at 25 ºC for 5 min and then the absorbance was measured in a spectrophotometer (Eppendorf BioSpectrometer Germany) at 560 nm against a blank sample following the equation 3.8.

$$\% \text{ Inhibition of Superoxide radical } = \frac{Ao - Ai}{Ao} X\ 100 \quad (3.8)$$

Where, Ao is the absorbance without adding the sample and

Ai is the absorbance after addition of the sample

3.2.8.7.3. Reducing power (RP) assay

The RP assay was performed following the protocol described previously [25]. Briefly, 1.0 ml of sample (1.0 mg/ml) dissolved in distilled water was mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of $K_3Fe(CN)_6$ (1% w/v). The resulting mixture was incubated at 50 ºC for 20 min, followed by the addition of 2.5 ml of 10% trichloroacetic acid. The mixture was centrifuged at 3000 rpm for 10 min. The supernatant was mixed with 2.5 ml water and 0.5 ml of $FeCl_3$ (0.1%, w/v). The absorbance was then measured in a spectrophotometer (Eppendorf BioSpectrometer Germany) at 700 nm against blank sample following the equation 3.9.

$$\% \text{ Inhibition of reducing power} = \frac{Ao - A\,i}{A\,o} X\ 100 \quad (3.9)$$

Where, Ao is the absorbance without the sample and

Ai is the absorbance after addition of the sample

3.2.9. Assessment of stability and shelf life of BGJ

Micro-centrifuge plastic tubes containing MW-US processed juice and conventional juice were kept at room temperature (~ 23 ± 2 ºC) and at 4ºC. At different time points (0, 3, 7, 10, 14, 21, 28, 60, 90 and 120 days) the pH and antioxidant activities (in terms of DPPH) were assayed. The activity at the beginning (0 day) was considered as 100% activity and the other values were determined from comparisons. All the experiments were performed in triplicate to ensure reproducibility. Yeast, mould, and bacteria were checked during the storage period.

3.2.10. Statistical analysis

Statistical analysis was performed to determine the effect of different independent variables (MP, MT, UA, and UT) on the responses (cell viability, total phenolic content, total terpenoids and DPPH free radical scavenging activity). Regression analysis was done to determining the accuracy of the developed mathematical model. Analysis of variance (ANOVA) on response was done in order to determine the significant terms of experimental data. Coefficient of determination (R^2) and lack of fit was determined for checking the accuracy of the developed model.

3.3. Results and Discussion

3.3.1. Effect of microwave and ultrasound treatment on various responses of pasteurized BGJ

The reduction of *L. monocytogenes* within the juice samples was derived significantly by using the assumed quadratic model. The significance of model developed for the responses are shown in Table 3.2. From the Table 3.2 (2.1, 2.2, 2.3, 2.4), it can be observed that models developed for the cell viability, total phenolic content, total terpenoids and DPPH content of the sample was significant, whereas lack of fit was

insignificant showing accuracy of the developed model. The coefficient of determination (R^2) of the developed model was 0.92, 0.93, 0.95, 0.91 for cell viability, total phenolic content, total terpenoids and DPPH content of the sample.

Table 3.2: ANOVA tables of the fitted model for the response variables.

Table 3.2.1: Cell Viability

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	45.52	14	3.25	14.14	<0.0001	significant
A-Microwave Power	7.105E-15	1	7.105E-15	3.090E-14	1.0000	
B-Microwave Temperature	20.06	1	20.06	87.23	<0.0001	
C-Ultrasound Amplitude	8.00	1	8.00	34.79	<0.0001	
D-Ultrasound Time	8.00	1	8.00	34.79	<0.0001	
AB	0.0000	1	0.0000	0.0000	1.0000	
AC	0.0000	1	0.0000	0.0000	1.0000	
AD	0.0000	1	0.0000	0.0000	1.0000	
BC	0.2500	1	0.2500	1.09	0.3136	
BD	0.2500	1	0.2500	1.09	0.3136	
CD	6.25	1	6.25	27.18	0.0001	
A^2	0.0032	1	0.0032	0.0139	0.9078	
B^2	0.7418	1	0.7418	3.23	0.0926	
C^2	0.0032	1	0.0032	0.0139	0.9078	
D^2	0.0032	1	0.0032	0.0139	0.9078	
Residual	3.45	15	0.2299			
Lack of Fit	1.45	10	0.1449	0.3622	0.9193	not significant
Pure Error	2.00	5	0.4000			
Cor Total	48.97	29				

Factor coding is **Coded**.

Sum of squares is **Type III - Partial**

The **Model F-value** of 14.14 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case B, C, D, CD are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The **Lack of Fit F-value** of 0.36 implies the Lack of Fit is not significant relative to the pure error. There is a 91.93% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit.

Table 3.2.2: TPC

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	802.72	14	57.34	15.36	< 0.0001	significant
A-Microwave Power	2.22	1	2.22	0.5943	0.4527	
B-Microwave Temperature	2.14	1	2.14	0.5720	0.4612	
C-Ultrasound Amplitude	293.95	1	293.95	78.73	< 0.0001	
D-Ultrasound Time	132.46	1	132.46	35.48	< 0.0001	
AB	19.58	1	19.58	5.24	0.0369	
AC	40.77	1	40.77	10.92	0.0048	
AD	16.89	1	16.89	4.52	0.0504	
BC	29.16	1	29.16	7.81	0.0136	
BD	60.14	1	60.14	16.11	0.0011	
CD	0.4422	1	0.4422	0.1184	0.7355	
A^2	20.11	1	20.11	5.39	0.0348	
B^2	87.28	1	87.28	23.37	0.0002	
C^2	5.40	1	5.40	1.45	0.2477	
D^2	117.74	1	117.74	31.53	< 0.0001	
Residual	56.01	15	3.73			
Lack of Fit	11.54	10	1.15	0.1298	0.9967	not significant
Pure Error	44.46	5	8.89			
Cor Total	858.72	29				

Factor coding is **Coded**.

Sum of squares is **Type III - Partial**

The **Model F-value** of 15.36 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case C, D, AB, AC, BC, BD, A^2, B^2, D^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The **Lack of Fit F-value** of 0.13 implies the Lack of Fit is not significant relative to the pure error. There is a 99.67% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit.

Table 3.2.3: Terpenoids

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1.017E+05	14	7265.26	21.26	< 0.0001	significant
A-Microwave Power	61542.01	1	61542.01	180.06	< 0.0001	
B-Microwave Temperature	11375.35	1	11375.35	33.28	< 0.0001	
C-Ultrasound Amplitude	1750.35	1	1750.35	5.12	0.0389	
D-Ultrasound Time	5168.06	1	5168.06	15.12	0.0015	
AB	5814.06	1	5814.06	17.01	0.0009	
AC	0.3906	1	0.3906	0.0011	0.9735	
AD	6703.52	1	6703.52	19.61	0.0005	
BC	3.52	1	3.52	0.0103	0.9206	
BD	6909.77	1	6909.77	20.22	0.0004	
CD	25.00	1	25.00	0.0731	0.7905	
A^2	18.55	1	18.55	0.0543	0.8190	
B^2	1596.67	1	1596.67	4.67	0.0472	
C^2	927.99	1	927.99	2.72	0.1202	
D^2	268.26	1	268.26	0.7849	0.3896	
Residual	5126.85	15	341.79			
Lack of Fit	3562.26	10	356.23	1.14	0.4709	not significant
Pure Error	1564.58	5	312.92			
Cor Total	1.068E+05	29				

Factor coding is **Coded**.

Sum of squares is **Type III - Partial**

The **Model F-value** of 21.26 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case A, B, C, D, AB, AD, BD, B^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The **Lack of Fit F-value** of 1.14 implies the Lack of Fit is not significant relative to the pure error. There is a 47.09% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit.

Table 3.2.4: DPPH

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1381.18	14	98.66	11.05	< 0.0001	significant
A-Microwave Power	30.19	1	30.19	3.38	0.0858	
B-Microwave Temperature	187.08	1	187.08	20.96	0.0004	
C-Ultrasound Amplitude	229.41	1	229.41	25.70	0.0001	
D-Ultrasound Time	237.91	1	237.91	26.65	0.0001	
AB	2.16	1	2.16	0.2420	0.6299	
AC	4.56	1	4.56	0.5106	0.4859	
AD	32.43	1	32.43	3.63	0.0760	
BC	256.48	1	256.48	28.73	< 0.0001	
BD	1.58	1	1.58	0.1764	0.6804	
CD	24.90	1	24.90	2.79	0.1156	
A^2	7.67	1	7.67	0.8588	0.3687	
B^2	0.1145	1	0.1145	0.0128	0.9113	
C^2	117.86	1	117.86	13.20	0.0025	
D^2	14.24	1	14.24	1.60	0.2258	
Residual	133.91	15	8.93			

Lack of Fit	101.95	10	10.19	1.59	0.3164	not significant
Pure Error	31.97	5	6.39			
Cor Total	1515.10	29				

Factor coding is **Coded**.

Sum of squares is **Type III - Partial**

The **Model F-value** of 11.05 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case B, C, D, BC, C^2 are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

The **Lack of Fit F-value** of 1.59 implies the Lack of Fit is not significant relative to the pure error. There is a 31.64% chance that a Lack of Fit F-value this large could occur due to noise. Non-significant lack of fit is good -- we want the model to fit.

The developed quadratic models in terms of coded values of all the factors are as follows:

$$\textbf{Cell viability} = 5.02 + 0.0000\,A - 1.06\,B - 0.6667\,C - 0.6667\,D + 0.0000\,AB + 0.0000\,AC + 0.0000\,AD - 0.1250\,BC - 0.1250\,BD - 0.6250\,CD - 0.0351\,A^2 - 0.5351\,B^2 - 0.0351\,C^2 - 0.0351\,D^2 \quad (3.10)$$

$$\textbf{TPC} = 281.24 - 0.3511\,A - 0.3444\,B + 4.04\,C + 2.71\,D - 1.11\,AB + 1.60\,AC + 1.03AD - 1.35\,BC + 1.94\,BD - 0.1662\,CD + 2.79\,A^2 - 5.80\,B^2 - 1.44\,C^2 + 6.74\,D^2 \quad (3.11)$$

$$\textbf{Terpenoids} = 513.00 + 58.47A + 25.14\,B + 9.86\,C + 16.94\,D + 19.06\,AB - 0.1562\,AC + 20.47\,AD + 0.4688\,BC + 20.78\,BD - 1.25\,CD - 2.68\,A^2 + 24.82\,B^2 - 18.93\,C^2 - 10.18\,D^2 \quad (3.12)$$

$$\mathbf{DPPH} = 26.37 + 1.29\,\mathrm{A} + 3.22\,B + 3.57\,C + 3.64\,D - 0.3675\,AB - 0.5338\,AC + 1.42\,AD - 4.00BC + 0.3138\,BD + 1.25\,CD - 1.72\,\mathrm{A}^2 - 0.2103\,\mathrm{B}^2 + 6.74\,\mathrm{C}^2 + 2.34\mathrm{D}^2$$

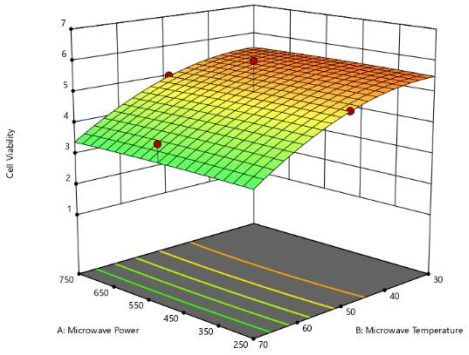
(3.13)

3.3.1.1. Effect of interaction of various factors on *L. monocytogenes* cell viability

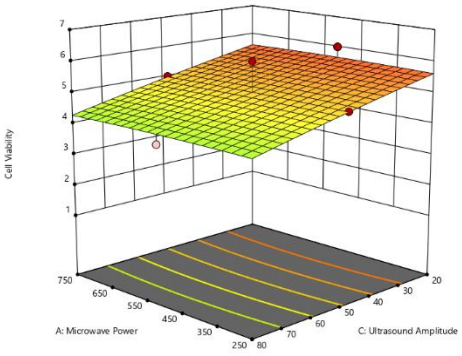
MT had significant effect on *L. monocytogenes* reduction. The negative effects of MT can be seen in the reducing slope of *L. monocytogenes* count (Figures 3.2u, x, y). This may be due to the thermal effects of microwave as also reported by other studies [64]. The other reason may also be due to the non-thermal effects of microwave which might have brought electroporation in the microbial cell wall leading to the bacterial cell lysis [39, 64].

UA had negative effect on *L. monocytogenes* reduction. It was seen that when the UA was raised the destruction effect of ultrasonic waves on *L. monocytogenes* was more (Figures 3.2v, x, z)**.** UA increases the number of formed bubbles and results in increased cavitation in the sample. The increase in cavitation in the sample leads to the reduction in microbial cell viability. This is similar with the studies reported previously [64, 76]. UT also was seen to have significant effect on *L. monocytogenes* reduction (Figures 3.2w, y, z)**.** Increase of UT leads to the increase of sonic stream in reactor resulting in higher contributions of ultrasonic waves to the microorganism cell. Related literature also showed the decrease in microbial count with increase in the time of ultrasound exposure [64, 78]. The increase of ultrasound treatment duration increases the sonic cycles in the reactor, which in turn brings better impacts of ultrasound on microbial population reduction. The Figures 3.2(u, v, w) clearly shows MP does not have any significant effect on cell viability of *L. monocytogenes***.** The same results can be observed from Table 3.2 too. The developed equation also shows the negative effect on cell viability with the increase of MT, UA and UT, whereas MP has no significant effect on *L. monocytogenes* reduction. Even though MP didn't have any significant effect on the microbial reduction, it was observed that the increase of MP effected in the time to reach the desired temperature. Higher MP was more efficient for the sample to reach the desired temperature in short time.

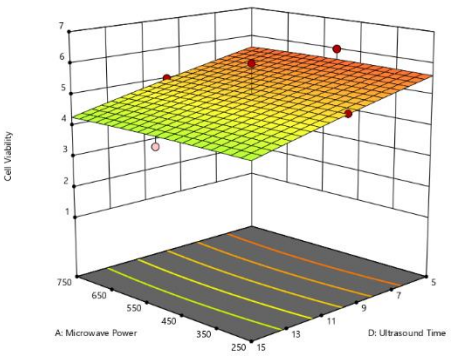
It was also noticed that after microwave treatment alone, a log reduction of 1 - 1.5 of *L. monocytogenes* count was noticed, while after ultrasound a log reduction of 2-3 was noticed. This also supports the effectiveness of the combined process to reduce the desired microbial count (5 log reductions).



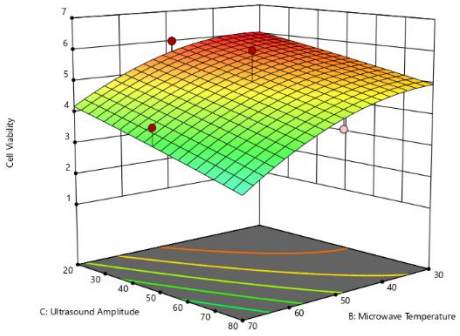
u



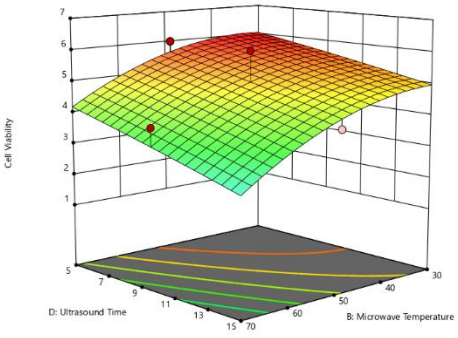
v



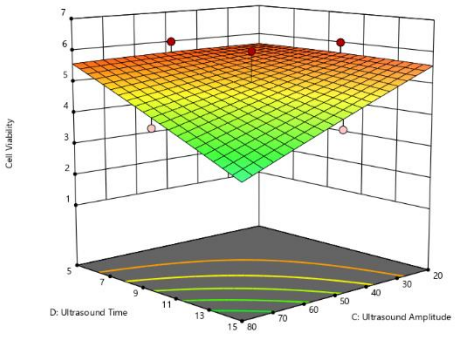
w



x



y



z

Figure 3.2: Effect of interaction of (u) Microwave power and microwave temperature, (v) Microwave power and ultrasound amplitude percentage, (w) Microwave power and ultrasound exposure time, (x) Microwave temperature and ultrasound amplitude percentage, (y) Microwave temperature and ultrasound exposure time, (z) Ultrasound amplitude percentage and ultrasound exposure time on cell viability.

3.3.1.2. Effect of interaction of various factors on total phenolic contents and total terpenoid contents of the juice

The total phenolic content of the BGJ sample can be seen to increase with the increase of UA (Figures 3.3v, x, z). Similar studies were reported by [72] where the retention of plant compounds were positively affected by ultrasound amplitude levels. The significant effects of UT on total phenolic content of the BGJ was also noticed (Figures 3.3w, y, z)**.** With the increase of UT an increase in the total phenolic content of the sample was noticed. This may be due to the reason that due to sonication disruption of cell walls occurs, which further contributes in the release of bound phenolics from the cell. Also another reason as can be due to the generation of hydroxyl radicals (OH·) by ultrasound which gets added to the aromatic ring present in the phenolic compounds [7]. However, no significant effect was observed on phenolic content by MP and MT (Figures 3.3 u, v, w, and 3y)**.** The total terpenoids content of the pasteurized juice was seen to have been positively affected by all the independent variables. It is noteworthy to mention that MP and MT have more significant effects on terpenoids content of the juice (Figure. 3.4, Table 3.2). This may be mainly due to the thermal effects of microwave. The increase in MT might have caused cell ruptures causing extraction of terpenoids in the sample and increase in detection [20, 37]. These findings were also ascertained by ANOVA analysis (Table 3.2). Similar behavior of the corresponding factors was also noticed.

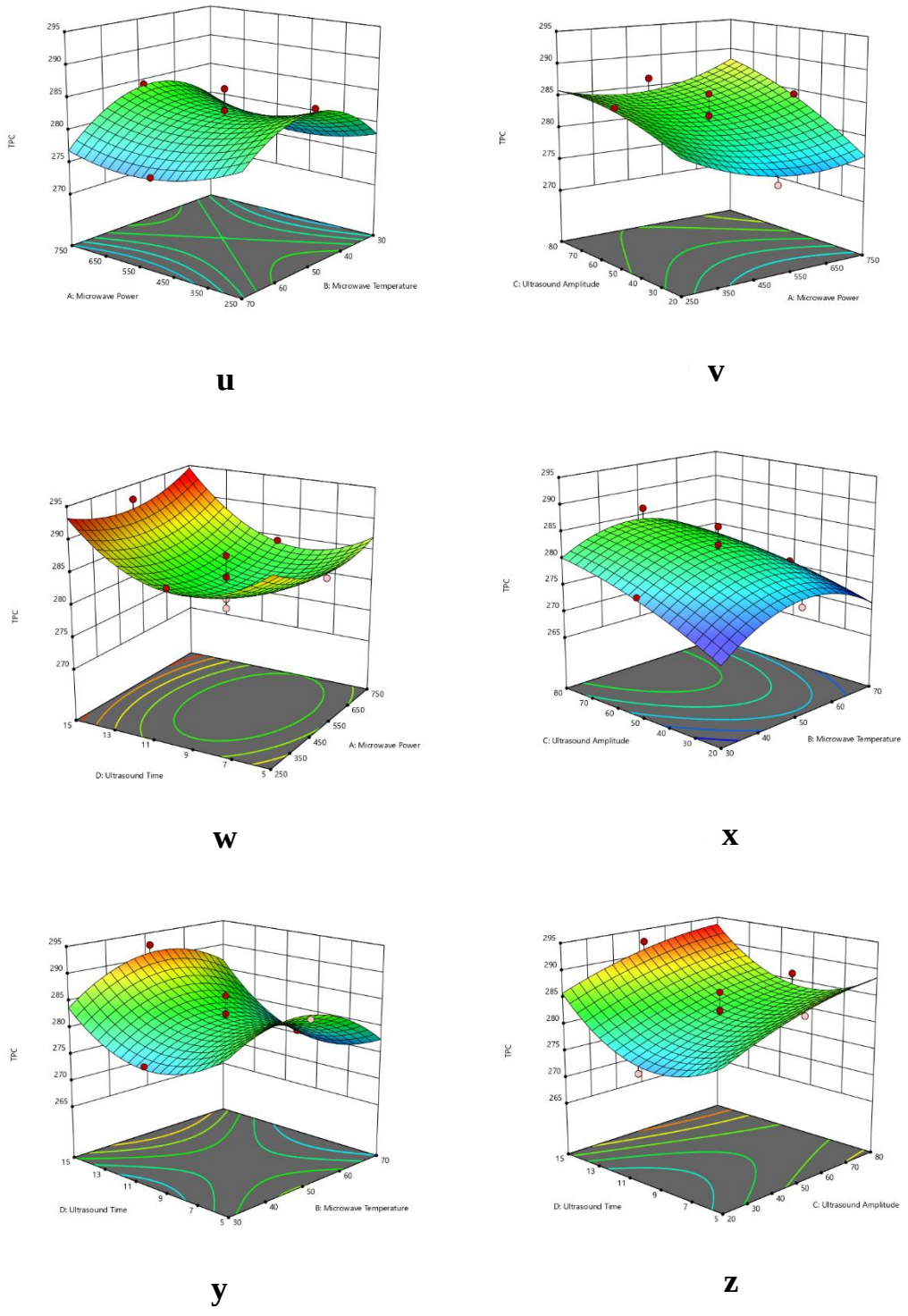
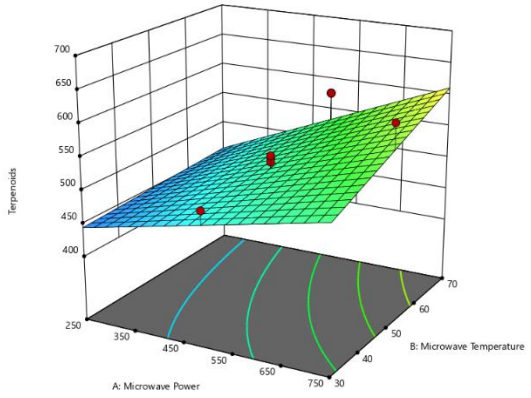
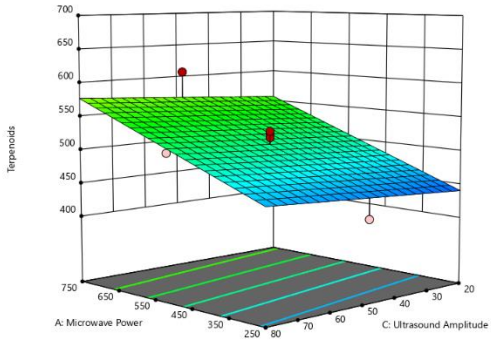


Figure 3.3. Effect of interaction of (u) Microwave power and microwave temperature, (v) Microwave power and ultrasound amplitude percentage, (w) Microwave power and ultrasound exposure time, (x) Microwave temperature and ultrasound amplitude

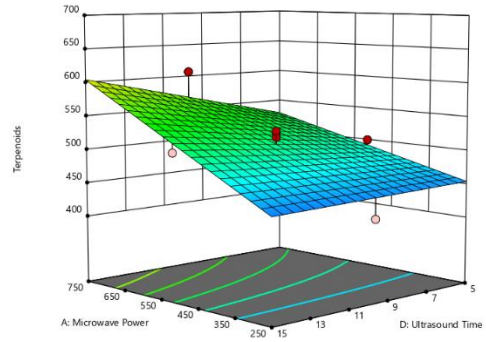
percentage, (y) Microwave temperature and ultrasound exposure time, (z) Ultrasound amplitude percentage and ultrasound exposure time on total phenolic contents of the juice



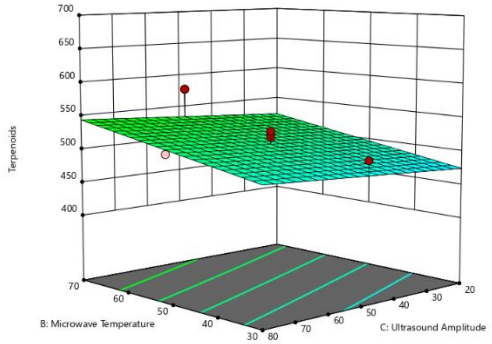
u



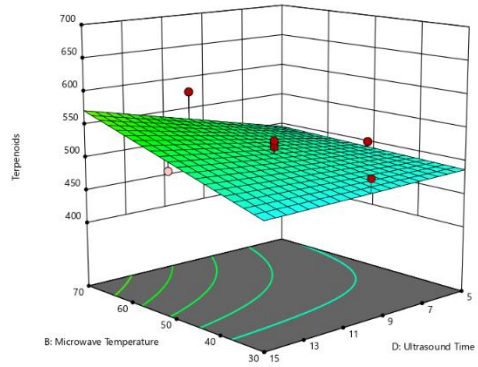
v



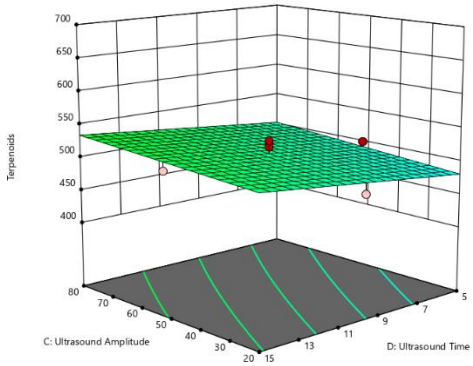
w



x



y

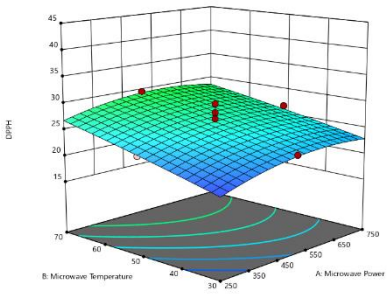


z

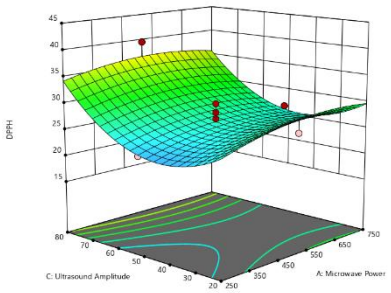
Figure 3.4. Effect of interaction of (u) Microwave power and microwave temperature, (v) Microwave power and ultrasound amplitude percentage, (w) Microwave power and ultrasound exposure time, (x) Microwave temperature and ultrasound amplitude percentage, (y) Microwave temperature and ultrasound exposure time, (z) Ultrasound amplitude percentage and ultrasound exposure time on total terpenoid contents of the juice

3.3.1.3 Effect of interaction of various factors on the DPPH free radical scavenging activity of the juice

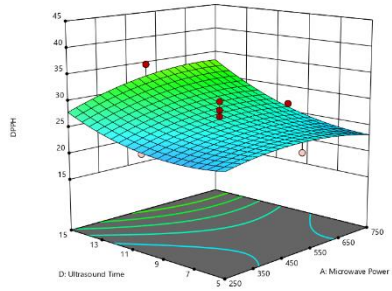
The DPPH free radical scavenging activity of the BGJ sample can be seen to be increased with the increase of UA (Figures 3.5v, x, z). Also, with the increase of UT an increase in the antioxidant activity of the sample was noticed (Figures 3.5w, y). The reason behind the increase in DPPH scavenging activity of the sample may be because of the polyphenolic compound of the BGJ. As explained in section 3.3.1.2, the total phenolic content of the sample was increased with the increase of UA and UT. The increase in the total phenolic content might have increased the antioxidant property of the sample. This is in accordance with the studies reported previously [2, 61]. The Figures 5u, v, w shows that MP does not have any significant effect on free radical scavenging activity whereas MT increases the activity (Figures 3.5u, x, y)**.** The positive effects of MT in the free radical scavenging activity may be due to the extraction of antioxidant compound in the BGJ as a result of the thermal effects of microwave [49, 51].



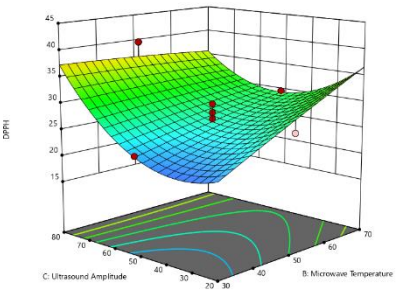
u



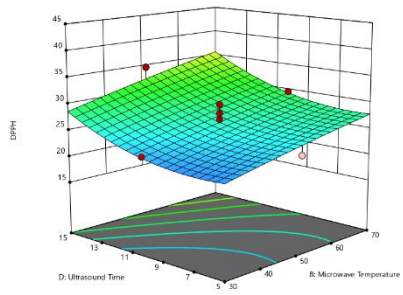
v



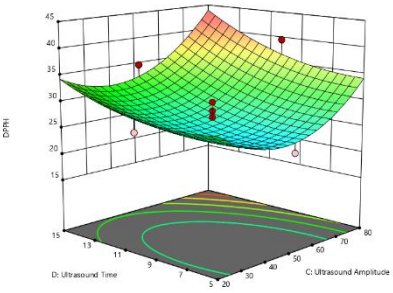
w



x



y



z

Figure 3.5: Effect of interaction of (u) Microwave power and microwave temperature, (v) Microwave power and ultrasound amplitude percentage, (w) Microwave power and ultrasound exposure time, (x) Microwave temperature and ultrasound amplitude percentage, (y) Microwave temperature and ultrasound exposure time, (z) Ultrasound amplitude percentage and ultrasound exposure time on DPPH free radical scavenging activity of the juice

3.3.1.4 Optimum conditions from the design and validation

The aim of the optimization was to achieve a 5 log reduction in the *L. monocytogenes* count using the independent variables (MP, MT, UA, and UT). The other conditions of the optimization were to keep the physicochemical, functional, antioxidant and phytochemical properties of the juice intact with the decrease of microbes. The optimum process conditions were 750 W MP, 70 ºC MT, 80% UA and 15 min of UT (Table 3.3). Under the optimized conditions the experimental values as obtained were 1.30 of log c.f.u/ml of *L. monocytogenes* count (which is near to the desired 5 log reduction of the microbial count), 292.70 (mg/g) of TPC, 682.5 (µg/g) of total terpenoid content and 43.67 % of DPPH scavenging activity. The percent of error between the experimental and predicted values of cell viability, TPC, DPPH scavenging activity and total terpenoid were 2.25, 0.39, 1.75 and 3.21 %.

Table 3.3. Optimized condition for the pasteurization technique.

	Microwave power (W)	Microwave temperature (ºC)	Ultrasound amplitude percentage (%)	Ultrasound exposure time (min)	Cell viability (Log c.f.u/ml)	TPC (µg/ml)	Terpenoids (µg / ml)	DPPH (%)	Desirability
Predicted values	750.00	70.00	80.00	15.00	1.33	291.55	670.74	42.31	0.96
Experimental values	750.00	70.00	80.00	15.00	1.30	292.70 ± 0.62	682.5 ± 0.08	43.67 ± 0.86	
%Error					2.25	0.39	1.75	3.21	

* Experimental values are mean ± S.D. of triplicate determinations.

TPC (Total phenolic contents)

3.3.1.5 Interpretation of optimized results in terms of death and reaction order kinetics

For the interpretation of optimized results in terms of death and reaction order kinetics, microwave-ultrasound based pasteurization was conducted under the MP of 750 W, MT of 70ºC and UA of 80%. For each set of MP, MT and UA the ultrasound exposure time was varied from 0 to 15 min with an interval of 3 min.

Table 3.4: Statistical and model parameters of death and reaction order kinetics.

Responses	Coefficient of determination (R^2)	Model parameters	
		D (min)	k (min^{-1})
Cell viability	0.90	3.03	0.760
TPC	0.96	-	0.004
DPPH	0.94	-	0.014
Terpenoids	0.93	-	0.027

*Death kinetics of cell viability alone have been checked

From Table 3.4, adequacy of model fitting for death and reaction order kinetics was observed in terms of R^2 (coefficient of determination), RMSE (root mean square error) and SSE (sum of square error). The values of reaction order rate (k) were observed to be 0.76, 0.004, 0.014 and 0.027 min^{-1} respectively for cell viability, TPC, DPPH and terpenoids. The decimal reduction time for the pasteurization process was 3.03 min. The plots between the experimental and predicted results for various responses are represented in Figure. 3.6.

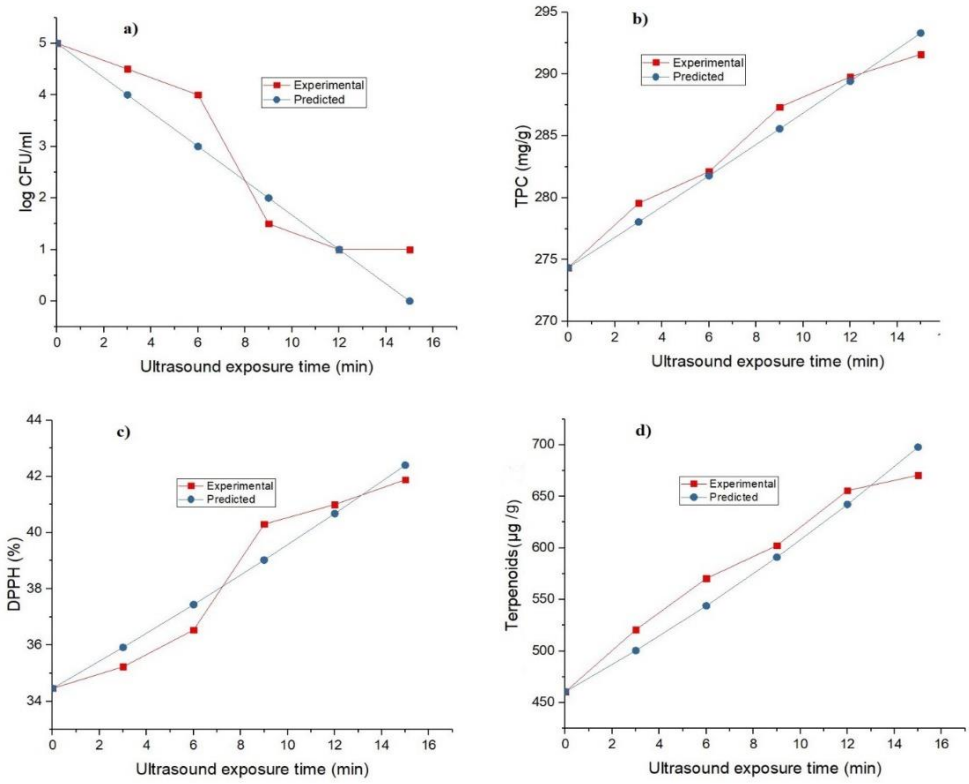


Figure 3.6: Comparison between experimental and predicted values for (a) cell viability (b) TPC (c) DPPH and (d) terpenoids

3.3.2 MW-US processed juice showed superior TS, TSS, TA and pH as compared to conventionally processed juice

The pasteurization was carried out on BGJ by conventional technique and MW-US technique (Table 3.5). The non-significant change was seen in the TS content of the treated samples with respect to the raw. However, the TSS content was seen to be slightly increased in the MW-US (Table 3.5) sample and reached up to 3.4º Brix in the optimized condition of the design with respect to the raw control (3.0 ºBrix). The ultrasonic exposure might have caused the breakdown of the cellular content of the juice which might be the responsible for increase in TSS of the sample. The titratable acidity and pH were found to be non-significant in both the treatments (Table 3.5). These results are in accordance with other previous reports [2, 61].

Table 3.5: Physicochemical and functional properties of MW-US treated juice as compared to conventionally processed and raw juice.

Sample	TS (%)	TSS (ºBrix)	TA (%)	pH	TPC (mg/g)	Terpenoids (µg/g)	Protein content (µg /mg) of dry weight
Control (raw)	6.03 ± 0.02	3.0 ± 0.10	0.038 ± 0.002	5.6 ± 0.23	272.29 ± 0.91	587.5 ± 0.05	25.47 ± 0.10
Conventionally treated (75^0C, 10 min)	6.02 ± 0.03	3.0 ± 0.05	0.038 ± 0.004	5.4 ± 0.48	279.96 ± 1.83	380.0 ± 0.09	22.13 ± 0.44
MW-US	6.03 ± 0.02	3.4 ± 0.14	0.038 ± 0.002	5.5 ± 0.16	292.70 ± 0.62	682.5 ± 0.08	28.10 ± 0.22

* Values are mean ± S.D. of triplicate determinations.

MW-US (Microwave followed by Ultrasound Pasteurization), TS (Total solid content), TSS, (Total soluble solids), TA (Titratable acidity), TPC (Total phenolic contents)

Table 3.6: HPLC analysis of phenolic compounds of MW-US treated juice as compared to conventionally processed and raw juice.

Phenolic compounds	Raw (%)	Conventional (%)	MW-US (%)
Caffeic acid	2.200	1.470	1.610
Catechin	5.590	3.690	3.430
Coumaric acid	BDL	BDL	BDL
Ferulic acid	0.004	0.010	0.010
Gallic acid	1.025	0.275	0.600
Quercetin	0.033	0.020	0.020
Rutin	0.060	0.040	0.050
Sinapic acid	BDL	BDL	BDL

BDL (Below Detectable Limit), MW-US (Microwave followed by Ultrasound Pasteurization)

3.3.3. MW-US processed juice demonstrated better phenolics and terpenoids content as compared to conventionally processed juice

The TPC and total terpenoids content of the MW-US processed BGJ were seen to be significantly increased than the raw BGJ (control) while in conventionally processed juice it was seen to be decreased than the raw BGJ (control) (Table 5). The reason for increase in phenolic compounds in MW-US processed juice has been explained in section 3.3.1.2. Phenolic profiling of MW-US shows the presence of 6 different phenolic compounds (Table 3.6). Catechin was found to be the highest phenolic compound followed by caffeic acid and gallic acid. There are no significant changes in the tested phenolics in MW-US and conventionally treated juice. However, both the pasteurization treatment demonstrated decrease in tested phenolic contents as compared with raw. It is noteworthy to mention that the total phenolic content was higher in MW-US as compared to raw and conventionally pasteurized juice. These results suggested that there may be other phenolic compounds present in the juice that increased the total phenolic content. The detail analysis will be our next goal of study.

3.3.4. MW-US processed juice retained better protein and amino acid content as compared to conventionally processed juice

The protein content was estimated to be 25.47, 22.13, 28.10 (µg /mg) of dry weight for raw, conventional and MW-US treated juice respectively (Table 5). Protein content of MW-US processed sample was seen to be increased. This may be due to the reason that sonication leads to disruption of cell walls, which further contributes to the release of plant compound from the cell [7]. Another reason may be due to the MT which causes cell ruptures leading to extraction of protein in the sample and increase in detection [20, 37]. Analysis of the amino acid composition of raw, conventional and microwave-treated BGJ are shown in Table 3.7. Amino acid compositional analysis of raw, conventional and microwave-treated BGJ revealed the presence of alanine as one of major amino acid in the BGJ followed by arginine and methionine. These amino acids are essential to regulate key metabolic pathways to improve health, survival and growth and development [77]. Apart from these, presence of glutamic acid, serine, tyrosine, phenylalanine, isoleucine and leucine were seen which are essential for various roles such as regulating gene expression, antioxidative responses, immunity etc [77]. Nevertheless, all the samples did not contain aspartic acid, histidine, cysteine, valine, lysine and proline. Our results demonstrated that MW-US treated juice did not affect the amino acid compositions, so that nutritional parameter remains intact. Further in some cases, there is significant increase in amino acid concentration in MW-US treated sample as compared to conventional method. Elaborating the detail concept will be one of our future study goals.

Table 3.7: Amino acids composition of MW-US treated juice as compared to conventionally processed and raw juice. (%, w/w).

Amino acids (% composition)	Raw (%)	Conventional (%)	MW-US (%)
Alanine	78.6	75.2	76.5
Aspartic acid	BDL	BDL	BDL
Histidine	BDL	BDL	BDL
Glutamic acid	2.2	1.9	2.1
Serine	0.8	0.5	0.7
Cystine	BDL	BDL	BDL
Arginine	8.4	7.5	7.8
Tyrosine	2.4	1.8	1.9
Valine	BDL	BDL	BDL

Methionine	6.1	5.3	5.7
Phenylalanine	2.5	1.7	2.1
Isoleucine	1.8	1.5	1.7
Leucine	1.5	0.9	1.1
Lysine	BDL	BDL	BDL
Proline	BDL	BDL	BDL

BDL (Below Detectable Limit), MW-US (Microwave followed by Ultrasound Pasteurization)

3.3.5. MW-US processed juice demonstrated superior vitamin retainment as compared to conventionally processed juice

Vitamin profiling of raw, conventional and MW-US processed juice are shown in Table 3.8, 3.9. From the tables, it was observed that most of the vitamins were intact in MW-US processed juice compared to conventionally processed juice. However both the pasteurization treatment demonstrated slight decrease in vitamin contents as compared with raw. Earlier reports also demonstrated a slight reduction of vitamins in various juices by ultrasound treatment and thermal processing [70].

Table 3.8. Water soluble vitamins in the MW-US treated juice as compared to conventionally processed and raw juice.

Sl. No.	Vitamins	Raw (mg/100g)	Conventional (mg/100g)	MW-US (mg/100g)
1	Riboflavin (B2)	0.40	0.20	0.31
2	Niacin (B3)	2.40	1.40	1.90
3	Pantothenic Acid (B5)	3.00	2.30	2.80
4	Pyridoxamine (PM)	BDL	BDL	BDL
5	Pyridoxal (PLP)	BDL	BDL	BDL
6	Pyridoxine (PN)	0.41	0.28	0.32
7	Total B6	0.30	0.20	0.30
8	Ascorbic Acid (Vit C)	2.10	1.70	1.90
9	Tetra Hydro Folate (THF)	0.02	0.01	0.02
10	Formyl THF (5 FTHF)	0.02	0.01	0.01
11	Formyl Folic Acid (10 FAA)	BDL	BDL	BDL
12	Total Folates (B9)	0.92	0.70	0.83

BDL (Below Detectable Limit), MW-US (Microwave followed by Ultrasound Pasteurization)

Table 3.9: Fat soluble vitamins in the MW-US treated juice as compared to conventionally processed and raw juice.

Sl. No.	Vitamins	Raw (µg/100g)	Conventional (µg/100g)	MW-US (µg/100g)
1	Lutein	BDL	BDL	BDL
2	Zeaxanthin	BDL	BDL	BDL
3	α - Carotene	29.20	19.70	26.10
4	β - Carotene	48.40	35.50	41.00
5	Total carotenoids	83.40	75.80	81.20
6	Tocopherols (α-T)	2300.00	1660.00	1800.00
7	Tocopherols (β-T)	270.00	170.00	200.00
8	Tocopherols (γ-T)	BDL	BDL	BDL
9	Tocopherols (δ-T)	510.00	420.00	470.00
10	Tocotrienols (α-T3)	BDL	BDL	210.00
11	Tocotrienols (γ-T3)	BDL	20.00	BDL
12	Tocotrienols (δ-T3)	BDL	BDL	BDL

BDL (Below Detectable Limit), MW-US (Microwave followed by Ultrasound Pasteurization)

3.3.6. MW-US processed juice demonstrated superior antioxidant activity as compared to conventionally processed juice

It was clearly observed that antioxidant activity of MW-US processed juice was significantly higher than raw and conventionally treated juice (Table 10). The total phenolic content could be the reason for the increase in antioxidant activity. As explained in section 3.3.1.2 the total phenolic compound in the MW-US processed juice was increased. The antioxidant activity

of the phenolic compounds is due their redox potential properties [5, 67]. Presence of hydroxyl groups facilitated the radical scavenging ability; and thereby free radicals and responsive oxygen species (ROS) oxidize significant cell segments continually in physiological system [14]. Then again antioxidants can dispose of ROS and protect significant cell parts from oxidation [28]. Likewise, ROS assume critical preventive jobs in the advancement of numerous ceases less maladies including cardiovascular ailments, maturing, coronary illness, diabetes, weakness, degenerative infections, ischemia and cancer [28,29, 34]. Additionally, there is a developing pattern in purchaser inclinations towards characteristic cell reinforcements. In this study, apart from DPPH assay, antioxidant activities were also assayed by RP, SOD, NO assays (Figure. 3.7) that vouched for the potential antioxidant property of MW-US processed juice. From our results, the antioxidant property may be corroborated due to presence of phenolic compounds; however, a detail study will provide deeper understanding of the active component(s) responsible for the activity that will be our next goal of interest.

Table 3.10: DPPH free radical scavenging activity of MW-US treated juice as compared to conventionally processed and raw juice.

Sample	DPPH (%)
Control (raw)	36.54±0.73
Conventionally treated	34.32 ± 0.59
MW-US	43.67±0.86

*Values are mean of triplicate determinations. Conventional Pasteurization (75^0C, 10 min), MW-US (Microwave followed by Ultrasound Pasteurization)

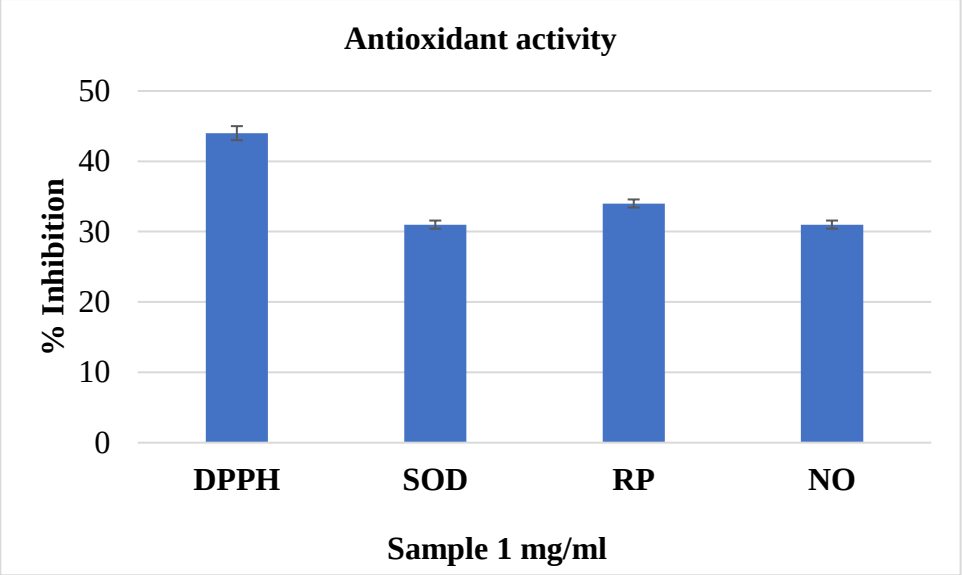


Figure 3.7: Antioxidant activity of MW-US processed juice, values are mean ± S.D. of triplicate determination

3.3.7. MW-US processed juice showed better retainment of colour as compared to conventionally processed juice

Hunter scales L^*, a^*, b^* were used. L^*express whiteness or brightness/darkness, a^* represents the variation between red and green and b^* represents the variation between yellow and blue. The total color differences (ΔE) gives the magnitude of color change in the sample after the treatment with respect to the standards. The differences in color are classified as not noticeable (0–0.5), slightly noticeable (0.5–1.5), noticeable (1.5–3.0), well visible (3.0–6.0), and great (6.0–12.0) [19]. Noticeable change was observed in conventionally treated juice sample and while slightly noticeable change was observed in MW-US treated juice sample with a ΔE value of 2.0 and 0.6 with respect to the raw as standard (Table 11). The result showed minimal change in the color of the MW-US treated juice than the conventionally treated.

Table 3.11: Total color change in the MW-US treated juice as compared to conventionally processed and raw juice.

	L*	a*	b*	ΔE
Control (raw)	9.020 ± 0.126	2.110 ± 0.041	12.250 ± 0.275	-
Conventionally treated	9.880 ± 0.157	1.060 ± 0.082	13.800 ± 0.463	2.063 ± 0.109
MW-US	9.130 ± 0.183	2.080 ± 0.056	12.880 ± 0.332	0.642 ± 0.053

*Values are mean of triplicate determinations.

Conventional Pasteurization (75^0C, 10 min)

MW-US (Microwave followed by Ultrasound Pasteurization)

3.3.8. MW-US processed juice exhibited prevalent post storage activity

In this study, MW-US was evaluated in BG juice through storage time, where significant reductions on microbial growth rate were achieved. Post-storage MW-US processed juice which was 120 days old at 4°C displayed approximately 76% of antioxidant activity (Figure.3.8). Nevertheless, these activities of MW-US processed juice showed less stability at room temperature (Figure.3.8). There was no significant change in the pH post 4 months storage (Figure3..9). Moreover, no yeast and mold growth was observed during the storage period (data not shown). There are several studies showing storage stability using ultrasound and HTST technologies [73, 79]. Our results demonstrated the potential application of MW-US processing of juices as the shelf-life of the juice was increased compared to raw and conventionally treated juice and the nutritional parameters were all intact and therefore, this method may be utilized in food industry.

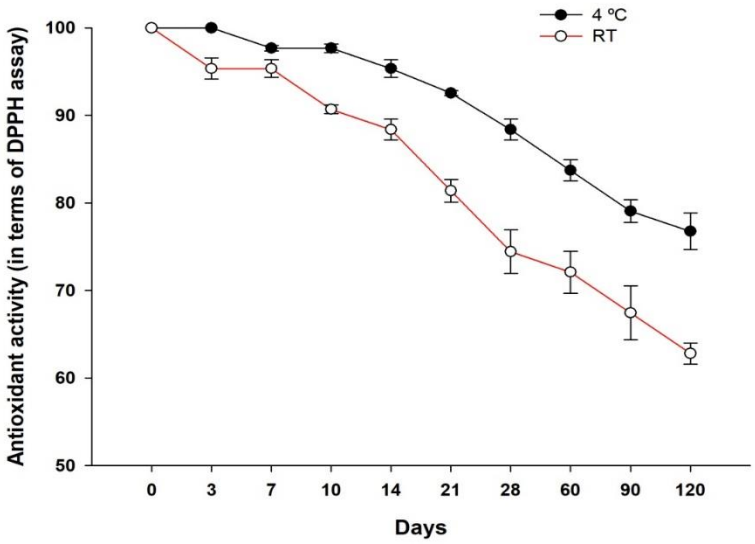


Figure 3.8: Storage study of MW-US processed juice (in terms of DPPH free radical scavenging activity), values are mean ± S.D. of triplicate determination.

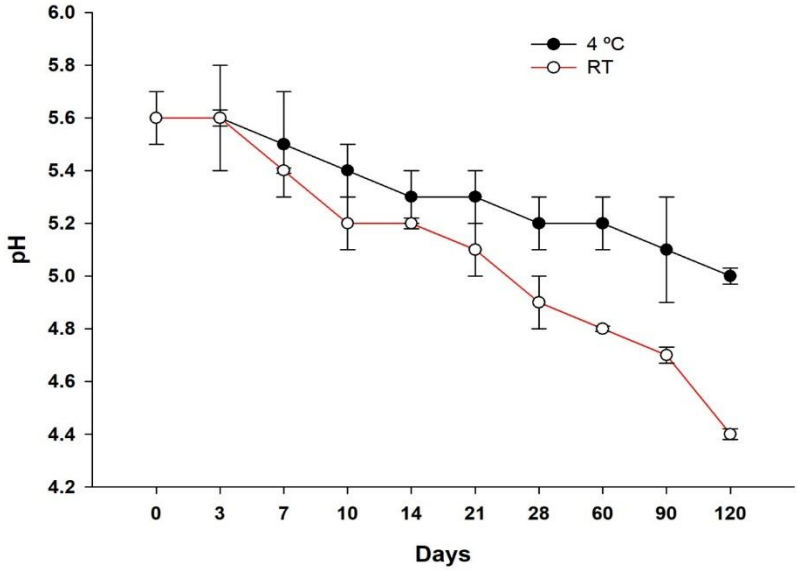


Figure 3.9: Storage study of MW-US processed juice (in terms of pH values), values are mean ± S.D. of triplicate determination

3.3.4. Conclusions

The study revealed the significant effects of microwave and ultrasound in the pasteurized BGJ sample. MT with UA and UT time was significant in reducing *L. monocytogenes* count from the spiked juice. Microwave and ultrasound processing were also significant in retaining key properties like TPC, total terpenoids content and antioxidant activity of the bottle gourd juice. The combined technique was found to be superior to the conventional methods of bottle gourd juice pasteurization. Retention of the desirable properties were much higher in the MW-US pasteurized juice in terms of phenolics, terpenoids, antioxidant, vitamins, total protein, amino acids, and color values over the conventional method.

Bibliography

[1]. Aadil, R. M., Zeng, X.-A., Han, Z., & Sun, D.-W. Effects of ultrasound treatments on quality of grapefruit juice. *Food Chemistry*, 141(3): 3201-3206, 2013.

[2]. Abid, M., Jabbar, S., Wu, T., Hashim, M. M., Hu, B., Lei, S., & Zeng, X. Effect of ultrasound on different quality parameters of apple juice. *Ultrasonics Sonochemistry*, 20(5): 1182-1187, 2013.

[3]. Aneja, K. R., Dhiman, R., Aggarwal, N. K., Kumar, V., & Kaur, M. Microbes associated with freshly prepared juices of citrus and carrots. *International Journal of Food Science*, 2014.

[4]. Arella, F., Lahely, S., Bourguignon, J. B., & Hasselmann, C. Liquid chromatographic determination of vitamins B1 and B2 in foods. A collaborative study. *Food Chemistry*, 56(1): 81-86, 1996.

[5]. Baba, S. A., & Malik, S. A. Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of *Arisaema jacquemontii* Blume. *Journal of Taibah University for Science*, 9(4): 449-454, 2015.

[6]. Berrios-Rodriguez, A., Olanya, O. M., Ukuku, D. O., Niemira, B. A ., Orellana, L. E., Mukhopadhyay, S., Cassidy, J. M., Boyd, G. Inactivation of *Listeria monocytogenes* on post-harvest carrot and tomato by gamma radiation, sanitizer, biocontrol treatments and their combinations. *LWT*, 118: 108805, 2020. https://doi.org/10.1016/j.lwt.2019.108805

[7]. Bhat, R., Kamaruddin, N. S. B. C., Min-Tze, L., & Karim, A.A. Sonication improves kasturi lime (*Citrus microcarpa*) juice quality. *Ultrasonics Sonochemistry*, 18(6): 1295-1300, 2011.

[8]. Bhat, S., Saini, C. S., Kumar, M., & Sharma, H. K. Effect of thermal and alternate thermal processing on bottle gourd (*Lagenaria siceraria*) Juice. *Journal of Food Processing and Preservation*, 41(3): e12911, 2017.

[9]. Bhat, S., & Sharma, H. K. Combined effect of blanching and sonication on quality parameters of bottle gourd (*Lagenaria siceraria*) juice. *Ultrasonics Sonochemistry*, 33: 182-189, 2016.

[10]. Bhattacharya, M., & Basak, T. A comprehensive analysis on the effect of shape on the microwave heating dynamics of food materials. *Innovative Food Science & Emerging Technologies*, 39: 247-266, 2017.

[11]. Bonvehi, J. S., Coll, F. V., & Rius, I. A. Liquid chromatographic determination of tocopherols and tocotrienols in vegetable oils, formulated preparations, and biscuits. *Journal of AOAC International*, 83(3): 627-634, 2000.

[12]. Bornhorst, E. R., Liu, F., Tang, J., Sablani, S. S., & Barbosa-Cánovas, G. V. Food quality evaluation using model foods: a comparison study between microwave-assisted and conventional thermal pasteurization processes. *Food and Bioprocess Technology*, 10(7): 1248-1256, 2017.

[13]. Brownmiller, C., Howard, L. R., & Prior, R. L. Processing and storage effects on monomeric anthocyanins, percent polymeric color, and antioxidant capacity of processed blueberry products. *Journal of Food Science*, 73(5): 72-79, 2008.

[14]. Çetinkaya, Y., Göçer, H., Menzek, A., & Gülçin, İ. Synthesis and antioxidant properties of (3, 4 dihydroxyphenyl)(2, 3, 4 trihydroxyphenyl) methanone and its derivatives. Archiv der Pharmazie, 345(4): 323-334, 2012.

[15]. Chandrasekaran, S., Ramanathan, S., & Basak, T. Microwave food processing—A review. *Food Research International*, 52(1): 243-261, 2013.

[16]. Chyne, D. A. L., Ananthan, R., & Longvah, T. Food compositional analysis of Indigenous foods consumed by the Khasi of Meghalaya, North-East India. *Journal of Food Composition and Analysis*, 77: 91-100, 2019.

[17]. Contreras, C., Benlloch-Tinoco, M., Rodrigo, D., & Martínez-Navarrete, N. Impact of microwave processing on nutritional, sensory, and other quality attributes. In *The Microwave Processing of Foods* (pp. 65-99). Woodhead Publishing, 2017.

[18]. Crépet, A., Albert, I., Dervin, C., & Carlin, F. Estimation of microbial contamination of food from prevalence and concentration data: application to *Listeria monocytogenes* in fresh vegetables. *Applied and. Environmental Microbiology*, 73(1): 250-258, 2007.

[19]. Cserhalmi, Z., Sass-Kiss, Á., Tóth-Markus, M., & Lechner, N. *Innovative Food Science &. Emerging Technologies*, 7: 49, 2006.

[20]. da Rosa, G. S., Vanga, S. K., Gariepy, Y., & Raghavan, V. Comparison of microwave, ultrasonic and conventional techniques for extraction of bioactive compounds from olive leaves (*Olea europaea L*.). *Innovative Food Science & Emerging Technologies*, 58: 102234, 2019.

[21]. De Brouwer, V., Storozhenko, S., Van De Steene, J. C., Wille, S. M., Stove, C. P., Van Der Straeten, D., & Lambert, W. E. Optimisation and validation of a liquid chromatography–tandem mass spectrometry method for folates in rice. *Journal of Chromatography A*, 1215(1-2): 125-132, 2008.

[22]. Doyle, M. E., Mazzotta, A. S., Wang, T., Wiseman, D. W., & Scott, V. N. Heat resistance of *Listeria monocytogenes*. *Journal of Food Protection*, 64(3): 410-429, 2001.

[23]. Ekezie, F.-G. C., Sun, D.-W., Han, Z., & Cheng, J.-H. Microwave-assisted food processing technologies for enhancing product quality and process efficiency: A review of recent developments. *Trends in Food Science & Technology*, 67: 58-69, 2017.

[24]. Farber, J., & Peterkin, P. *Listeria monocytogenes*, a food-borne pathogen. *Microbiology and Molecular Biology Reviews*, 55(3): 476-511, 1991.

[25]. Ferreira, I. C. F. R., Baptista, P., Vilas-Boas, M., & Barros, L. Free-radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal: Individual cap and stipe activity. *Food Chemistry*, 100(4): 1511-1516, 2007.

[26]. Ghorai, N., Chakraborty, S., Gucchait, S., Saha, S. K., & Biswas, S. Estimation of total Terpenoids concentration in plant tissues using a monoterpene, *Linalool as standard reagent. Protocol Exchange*, 5(10): 1038, 2012.

[27]. Ghosh, K., Chandra, K., Ojha, A. K., Sarkar, S., & Islam, S. S. Structural identification and cytotoxic activity of a polysaccharide from the fruits of *Lagenaria siceraria* (Lau). *Carbohydrate Research*, 344(5): 693-698, 2009.

[28]. Göçer, H., Akıncıoğlu, A., Öztaşkın, N., Göksu, S., & Gülçin, İ. Synthesis, Antioxidant, and Antiacetylcholinesterase Activities of Sulfonamide Derivatives of Dopamine-Related Compounds. *Archiv der Pharmazie*, 346(11): 783-792, 2013.

[29]. Göçer, H., & Gülçin, İ. Caffeic acid phenethyl ester (CAPE): correlation of structure and antioxidant properties. *International Journal of Food Sciences and Nutrition*, 62(8): 821-825, 2011.

[30]. Gogoi, D., Arora, N., Kalita, B., Sarma, R., Islam, T., Ghosh, S. S., Devi, R., & Mukherjee, A. K. Anticoagulant mechanism, pharmacological activity, and assessment of preclinical safety of a novel fibrin (ogen) olytic serine protease from leaves of *Leucas indica*. *Scientific Reports*, 8(1): 1-17, 2018.

[31]. Gogoi, D., Ramani, S., Bhartari, S., Chattopadhyay, P., & Mukherjee, A. K. Characterization of active anticoagulant fraction and a fibrin (ogen) olytic serine protease from leaves of *Clerodendrum colebrookianum*, a traditional ethno-medicinal plant used to reduce hypertension. *Journal of Ethnopharmacology*, 243: 112099, 2019.

[32]. González-Tejedor, G. A., Garre, A., Esnoz, A., Artés-Hernández, F., & Fernández, P.S. Effect of storage conditions in the response of *Listeria monocytogenes* in a fresh purple vegetable smoothie compared with an acidified TSB medium. *Food Microbiology*, 72: 98-105, 2018.

[33]. Gouma, M., Álvarez, I., Condón, S., & Gayán, E. Modelling microbial inactivation kinetics of combined UV-H treatments in apple juice. *Innovative Food Science & Emerging Technologies*, 27: 111-120, 2015.

[34]. Gülçin, İ. Antioxidant activity of caffeic acid (3, 4-dihydroxycinnamic acid). *Toxicology*, 217(2-3): 213-220, 2006.

[35]. Guo, Q., Sun, D.-W., Cheng, J.-H., & Han, Z. Microwave processing techniques and their recent applications in the food industry. *Trends in Food Science & Technology*, 67: 236-247, 2017.

[36]. Jakob, E., & Elmadfa, I. Application of a simplified HPLC assay for the determination of phylloquinone (vitamin K1) in animal and plant food items. *Food Chemistry*, 56(1): 87-91, 1996.

[37]. Khajeh, M., Moghaddam, A. R. A., & Sanchooli, E. Application of Doehlert design in the optimization of microwave-assisted extraction for determination of zinc and copper in cereal samples using FAAS. *Food Analytical Methods*, 3(3): 133-137, 2010.

[38]. Kitts, D. D., Wijewickreme, A. N., & Hu, C. Antioxidant properties of a North American ginseng extract. *Molecular and Cellular Biochemistry*, 203(1-2): 1-10, 2000.

[39]. Kozempel, M. F., Annous, B. A., Cook, R. D., Scullen, O., & Whiting, R. C. Inactivation of microorganisms with microwaves at reduced temperaturas. *Journal of Food Protection*, 61(5): 582-585, 1998.

[40]. Kuldiloke, J. Effect of ultrasound, temperature and pressure treatments on enzyme activity and quality indicators of fruit and vegetable juices. Doctoral dissertation, Technical University of Berlin, 2002.

[41]. Li, J., Walker, C. E., & Faubion, J. M. Acidulant and oven type affect total anthocyanin content of blue corn cookies. *Journal of the Science of Food and Agriculture*, 91(1): 38-43, 2011.

[42]. Majumdar, S., Sarmah, B., Gogoi, D., Banerjee, S., Ghosh, S. S., Banerjee, S., Chattopadhyay, P., Mukherjee, A. K. Characterization, mechanism of anticoagulant action, and assessment of therapeutic potential of a fibrinolytic serine protease (Brevithrombolase) purified from *Brevibacillus brevis* strain FF02B. *Biochimie*, 103: 50-60, 2014.

[43]. Marcocci, L., Maguire, J. J., Droylefaix, M. T., & Packer, L. The nitric oxide-scavenging properties of Ginkgo biloba extract EGb 761. *Biochemical and Biophysical Research Communications*, 201(2): 748-755, 1994.

[44]. Mason, T. J. (1996). *Advances in Sonochemistry*: JAI PRESS INC, 1996.

[45]. Matsui, K. N., Granado, L. M., De Oliveira, P. V., & Tadini, C.C. Peroxidase and polyphenol oxidase thermal inactivation by microwaves in green coconut water simulated solutions. *LWT-Food Science and Technology*, 40(5): 852-859, 2007.

[46]. Mendes-Oliveira, G., Deering, A. J., San Martin-Gonzalez, M. F., & Campanella, O. H. Microwave pasteurization of apple juice: Modeling the inactivation of Escherichia coli O157: H7 and *Salmonella typhimurium* at 80–90° C. *Food Microbiology*, 87: 103382, 2020.

[47]. Muñoz-Cuevas, M., Fernández, P. S., George, S., & Pin, C. Modeling the lag period and exponential growth of *Listeria monocytogenes* under conditions of fluctuating temperature and water activity values. *Applied & Environmental Microbiology*, 76(9): 2908-2915, 2010.

[48]. Nicoli, M., Anese, M., & Parpinel, M. Influence of processing on the antioxidant properties of fruit and vegetables. *Trends in Food Science & Technology*, 10(3): 94-100, 1999.

[49]. Patras, A., Brunton, N. P., Da Pieve, S., & Butler, F. Impact of high pressure processing on total antioxidant activity, phenolic, ascorbic acid, anthocyanin content and colour of strawberry and blackberry purées. *Innovative Food Science & Emerging Technologies*, 10(3): 308-313, 2009.

[50]. Peng, J., Tang, J., Luan, D., Liu, F., Tang, Z., Li, F., & Zhang, W. Microwave pasteurization of pre-packaged carrots. *Journal of Food Engineering*, 202: 56-64, 2017.

[51]. Pérez-Grijalva, B., Herrera-Sotero, M., Mora-Escobedo, R., Zebadúa-García, J. C., Silva-Hernández, E., Oliart-Ros, R., Pérez-Cruz, C., & Guzmán-Gerónimo, R. Effect

of microwaves and ultrasound on bioactive compounds and microbiological quality of blackberry juice. *LWT*, 87: 47-53, 2018.

[52]. Phillips, K. M., Tarrago-Trani, M. T., Gebhardt, S. E., Exler, J., Patterson, K. Y., Haytowitz, D. B., Pehrsson, P. R., & Holden, J. M. Stability of vitamin C in frozen raw fruit and vegetable homogenates. *Journal of Food Composition and Analysis*, 23(3): 253-259, 2010.

[53]. Prajapati, R. P., Kalariya, M., Parmar, S. K., & Sheth, N. R. Phytochemical and pharmacological review of *Lagenaria sicereria*. *Journal of Ayurveda and Integrative Medicine*, 1(4): 266, 2010.

[54]. Rahman, A. H. Bottle gourd (*Lagenaria siceraria*) a vegetable for good health. *Natural Product Radiance*, 2(5): 249-250, 2003.

[55]. Raju, S., & Deka, S. C. Influence of thermosonication treatments on bioactive compounds and sensory quality of fruit (*Haematocarpus validus*) juice. *Journal of Food Processing and Preservation*, 42(8): e13701, 2018.

[56]. Ranganna, S. *Handbook of Analysis and Quality Control for Fruit and Vegetable Products*: Tata McGraw-Hill Education, 1986.

[57]. Robak, J., & Gryglewski, R. J. Flavonoids are scavengers of superoxide anions. *Biochemical Pharmacology*, 37(5): 837-841, 1988.

[58]. Rodriguez-Amaya, D. B., & Kimura, M. *HarvestPlus handbook for Carotenoid Analysis (Vol. 2)*: International Food Policy Research Institute (IFPRI) Washington, DC, 2004.

[59]. Roucher, V. F., Desnots, E., Naël, C., Agnoux, A. M., M.-C., A-G., Darmaun, D., & Boquien, C.-Y. Use of UPLC-ESI-MS/MS to quantitate free amino acid concentrations in micro-samples of mammalian milk. *SpringerPlus*, 2(1): 622, 2013.

[60]. Sabliov, C. M., Boldor, D., Coronel, P., & Sanders, T. H. Continuous microwave processing of peanut beverages. *Journal of Food Processing and Preservation*, 32(6): 935-945, 2008.

[61]. Saeeduddin, M., Abid, M., Jabbar, S., Wu, T., Hashim, M. M., Awad, F. N., Hu, B., Lei, S., & Zeng, X. Quality assessment of pear juice under ultrasound and commercial pasteurization processing conditions. *LWT-Food Science and Technology*, 64(1): 452-458, 2015.

[62]. Saeeduddin, M., Abid, M., Jabbar, S., Wu, T., Yuan, Q., Riaz, A., Hu, B., Zhou, L., & Zeng, X. Nutritional, microbial and physicochemical changes in pear juice under

ultrasound and commercial pasteurization during storage. *Journal of Food Processing and Preservation*, 41(6): e13237, 2017.

[63]. Saikia, S., Mahnot, N. K., & Mahanta, C. L. Optimisation of phenolic extraction from *Averrhoa carambola* pomace by response surface methodology and its microencapsulation by spray and freeze drying. *Food Chemistry*, 171: 144-152, 2015.

[64]. Samani, B. H., Khoshtaghaza, M. H., Lorigooini, Z., Minaei, S., & Zareiforoush, H. Analysis of the combinative effect of ultrasound and microwave power on *Saccharomyces cerevisiae* in orange juice processing. *Innovative Food Science & Emerging Technologies*, 32: 110-115, 2015.

[65]. Simsek, M., Sumnu, G., & Sahin, S. Microwave assisted extraction of phenolic compounds from sour cherry pomace. *Separation Science and Technology*, 47(8): 1248-1254, 2012.

[66]. Singleton, V. L., & Rossi, J. A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16(3): 144-158, 1965.

[67]. Soobrattee, M. A., Neergheen, V. S., Luximon-Ramma, A., Aruoma, O. I., & Bahorun, T. Phenolics as potential antioxidant therapeutic agents: mechanism and actions. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis*, 579(1-2): 200-213, 2005.

[68]. Sun, D.-W. *Thermal Food Processing: New Technologies and Quality Issues*: CRC Press, 2012.

[69]. Tang, J., Hong, Y.-K., Inanoglu, S., & Liu, F. Microwave pasteurization for ready-to-eat meals. *Current Opinion in Food Science*, 23: 133-141, 2018.

[70]. Tiwari, B. K., Muthukumarappan, K., O'Donnell, C. P., & Cullen, P. J. Effects of sonication on the kinetics of orange juice quality parameters. *Journal of Agricultural & Food Chemistry*, 56(7): 2423-2428, 2008.

[71]. Tiwari, B. K., O'donnell, C. P., Patras, A., Brunton, N., & Cullen, P. J. Stability of anthocyanins and ascorbic acid in sonicated strawberry juice during storage. *European Food Research and Technology*, 228(5): 717-724, 2009.

[72]. Tiwari, B. K., Patras, A., Brunton, N., Cullen, P. J., & O'donnell, C. P. Effect of ultrasound processing on anthocyanins and color of red grape juice. *Ultrasonics Sonochemistry*, 17(3): 598-604, 2010.

[73]. Tomadoni, B., Cassani, L., Viacava, G., Moreira, M. D. R., & Ponce, A. Effect of ultrasound and storage time on quality attributes of strawberry juice. *Journal of Food Process Engineering*, 40(5): e12533, 2017.

[74]. Ugarte-Romero, E., Feng, H., Martin, S. E., Cadwallader, K. R., & Robinson, S. J. Inactivation of *Escherichia coli* with power ultrasound in apple cider. *Journal of Food Science*, 71(2): E102-E108, 2006.

[75]. Valls, F., Sancho, M. T., Fernández-Muiño, M. A., & Checa, M. A. Determination of vitamin B6 in cooked sausages. *Journal of Agricultural & Food Chemistry*, 49(1): 38-41, 2001.

[76]. Vichare, N. P., Gogate, P. R., Dindore, V. Y., & Pandit, A. B. Mixing time analysis of a sonochemical reactor. *Ultrasonics Sonochemistry*, 8(1): 23-33, 2001.

[77]. Wu, G. Functional amino acids in growth, reproduction, and health. *Advances in Nutrition*, 1(1): 31-37, 2010.

[78]. Wu, J., Gamage, T. V., Vilkhu, K. S., Simons, L. K., & Mawson, R. Effect of thermosonication on quality improvement of tomato juice. *Innovative Food Science & Emerging Technologies*, 9(2): 186-195, 2008.

[79]. Yildiz, G. Application of ultrasound and high-pressure homogenization against high temperature-short time in peach juice. *Journal of Food Process Engineering*, 42(3): e12997, 2019.

[80]. Yue, J., Xu, J., Cao, J., Zhang, X., & Zhao, Y. Cucurbitane triterpenoids from *Momordica charantia L*. and their inhibitory activity against α-glucosidase, α-amylase and protein tyrosine phosphatase 1B (PTP1B). *Journal of Functional Foods*, 37: 624-631, 2017.