

Design consideration and modelling studies of ultrasound and ultraviolet combined approach for shelf-life enhancement of pine apple juice

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ABSTRACT

Although both ultraviolet (UV) radiation and ultrasound (US) treatment have their capabilities in microbial inactivation, applying any one method alone may require a high dose for complete inactivation, which may affect the sensory and nutritional properties of pineapple juice. Hence, this study was intended to analyse and optimise the effect of combined US and UV treatments on microbial inactivation without affecting the selected quality parameters of pineapple juice. US treatment (33 kHz) was done at three different time intervals, viz. 10 min, 20 min and 30 min., after which, juice samples were subjected to UV treatment for 10 min at three UV dosage levels, viz. 1 J/cm², 1.3 J/cm², and 1.6 J/cm². The samples were evaluated for total colour difference, pH, total soluble solids (TSS), titrable acidity (TA), and ascorbic acid content; total bacterial count and total yeast count; and the standardization of process parameters was done using Response Surface Methodology and Artificial Neural Network. The results showed that the individual, as well as combined treatments, did not significantly impact the physicochemical properties while retaining the quality characteristics. It was observed that combined treatment resulted in 5 log cycle reduction in bacterial and yeast populations while the individual treatment failed. From the optimization studies, it was found that combined US and UV treatments with 22.95 min and 1.577 J/cm² ensured a microbiologically safe product while retaining organoleptic quality close to that of fresh juice.

1. Introduction

Pineapple (*Ananas comosus*) is an important fruit having immense commercial value and is liked by most people regardless of their age due to its exceptional juiciness, health benefits and vibrant tropical flavour. Pineapple stands third in the overall production of tropical fruits after banana and mango [11]. According to FAO [21], in 2020 the production of pineapple is around 27.82 million metric tons globally. Pineapple is a highly potential fruit that contains many nutrients. It is an abundant

source of vitamin A, C and organic acids [16]. It contains carbohydrates, water, crude fibre, protein, and other micronutrients such as calcium, potassium, and manganese, essential for maintaining balanced nutrition. Pineapple can be recommended to people suffering from certain disorders as a medical diet [26]. Thermal treatment affects the sensory properties like texture, flavour, and colour, as well as nutritional quality adversely [44,43]. Pasteurization is reported [27] to cause 94% reduction in Vitamin C content, 50% reduction in calcium and potassium content, nonenzymatic browning, pigment destruction, and formation of

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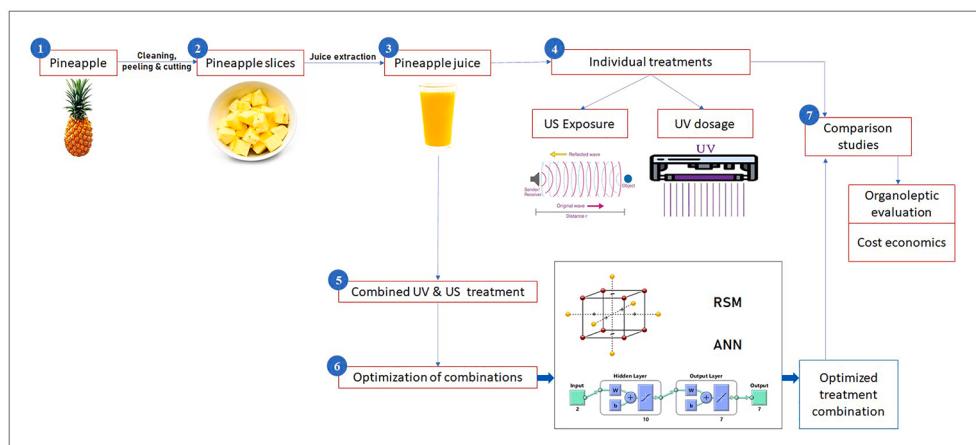


Fig. 1. Process flow diagram for combined UV and US treatment for shelf-life enhancement of pineapple juice.

hydroxy methyl furfural. Nowadays, consumers are highly conscious of the nutritional qualities of processed foods. For that reason, there is an enhanced interest in non-thermal techniques having a limited effect on the organoleptic quality of fresh juices. The application non thermal techniques like high pressure processing, pulsed electric field, ultraviolet processing, ultrasonication etc. for processing beverages is increasing in an impressive rate, owing to the retention of fresh-like qualities, while ensuring microbial inactivation [39,25] (Fig 1).

Ultraviolet (UV) radiation is a tool capable of destroying pathogens in food, without causing any thermally induced changes. UV radiation can be classified into three types according to their wavelength, viz. UV-A, B and C have wavelengths of 315 to 400 nm, 280–315 nm, and 200–280 nm respectively. Among these three types, UV – C radiation possesses a significant germicidal effect at 254 nm, which modifies the genetic makeup of the microorganisms making them dormant and unable to reproduce [49]. Even though UV radiation has considerable potential for surface disinfection of fresh produce, drinking water, and liquid foods, the limitation is its low penetration depth due to the shadow effect as suggested by Shen & Singh [50]. UV- C technology is not only cheaper but also safer as it does not generate any hazardous chemicals [35]. UV – C radiation is a technique having great potential in the destruction of bacteria and spores in different fruit juices [42]. UV-C radiation acts by causing light induced chemical reactions in the DNA of the microorganisms, leading to their destruction [51].

Also, UV-C radiation can suppress oxidative enzymes like polyphenol oxidases and peroxide reductases, thereby preventing the browning reaction [14]. Ultrasound (US) treatment is another non-thermal technology widely applied in food processing for preservation, extraction, homogenisation, filtration, degassing, non-destructive testing, cutting, emulsification, crystallisation, and other operations [12,10]. Ultrasound refers to sound waves having frequencies beyond the audible range of human beings, i.e. greater than 20 kHz. High-intensity ultrasound having frequencies between 20 and 100 kHz is commonly used in food preservation. In this technique, the inactivation of microorganism takes place by way of cavitation, which includes the formation, growth, and eventual collapse of bubbles during the propagation of US waves through the product [54]. Cavitation destroys the cells, releases free radicals, creates shock waves, and inactivates the microbes and denatures the enzymes while retaining the nutritional and sensory quality of foods. The effect of ultrasonication is affected by the type of microbes present in the food, with Gram-positive bacteria and spores showing increased resistance due to the presence of cell walls. Hence, to ensure a safe product US treatment need to be done for a longer time [47].

Novel technologies like pulsed electric field, pulsed light, ultrasonication, ultraviolet, high-pressure processing, etc. along with their combinations are widely used today for the efficient preservation of food

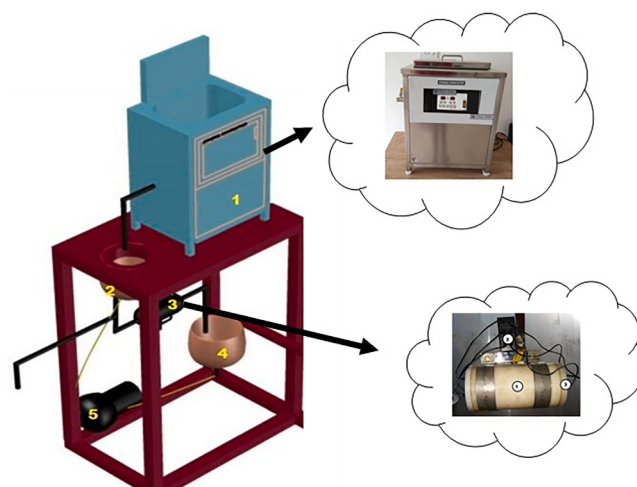


Fig. 2. Design of Combined Ultrasound with UV 1) US bath with chiller 2) Intermediate storage tank 3) UV treatment system 4) Recirculation tank 5) Motor.

products [29]. Although both UV radiation and US treatment have their capabilities in microbial inactivation, applying any one method alone may require a high dose for complete inactivation, which may affect the sensory and nutritional properties of pineapple juice. The synergistic effect of US and UV treatment of pineapple juice has not been reported anywhere in the literature as a pasteurization method. Therefore, developing a combined ultrasound and ultraviolet radiation treatment system as consecutive hurdles for the treatment of pineapple juice could result in a hybrid effect in which microbial destruction is achieved by a synergic mechanism of each technology. Such a process could produce pineapple juice with increased shelf life while retaining freshness like nutritional and organoleptic characteristics and ensuring safety, which can attract remunerative prices in the market. Hence, this study is intended to analyze and optimize the effect of combined ultrasound and ultraviolet radiation treatments on microbial inactivation without affecting the selected quality parameters of pineapple juice.

2. Materials and methods

2.1. Materials and reagents

Fresh sound pineapples of the 'kew' variety were collected from the local market, Tavanur, Kerala, India, after visual inspection. Fruits were washed in running water. The crown and outer skin of fruits were

removed by using a sterilised knife. After peeling, fruit slices were cored and then chopped. The juice was extracted in a blender with juice extraction attachment (Wonderchef Nutri-Blend, India) and collected in a sterile stainless-steel vessel.

The extracted juice was filtered using a muslin cloth into sterile Polyethylene Terephthalate (PET) bottles and maintained in refrigerated condition ($4 \pm 2^\circ\text{C}$) for conducting experiments. All chemicals and reagents used were of analytical grade.

2.2. Design of experimental setup (a combined ultrasound and ultraviolet treatment system)

The experimental setup was conceptualized and fabricated based on a thorough review of literature on ultrasound and UV systems for pumpable fluids. The Schematic setup of the unit is shown in Fig. 2. It consists of the following components: 1. Ultrasonic bath with cooling mechanism, 2. an intermediate storage tank, 3. a UV treatment system, and 4. a circulation system. The ultrasound production system comprises a generator, transducer and the application system. The generator generates electrical or mechanical energy and the transducer transform this energy into ultrasound of suitable frequencies. Ultrasound treatment was carried out in an ultrasound bath with the cooling mechanism (capable of maintaining temperature between 10 and 30°C) operating at a frequency of 33 kHz and output power of 250 W. The ultrasound bath was made up of stainless steel (AISI 304). The length, width and height of the bath are 445 mm, 420 mm and 545 mm respectively with a holding capacity of 10 L. The sonicator consisted of a metal oxide silicon transistor-based switched-mode power supply generator and five Lead Zirconate Titanate (PZT) transducers. High-frequency electrical energy is converted into ultrasound waves of the required frequency by using piezoelectric sandwich-type transducers attached to the base of the stainless steel tank. For understanding the behaviour of bubbles during ultrasound treatment, the following equations were used.

Rayleigh-Plesset equation as given by Gogate and Pandit [23] was used to study the bubble dynamics.

$$R \left(\frac{d^2 R}{dt^2} \right) + \frac{3}{2} \left(\frac{dR}{dt} \right)^2 = \frac{1}{\rho} \left[p_i - \frac{4\mu}{R} \left(\frac{dR}{dt} \right) - \frac{2\sigma}{R} - p_\infty \right] \quad (1)$$

Where R is the radius (m) of the bubble wall at time t (s), ρ_l is the density of the liquid (kg/m^3), p_i and p_∞ are the pressures (N/m^2) inside the bubble at time t and of the surrounding liquid respectively, μ is the viscosity (Ns/m^2) of surrounding liquid and σ is the surface tension (N/m) of bubble-liquid interphase.

The maximum cavitation is observed when the radius of the bubbles is closer to the linear resonance radius (R_r) as given below [19]:

$$R_r = \frac{1}{2\pi f} \sqrt{\frac{2\gamma P}{\rho}} \quad (2)$$

Where, f is the ultrasonic frequency (Hz), γ is the heat capacity ratio of the gas, and P and ρ are the pressure (N/m^2) and density (kg/m^3) of the liquid respectively.

The amplitude of ultrasound pressure (MPa) can be expressed as [55]:

$$P_A = \sqrt{2I\rho C} \quad (3)$$

Where I is the ultrasound intensity (W/m^2), ρ is the density of the medium (kg/m^3) and C is the velocity of sound in that medium (m/s).

The collapse pressure of the bubbles during cavitation was considered according to the equation given by Gogate and Pandit [23].

$$P_c = 114(R_0)^{-1.88}(I)^{-0.17}(f)^{0.11} \quad (4)$$

Where, R_0 is the initial cavity size (cm), I is the intensity (W/cm^2) and f is the frequency (Hz).

The intermediate storage tank made of stainless steel (AISI 304) is

meant to store the pineapple juice after US treatment and supply the treated juice for further subjecting to UV radiation. The cylindrical tank having an inner diameter of 200 mm, depth of 180 mm and wall thickness 0.6 mm was designed to hold 5 L at a time, using the formula for the volume of cylinder (V).

$$V = \frac{\pi}{4} d^2 h \quad (5)$$

Where, d and h are the diameter and height of the tank respectively.

The ultraviolet treatment system consists of three low pressure mercury lamps of 3 W output power and wavelength 254 nm, a quartz tube and nylon treatment chamber. The treatment chamber was drilled out from a cylindrical nylon block so that it has 130 mm and 100 mm outer and inner diameter respectively. The length of the chamber is 230 mm and its wall thickness 15 mm. The cylindrical chamber accommodates the quartz tube and UV lamps. Both the ends of cylindrical treatment chamber were closed with nylon end caps of 40 mm thickness and 130 mm diameter. At the centre of both end caps, 14 mm holes were drilled which in turn supports the quartz tube.

The Bunsen-Roscoe reciprocity law for photochemical processes stated that the intensity of a photochemical reaction, such as the impact of UV-C radiation on nucleic acid of micro-organism is directly proportional to the total radiant energy dose that reaches the object. The dosage was calculated by using the formula suggested by Pedrós-Garrido et al. [45].

$$D = I \times t \quad (6)$$

Where, I is the intensity of UV radiation and t is the treatment time.

The ultraviolet dosage required is heavily dependent on the absorbance of UV light by the food product, which can be expressed using Beer-Lambert Law as [30]:

$$A = \sum_{i=1}^n (\epsilon_i \times c_i \times d) \quad (7)$$

Where, ϵ is the molar absorptivity, c is the concentration, d is the distance travelled by the UV light and n is the number of different constituents having varying absorption within the liquid food sample. The penetration depth (λ) is the distance at which the initial irradiance is reduced by 90%, and may be expressed as:

$$\lambda = \frac{1}{\alpha} \quad (8)$$

Where, the absorption coefficient α can be defined as:

$$\alpha = 2.303 \frac{A}{d} \quad (9)$$

Where, A is the absorbance as calculated in Eqn 6, and d is the distance travelled by the UV light.

Most of the studies make use of transmittance (T) of the UV light in the food products, which can be described as:

$$T = \frac{I_1}{I_0} \quad (10)$$

Where, I_1 and I_0 are the irradiances of transmitted and incident light respectively.

Circulation system consists of a 5 L stainless steel recirculation tank with diameter and depth of 200 mm and 180 mm respectively, a 24 V DC pump and associated connecting pipes and valves. This system circulates the pineapple juice through the UV- treatment chamber until it subjected to the required dosage for the required time as per the experimental design.

2.3. Experimental procedure

The filtered fresh pineapple juice was fed to an ultrasound bath with a chiller at a temperature adjusted to 20°C to maintain the quality of

Table 1

Experimental Design for optimization of process parameters using Central composite design of response surface methodology.

Run	Independent variables		Dependent variables						
	US Exposure time (min)	UV dosage (J/cm ²)	Total colour change	pH	Total Soluble Solids (°Brix)	Titration Acidity (% citric acid)	Vitamin C (mg of ascorbic acid /100 g of sample)	Bacterial log reduction	Yeast log reduction
1	20	1.3	0.325	3.82	11.68	0.3328	45.6	5.09	4.89
2	20	1.3	0.495	3.82	11.68	0.3332	45.6	5.31	5.08
3	20	1.724	0.362	3.83	11.69	0.3333	45.3	5.13	4.94
4	30	1.6	0.547	3.83	11.69	0.3325	45.6	5.31	5.08
5	20	1.3	0.367	3.81	11.69	0.3334	45.3	5.13	4.94
6	10	1.0	0.365	3.80	11.67	0.3326	45.7	4.52	4.52
7	5.8	1.3	0.378	3.80	11.68	0.3324	45.5	4.02	4.41
8	20	0.876	0.364	3.80	11.67	0.3333	45.3	5.13	4.94
9	34.14	1.3	0.768	3.83	11.68	0.3324	45.0	5.33	5.10
10	20	1.3	0.367	3.80	11.69	0.3333	45.3	5.13	4.94
11	30	1.0	0.642	3.81	11.67	0.3335	44.6	5.38	5.18
12	10	1.6	0.586	3.84	11.69	0.3333	44.9	5.03	4.81
13	20	1.3	0.369	3.81	11.68	0.3331	45.3	5.13	4.94

juice upon US treatment. The studies were done at three different time intervals, viz. 10 min, 20 min and 30 min. After ultrasound processing, juice samples were collected in the intermediate storage tank and subsequently subjected to ultraviolet treatment for 10 min at three UV dosage levels, viz. 1 J/cm², 1.3 J/cm², and 1.6 J/cm².

Individual effects of US and UV on the juice samples were also investigated for comparison studies. Further decisions on dosage levels and their effect on the quality attributes for efficient treatment of juice samples were also investigated. The processed samples were stored in amber coloured PET bottles and kept in refrigerated conditions (4 ± 2 °C) for further study.

2.4. Physicochemical characteristics of treated pineapple juice

The physicochemical characteristics of treated samples such as total colour difference, pH, total soluble solids (TSS), titration acidity (TA), and ascorbic acid content were evaluated. Hunter Lab colourimeter (Hunter-Lab's Colour Flex EZ, USA) was used to quantify the total colour difference (ΔE) of fresh and treated samples. The colour of samples was measured based on three colour coordinates, namely L*, a*, and b*, where L (lightness), a (red/green), and b (yellow/blue) values were used to estimate the total colour difference (ΔE) using equation (11).

$$\Delta E = \sqrt{(\Delta L^2 + \Delta a^2 + \Delta b^2)} \quad (11)$$

The pH value of fruit juices depicts its characteristic flavour and influences consumer acceptance. A digital pH meter (MK VI, SYSTRONICS; Ahmedabad, India) was used to determine the pH of the juice samples. The total soluble solids (TSS) of juice indicate the presence of various soluble chemical substances in their insoluble form. TSS is an indication of sugars present in the juice samples. TSS of treated juice samples were measured using a digital refractometer (Erma, Italy). Titration acidity was calculated by using the standard titration method [9]. The juice sample (5 ml) was diluted in a standard flask up to 100 ml with distilled water, of which 10 ml was pipetted out into a conical flask. The sample was titrated with phenolphthalein indicator against 0.1 N

NaOH until the endpoint was reached. The percentage of titration acidity was calculated using equation 12.

$$\text{Titration acidity (\%)} = \frac{\text{Titre value (ml)} \times \text{Normality of alkali}}{\text{M}_{\text{eq}} \times \text{Weight of citric acid weight of sample}} \quad (12)$$

Ascorbic acid content was calculated by using the titrimetric method using 2, 6-dichlorophenol indophenol dye as described by Chakraborty et al [17]. The dye factor and ascorbic acid contents were calculated by using the formula.

$$\text{Dye factor} = \frac{0.5}{\text{Titre value}(V_1)} \quad (13)$$

Ascorbic acid (mg of ascorbic acid/100 ml of sample)

$$= \frac{0.5 \text{ mg}}{V_1} \times \frac{V_2}{5 \text{ ml}} \times \frac{100 \text{ ml}}{\text{Weight of the sample}} \times 100 \quad (14)$$

Where, V₂ is the volume of extract taken for estimation.

2.5. Microbial characteristics of treated pineapple juice

The microbial analyses of fresh as well as treated samples, both individual and combined treatments, were carried out by the standard plate count method. Total bacterial count and total yeast count of the juice samples were analyzed as the average number of colonies per ml of juice [41] was estimated using equation (15).

$$\text{Plate count (cfu/ml)} = \frac{\text{Average number of colonies from duplicate plate}}{\text{Dilution factor} \times \text{Volume plated}} \quad (15)$$

2.6. Statistical analysis

The standardization of process parameters for combined treatments of pineapple juice using ultrasound and UV dosages was carried out using Response Surface Methodology employing Stat-ease Design Expert (Version 7.0) software. The design of artificial neural network was

Table 2

Optimized combination and desirability analysis.

Number	US Exposure time (min)	UV dosage (J/cm ²)	Total colour change	pH	Total Soluble Solids (°Brix)	Titration Acidity (% citric acid)	Vitamin C (mg of ascorbic acid /100 g of sample)	Bacterial log reduction	Yeast log reduction	Desirability
Optimum solution	22.95	1.577	0.421	3.827	11.69	0.333	45.45	5.284	5.019	0.832
Tested result	22.95	1.577	0.391	3.83	11.68	0.331	44.61	5.02	5.11	

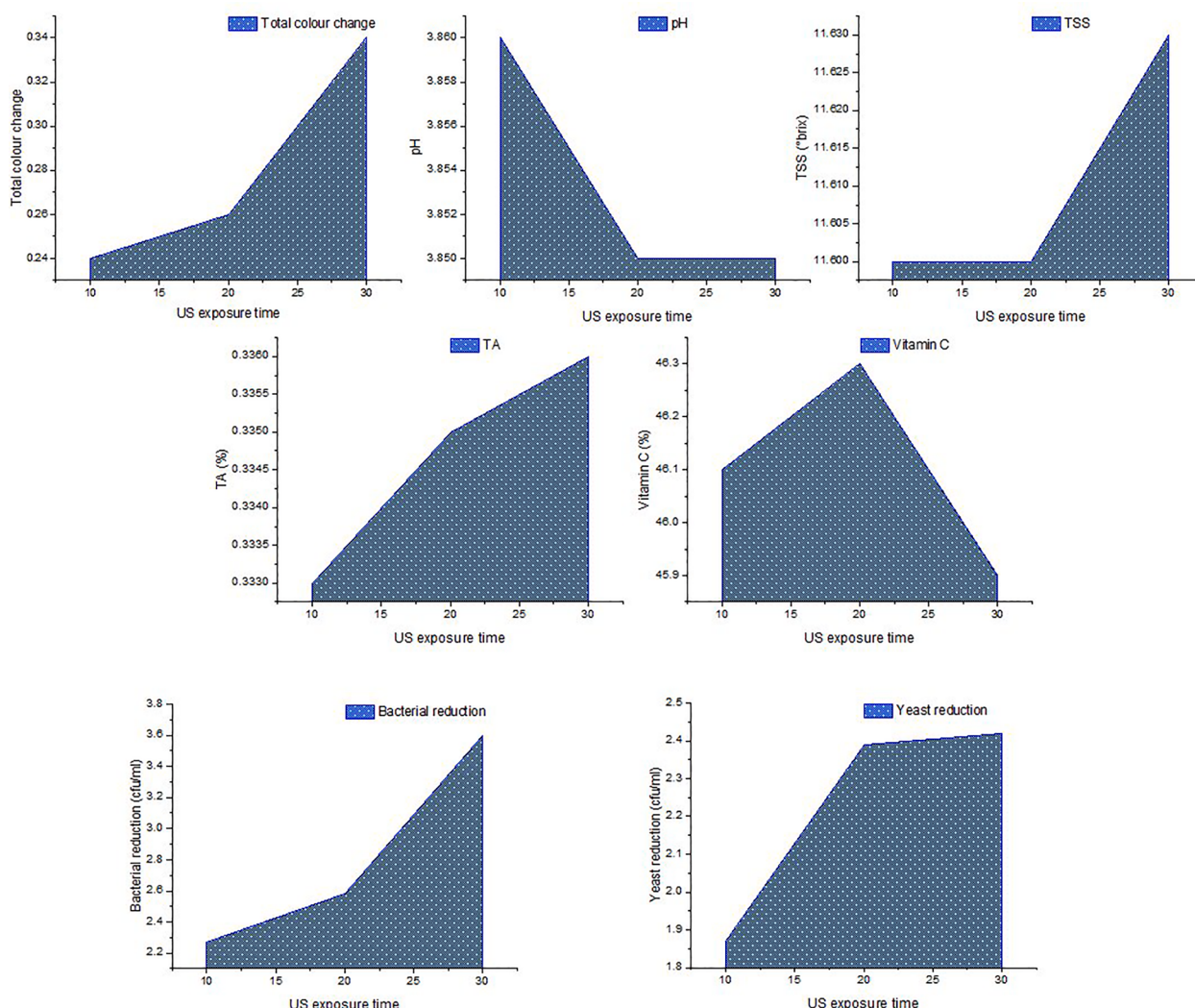


Fig. 3. Effects of US exposure time on physical (a) and microbial (b) characteristics of pineapple juice.

accomplished using “start” toolbox in MATLAB online R2020b.

2.6.1. Central composite design

Central Composite Design (CCD) method of Response Surface Methodology (RSM) was used for the optimization process and was analyzed using Stat-ease Design Expert (Version 7.0) software. The independent parameters chosen for the treatment of pineapple juice were US exposure time, ranging between 10 and 30 min, and UV dosage levels, ranging between 1 and 1.6 J/cm². The US exposure time and UV dosage levels were selected for individual and combined treatments based on a thorough literature review and preliminary studies conducted in the lab. The dependent parameters chosen for the analysis included total colour change, pH, TSS, Titratable acidity (TA), Vitamin C content, Bacterial log reduction and Yeast log reduction, which are significant parameters that determine the quality of treated pineapple juice. The experimental design observed is shown in Table 1. The effects were analysed to minimise colour change, maximise bacterial log reduction and yeast log reduction, and keep pH, TSS and TA in an acceptable range. 13 sets of experiments were performed, and analysis of variance (ANOVA) was performed along with graphical interpretation. Multiple regression analyses were performed to correlate independent variables and fitted to models using a second-order quadratic polynomial. The significance of the obtained model was determined at a magnitude of $p < 0.05$. The optimised combination was analysed for desirability of the experiments.

2.6.2. Artificial neural network

An artificial neural network is an advanced computational tool having excellent prediction abilities, influenced by the neurological systems of living things [28]. In the same way the brain consists of neurons and synapses, ANN is made up of nodes and connections. Without any prior understanding of the nature or relationships among factors, an ANN can discern patterns from the arrangement of input–output criteria. That is, one of the most significant benefits of ANN is that a prior awareness of the impact of variables under investigation is not necessary as the prediction is done based on the trained data [38]. For the present experiment, the ANN model comprised two input neurons (UV dose levels and US exposure time), ten hidden neurons, and seven output neurons (total colour change, pH, TSS, TA percent, Vitamin C, Total Plate Count, and Total Yeast Count). The number of hidden neurons was altered in the range of 2–10, and the ideal number was determined using a trial-and-error approach relying on statistical measures such as R^2 and MSE obtained during training, validation, and testing [34]. The Levenberg-Marquardt back propagation method was used for training computation. The experimental data consisting of 13 runs were replicated three times to obtain 39 entries, which were divided as 60% training, 20% validation and 20% testing. The entire modeling was done using MATLAB online R2020b.

A Feed forward network allows the flow from the input layer to the output layer through the hidden layer. The architecture of the network is shown in Fig. 6. The signal flows from the Input layer to the hidden

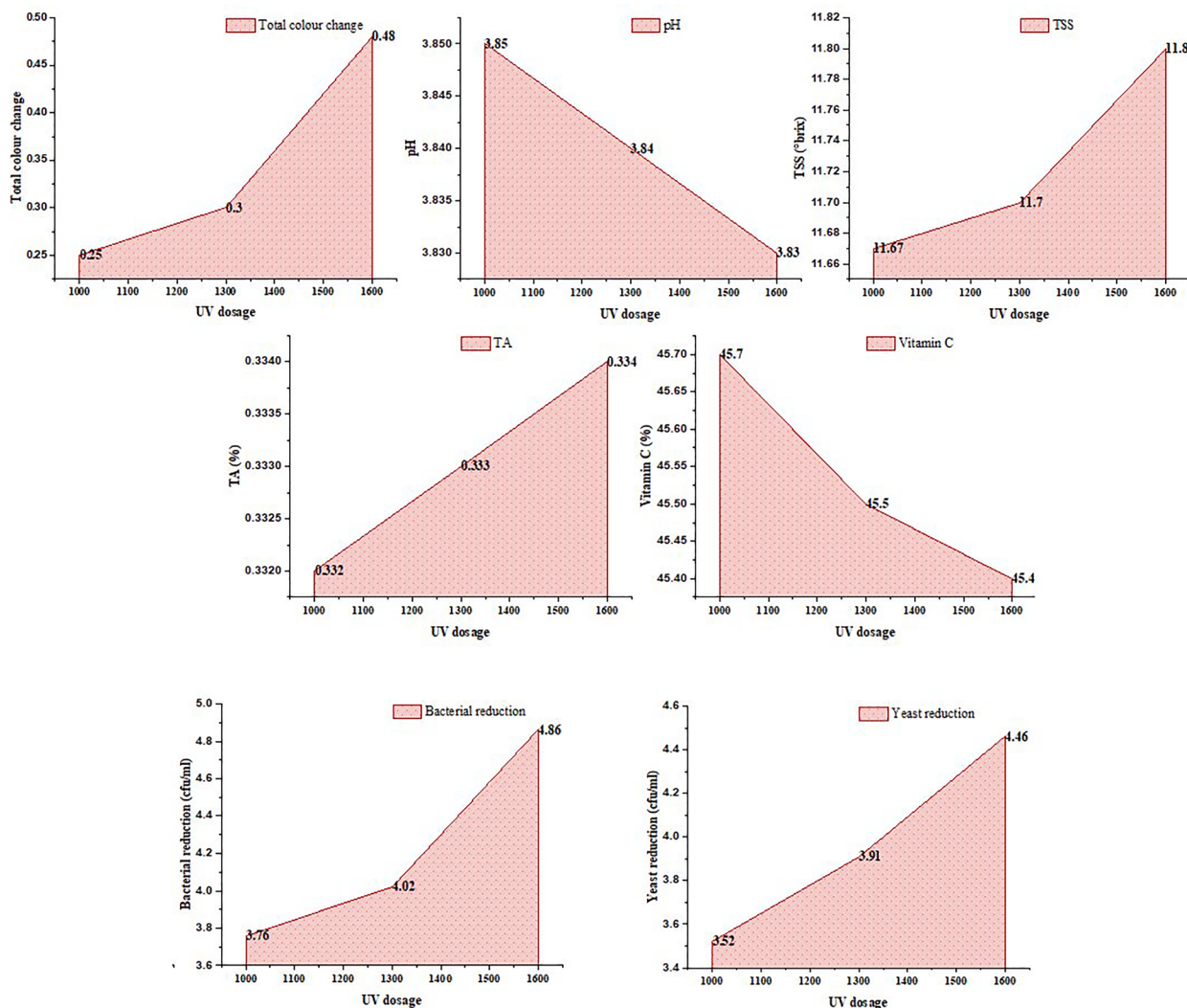


Fig. 4. Effects of UV dosage on physical (a) and microbial (b) characteristics of pineapple juice.

layer, and then to the output layer based on the below equations.

Purelin function was selected for the output layer as defined by below equation.

$$y = mx + c \quad (16)$$

signal flows from input factor to neurons as input function defined by below equation.

$$H_j = \sum_{i=1}^3 w_{ij} H_i + b_j \text{ where } j = 1 \text{ to } 3 \quad (17)$$

Tangent sigmoid (*tansig*) activation function was selected for the hidden layer.

$$y = \text{tansig}(x) = \left(\frac{2}{1 + e^{-2H_j}} \right) \quad (18)$$

signal moves from hidden layer to output layer as output function defined by below equations.

$$Y = \left(\frac{2}{1 + e^{-2H_j}} - 1 \right) w_j + b_2 \quad (19)$$

w_{ij} represents the weight from the i th input factor of the input layer to the j th neuron of the hidden layer. b_{1j} represents bias to the j th neuron of the hidden layer, w_j represents the weight from the j th neuron of the hidden layer to the output layer. b_2 represents bias to the output

layer.

2.7. Organoleptic characteristics of treated pineapple juice

After optimising and standardising parameters, the selected samples of combination treatments, individual treatments, and control were organoleptically evaluated by a panel of judges. Colour, flavour, taste and overall acceptance of samples were evaluated. The assessment was conducted by using 9 points hedonic scale of as per IS 6272: 1991. The sensory parameters of fresh and thermally pasteurised pineapple juice were evaluated. For thermal pasteurisation, pineapple juice was heated at 80 °C for 15 min and cooled to ambient temperature [31].

2.8. Cost economics

The total cost of production of combined treated pineapple juice was calculated using the procedure reported by Srikanth et al. [52] according to our specifications.

Table 3

Equations obtained by response surface methodology.

Parameter	Equation	R ²	MSE
Total colour change	$\Delta E = 0.38 + 0.099A + 0.015B - 0.079AB + 0.11A^2 + (5.950 \times 10^{-3})B^2$	0.827	0.004
pH	$pH = 3.81 + (5.303 \times 10^{-3})A + 0.013B - (5.000 \times 10^{-3})AB + (2.750 \times 10^{-3})A^2 + (2.750 \times 10^{-3})B^2$	0.744	7×10^{-5}
TSS	$TSS = 11.68 + 0.000A + (8.536 \times 10^{-3})B + 0.000AB - (2.000 \times 10^{-3})A^2 - (2.000 \times 10^{-3})B^2$	0.822	3×10^{-5}
Titration acidity	$TA = 0.33 + (1.250 \times 10^{-5})A - (3.750 \times 10^{-5})B - (4.250 \times 10^{-4})AB - (3.488 \times 10^{-4})A^2 + (1.013 \times 10^{-4})B^2$	0.871	5.3×10^{-8}
Vitamin C	$Vitamin\ C = 45.42 - 0.14A + 0.025B + 0.45AB - 0.10A^2 - 0.079B^2$	0.887	0.027
Total Plate Count	$TPC = 5.16 + 0.37A + 0.055B - 0.15AB - 0.20A^2 + 0.025B^2$	0.901	0.008
Total Yeast Count	$TYC = 4.96 + 0.24A + 0.024B - 0.098AB - 0.089A^2 + (3.500 \times 10^{-3})B^2$	0.948	0.005

3. Results & discussion

3.1. Effect of individual and combined ultrasound and ultraviolet treatments on pineapple juice

3.1.1. Colour

Colour is an essential visual indicator to determine juice quality and consumer acceptance of fruit juices [36]. The total colour difference in various samples was analysed and presented in Fig. 3a & 4a for US and UV respectively. The experimental results show that individual treatments did not cause any significant effect on colour change. During individual ultrasound treatments, the total colour difference varied between 0.24 and 0.34, implying that ultrasound treatment could not cause any noticeable colour changes in pineapple juice. Aadil et al. [1] who studied the ultrasonic treatment of grape juice, have also reported that the treatment-induced colour changes were not obvious to the naked eyes. It did not promote any non-enzymatic browning reaction or degradation of pigments [24]. Similarly, as shown in Fig. 4 (a), individual UV treatments also could not induce any discernible changes in colour values. Choi & Nielsen [18] observed that the UV pasteurisation

maintained superior colour of apple cider than thermal treatment. It could be hypothesised that UV treatment up to a dosage of 1.6 J/cm² could not have any harmful effects on conjugate bonds, disulphide bonds and could also avoid pigment photodegradation [8]. Therefore, individual UV treatments maintained the colour of juice samples. Prolonged UV treatment could accelerate pigment degradation and discolouration of fruit juices [40]. The total colour difference of combined treatments varied from 0.325 to 0.768. The combined effect of ultrasound and ultraviolet radiation resulted in an increased colour change compared to individual treatment, though the colour change was negligible and insignificant statistically. Therefore, the natural colour of the juice is maintained. Similar trend has been reported in case of mango juice and pomegranate juice [58,71].

3.1.2. pH

The pH of various samples was analysed and presented in Fig. 3(a) & 4(a). Fresh pineapple juice had a pH value of about 3.86 ± 0.0055 . The pH values of treated juices varied between 3.86 and 3.81. The results indicated a negligible effect on the pH of pineapple juice after performing both treatments individually. Combined US and UV treatments could not induce any significant changes in pH value ($p < 0.05$). It could be observed from Table 3 that combined treatment has a very subtle, negligible effect on the juice. The pH values were found to be stable even after different treatments (Tables 4 and 5).

Though some researchers have reported significant changes in the pH of US-treated fruit juices, which may be attributed to the generation of hydrogen peroxide or nitrate and nitrite in the aqueous medium, several other studies have noted no significant changes in pH [57,48]. Comparable results were reported by Adekunle et al. [5], who observed that ultrasound treatment of tomato juice could not induce any significant changes in pH of juice regardless of the strength and time of US treatment. Similarly, Bhat et al. [13] stated that ultraviolet treatment also could not produce any significant changes in the pH of starfruit juice compared to fresh samples.

3.1.3. Total soluble solids

The effects of individual treatments on TSS juice are represented in Fig. 3(a) & 4(a). The fresh pineapple juice had a TSS of 11.6 °Brix. No significant changes were observed in individual treatments. Individual

Table 4

F-value, P-value and significance of each variable on performance parameters of combined UV and US treatment on dependent parameters.

Source	Total colour change		pH		TSS		TA		Vitamin C		Bacterial reduction		Yeast reduction	
	F value	p-value	F value	p-value	F value	p-value	F value	p-value	F value	p-value	F value	p-value	F value	p-value
Model	6.692	0.0135	4.079	0.0470	6.452	0.0149	9.489	0.0051	11.044	0.0032	12.806	0.0021	25.602	0.0002
A-UV Exposure time	13.655	0.0077	2.653	0.1474	0	1.0000	0.034	0.8585	7.884	0.0262	46.820	0.0002	105.044	< 0.0001
B-UV dosage	0.332	0.5823	15.464	0.0057	29.746	0.0010	0.308	0.5962	0.257	0.6276	1.012	0.3479	1.044	0.3409
AB	4.375	0.0748	1.179	0.3135	0	1.0000	19.775	0.0030	41.679	0.0003	3.517	0.1029	8.798	0.0209
A ²	15.007	0.0061	0.620	0.4567	1.420	0.2722	23.158	0.0019	3.853	0.0904	11.886	0.0107	12.749	0.0091
B ²	0.043	0.8413	0.620	0.4567	1.420	0.2722	1.952	0.2051	2.220	0.1799	0.187	0.6782	0.019	0.8923

Table 5R² and Mean Square Error (MSE) values obtained from Artificial Neural Network.

	Training		Validation		Testing	
	R ²	MSE	R ²	MSE	R ²	MSE
Colour	0.918	0.0031	0.8738	0.0054	0.8285	0.0089
pH	0.8268	6.1709×10^{-5}	0.8277	9.5616×10^{-5}	0.8534	8.3361×10^{-5}
TSS	0.7794	3.4119×10^{-5}	0.7579	5.0643×10^{-5}	0.7547	3.8469×10^{-5}
TA	0.8752	3.4059×10^{-8}	0.8257	4.598×10^{-8}	0.8314	7.7263×10^{-8}
Vit-C	0.9261	0.0144	0.9158	0.0595	0.8758	0.0232
Bacterial reduction	0.9353	0.0165	0.9438	0.0086	0.9531	0.0333
Yeast reduction	0.952	0.0042	0.9606	0.0052	0.9652	0.0037

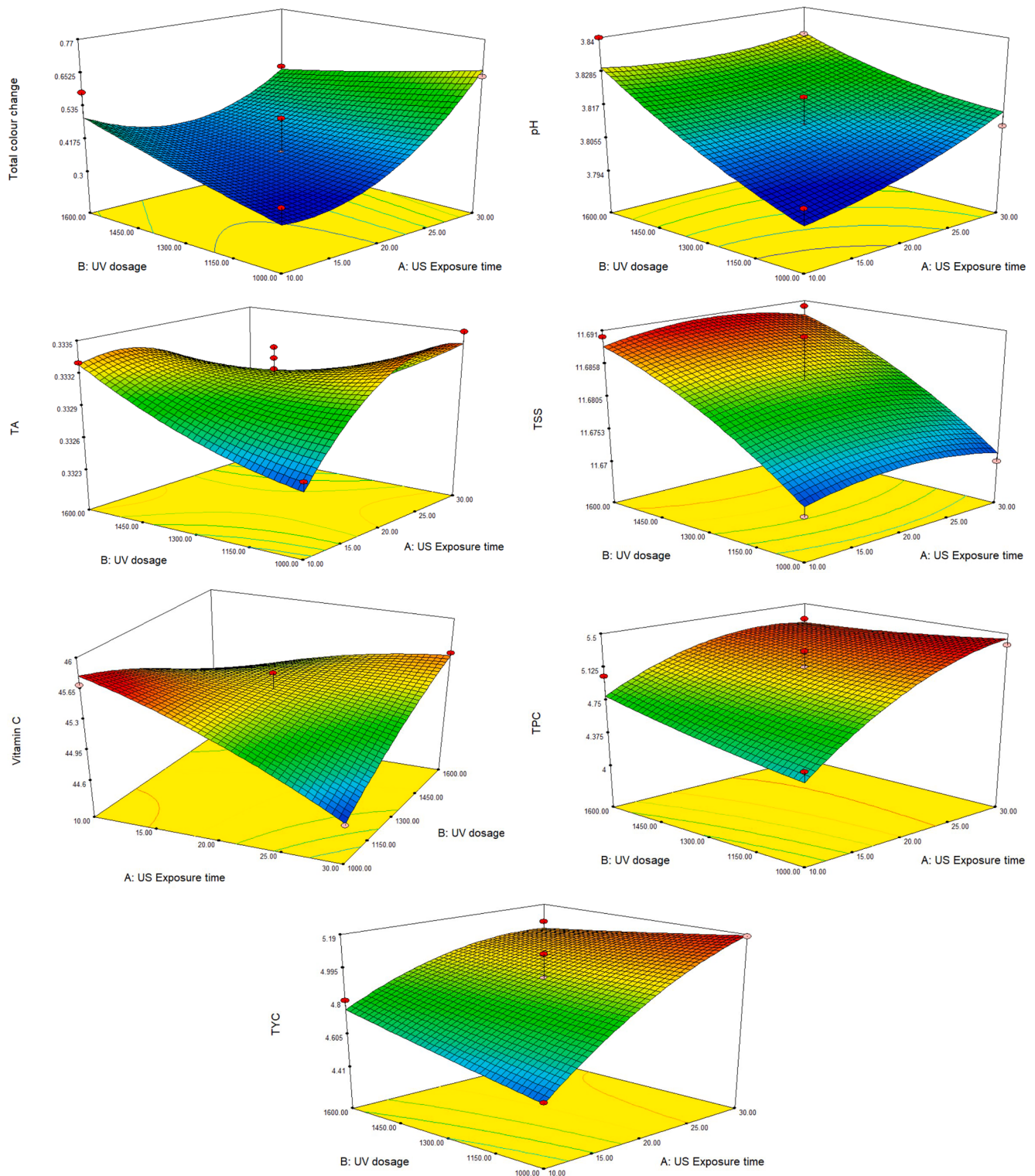


Fig. 5. Effect of process parameters on (a) Total colour change (b) pH (c) Titratable acidity (TA) (d) TSS (e) Vitamin C (f) TPC (g) TYC by Response Surface Methodology.

sonication changed TSS values from 11.6 to 11.63 °Brix. The results are in agreement with the observations reported by Tiwari et al. [56] during the sonication of orange juice using a 19 mm probe at a frequency of 20 kHz. It can be concluded that sonication was not sufficient enough to cause the breakdown of organic acids, chemical bonds and cell wall components for up to 30 min. Therefore, after treatment, the TSS of the samples do not show significant change [59]. UV treatment caused TSS

values to vary between 11.67 and 11.8 °Brix at different dosages. In combined treatments, TSS values were also found to increase from 11.63 to 11.87 °Brix with an increase in US treatment time and UV dosage though the changes were insignificant ($p < 0.05$). The equation obtained through regression analysis (Table 3) indicates that combined UV exposure and US treatment do not affect the TSS of pineapple juice. Aadil et al. [2] also reported similar findings during the combined

ultrasound and PEF treatment of fresh grape juice.

3.1.4. Titratable acidity

The titratable acidity of different samples is given in Fig. 3(a) and Fig. 4 (a). The fresh juice had titratable acidity of around $0.330 \pm 0.0003\%$ citric acid. The titratable acidity varied from 0.333 to 0.336% citric acid during individual US treatment and 0.332 to 0.334% citric acid during UV treatment. As per the experimental design, the acidity varied from 0.332 to 0.338% citric acid on combination treatments. Statistically, the variations were insignificant, and values were close to that of fresh pineapple juice, which is evident from the graph and the equation obtained through regression analysis. Similar observations were reported by Noci et al. [37] during the combined PEF and UV treatment of fresh apple juice, in which titratable acidity and pH showed no significant variation. Wang et al [58], opined that processing mango juice by US-UV combination treatment does not affect the titratable acidity significantly.

3.1.5. Vitamin-C

Ascorbic acid (Vitamin C) is a water-soluble vitamin present in fruit juices. It is an important nutritional quality indicator of fruit juices. Ascorbic acid consumption prevents cardiovascular and cancer diseases [3]. Vitamin C is very sensitive to heat and O_2 presence. The results of the experiments conducted to estimate ascorbic acid content during US and UV treatments are depicted in Fig. 3(a) & 4(a). During sonication, it may be observed that, ascorbic acid content increased from an initial value of 46.1 mg of ascorbic acid /100 g of the sample to 46.3 mg of ascorbic acid/100 g of sample when treatment time was increased from 10 min to 20 min. However, ascorbic acid content decreased to 45.9 mg of ascorbic acid/100 g of the sample when the treatment time was further increased to 30 min. Bhat et al. [13] also reported similar observations in sonication of Kasturi lime juice. During this process, entrapped oxygen in the juice gets expelled. The reduced oxygen content minimises the degradation reaction rate, which would be the reason for the increased ascorbic acid content per 100 g of juice sample, in comparison with the fresh juice. Sonication for more than 20 min could initiate an ascorbic acid content degradation reaction mainly attributed to the formation of hydrogen ions and free radicals due to sonolysis of water [4]. Though there is a decrease in ascorbic content after 20 min of sonication, it was found that the ascorbic acid content after 30 min sonication showed almost similar results as that of fresh juice. Individual UV treatments have reported an insignificant effect on ascorbic acid content.

Combination treatments using both ultrasound and UV exposure indicated an insignificant effect on ascorbic acid content in all treatment combinations of experimental design ($p < 0.05$). It may also be observed that all the treatment combinations preserve vitamin C content compared to conventional thermal pasteurisation wherein temperature increases to about 90°C would result in about 94% reduction in vitamin C [20]. Wang et al [58] reported an enhancement in ascorbic acid content in mango juice after US-UV treatment. However, the process parameters in that study was different like low processing temperature, lower dosage and longer preexposure to UV and treatment with lower frequency US as short pulses.

From the above results, it could be inferred that both individual and combination treatment could not cause an appreciable reduction in the physical quality characteristics of pineapple juice, and the treated juice maintained fresh like characteristics even at the highest US treatment time and UV dosage either at the individual as well as at combined treatment stages.

3.1.6. Bacteria and yeast reduction

The evaluation of bacterial load in individual US and UV treated samples were carried out as outlined in section 2.5, and the results are reported in Fig. 3(b) & 4(b). The bacterial and yeast population in fresh pineapple juice were also analyzed for comparison. When the pineapple juice was subjected to US alone, a bacterial $\log_{10}$ reduction of up to 3.6

± 0.051 was observed for 30 min treatment time. Though the bacterial reduction increased with an increase in treatment time, the treatment could not achieve a safe bacterial limit for human consumption. In the form of longitudinal waves, ultrasound passes through the material and induces cavitation. The bubbles' implosion creates shock waves that eventually cause the inactivation of microorganisms by the destruction of cell walls and cell membranes and DNA denaturation through sonolysis of water. Perdih et al. [46] reported that gram-positive bacteria are more resistant to ultrasonic waves than gram-negative bacteria. This is presumed due to cell wall thickness and peptidoglycan presence in the cell wall.

When pineapple juice samples were subjected to UV treatment alone, with an increase in UV dosage, the bacterial $\log_{10}$ reduction was found to be increased and reached a value of 4.86 ± 0.016 at a UV dosage of 1.6 J/cm^2 . Nevertheless, in these treatments also, the system could not reach a safe bacterial population. The microbial inactivation is mainly due to DNA denaturation. DNA absorbs UV lights, which leads to cross-linking between adjacent pyrimidine nucleotide bases such as thymine and cytosine in the same strand. This dimer formation inhibits the further replication and transcription process and inevitably leads to the death of cells. Bintsis et al. [15] reported that even though UV has bactericidal capabilities in liquid food media, it has low penetrating power, especially in turbid pumpable liquids.

It could be evident from the graph (Fig. 5) that the combined effect of ultrasound waves and UV exposure leads to increased bacterial load reduction, which could be ascribed to two levels of inactivation; ultrasound cavitation, leading to the destruction of cell membranes and structures, followed by UV exposure resulting in denaturation of DNA leading to cell death.

According to FDA specifications, a 5-log reduction in bacterial population in fruit juice could be considered safe for human consumption [22]. Lee et al. [33] reported that when apple juice was subjected to UV assisted ohmic heating at a wavelength of 254 nm, 65°C produced a high $\log_{10}$ reduction of *E. coli* (6.39 ± 1.30) compared to individual UV and ohmic heating treatments. It may be postulated that the synergic effect of combined US and UV treatment resulted in a higher level of inactivation than individual treatments.

It could be observed that the US treatment could only produce a yeast log reduction of 2.42 ± 0.036 , which is much less than the safe limit. It may be derived from the results that US treatment could only result in an insignificant reduction in yeast population when subjected to ultrasonication from 10 to 30 min at US frequency of 33 kHz. Yeast showed more resistance to ultrasound treatment than bacteria due to their higher cell wall thickness and composition [59]. UV treatment resulted in a significant yeast log reduction ($p < 0.05$) of up to 4.46 ± 0.036 , but could not achieve the safe yeast limit even at the highest experimental dosage of 1.6 J/cm^2 .

In order to counteract this reduction in microbial power, either the UV wattage has to be increased or the cross-sectional thickness of the flow has to be lowered to obtain a log reduction of acceptable safety. This would lead to increased power consumption or decreased throughput capacity, apart from compromising juice quality. Therefore, in order to achieve a safe microbial limit, a combination of US and UV was taken up for the study, which could lead to a reduction in bacterial load to a safe level, simultaneously reducing the intensity of treatment parameters preserving its quality at low energy and increasing throughput. When pineapple juice was subjected to combination treatment, it was revealed that a 5-log reduction could be achieved at a treatment combination of 10 min & 1.6 J/cm^2 itself. According to FDA specifications, a 5-log reduction in yeast population in liquid food could be considered safe for human consumption [6]. Combined treatment could reduce yeast load to a safer consumable limit.

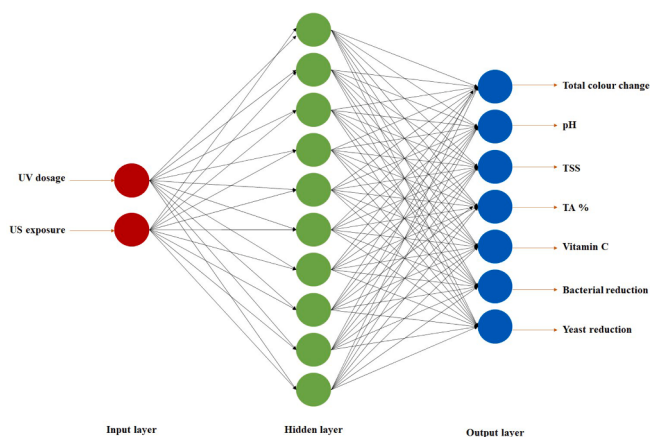


Fig. 6. Configuration of multilayer ANN model with two input neurons, ten hidden neurons and seven output neurons.

3.2. Feed forward back propagation neural network (FFBPNN)

A multilayer feed-forward neural network structure was designed for forecasting the effectiveness of the obtained model in terms of significant factors affecting the quality of pineapple juice, viz. Total colour change, pH, TSS, TA %, Vitamin C, TPC, and TYC. The efficacy of the model using an artificial neural network was also examined and the relationship between perceived and estimated values is expressed in Fig. 6 and Fig. 7. The obtained results indicated variation in R^2 value from 0.75 to 0.96, and Root Mean Square Error values between 3.456×10^{11} to 0.0595. Higher R^2 and minimum mean square error (MSE) values of ANN indicated higher accuracy and authenticity of the tested model. The R^2 values of tested microbial population-related parameters, i.e., total bacterial reduction and total yeast reduction, are above 0.9, indicating that the model is suitable for predicting the quality of treated pineapple juice. The R^2 values for physical characteristics were of the acceptable range.

According to the obtained results of these studies, the strength of the CCD and ANN models is highly dependent upon the considered application, the developed ANN model is stronger than CCD because significant parameters have been utilized to formulate the multiple linear regression equation for anticipating corresponding parameters.

3.3. Optimization and validation

Optimization and validation of the obtained models were performed using RSM and modelling was done using ANN. According to statistical analysis and optimization process using RSM, the best combination of process parameters is a US dosage of 22.95 min and a UV dosage of 1.577 J/cm^2 . The data obtained and validation results are shown in Table 2.

The obtained model showed a desirability level of 0.83, indicating the effectiveness of the model. The validation of the obtained combination was done, and the results of physical and microbial characteristics indicated closeness in value. From the observations made, it could be inferred that a combination of UV and US could reduce the microbial population to an acceptable limit. In the meantime, it does not alter the physical and nutritional characteristics of pineapple juice, which implies a positive effect of optimal treatment combination on the quality of juice. The results indicate that RSM and ANN are practical methodologies for the prediction of model and optimization process of combined UV dosage and US exposure time of pineapple juice. Similar reports on the effectiveness of ANN and RSM on optimization of parameters of novel technologies were reported by Srinivas et al. [53] and Lal et al. [32].

3.4. Organoleptic evaluation

Organoleptic evaluation is a research methodology that utilizes the senses such as sight, smell, taste, touch, and hearing to induce, quantify, analyse and comprehend responses to specific characteristics of food materials. It provides valid and reliable information about the product. The radar chart showing variations in scores is given in Fig. 8. It may be observed that the combination optimized by RSM had the sensory score and overall acceptability close to fresh juice. From the results, it could be confirmed that consumer acceptance of optimized treatment combinations was higher than in thermally pasteurized pineapple juice in terms of visual appearance, taste, flavour, and overall acceptance. Pineapple juice treated with an optimized treated combination using RSM yielded juices of quality comparable with that of fresh juice.

3.5. Cost economics

The production cost of pineapple juice employing the combined US and UV treatment under optimized treatment parameters of US treatment time and UV dosage was calculated based on standard procedure. It was found that the cost of production of one-litre pineapple juice was estimated to be Rs. 119.95, i.e., around 1.58 USD, the calculation of which is provided as Supplementary material. The cost of thermally processed pineapple juice available in the market is Rs. 175/litre, i.e., around 2.30 USD. Reduction in cost could imply the replacement of newly developed combined technology as a viable solution for replacing conventionally used thermal processing methods.

4. Conclusion

Generally, thermal treatments are used for the preservation of fruit juices. Non-thermal techniques can play a major role in fruit juice preservation in future. Even though UV radiation and US treatment has their own potential as a preservation method, the application of any single treatment would not be competent enough to kill all microorganisms. This study investigated the individual as well as combined effects of US and UV. The results showed that the individual, as well as combined treatments did not induce any significant impact on the physicochemical properties of pineapple juice samples, while retaining the quality characteristics. The changes in colour, pH, TSS, TA, and vitamin C content were not statistically significant. From the microbial analysis, it was concluded that combined treatment resulted in 5 log cycle reduction in bacterial and yeast populations whereas individual treatment failed to produce 5 log cycle reductions. From the optimization studies using RSM and ANN, it was found that combined US and UV treatments with 22.95 min ultrasound exposure and a UV dosage of 1.577 J/cm^2 ensured a microbiologically safe product while retaining fresh like qualities. Organoleptic evaluation of optimized samples, fresh juice and thermally treated samples revealed that combined US and UV treatments with obtained treatment combination retained all organoleptic qualities close to that of fresh juice. Therefore, combined US and UV treatments with 22.95 min ultrasound exposure and a UV dosage of 1.577 J/cm^2 were selected as the best treatment and therefore these process variables were selected as optimum parameters for the developed system.

Considering the quality parameters, organoleptic evaluation and cost analysis, it can be concluded that the combination of US and UV treatments is suitable for treating pineapple juice.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

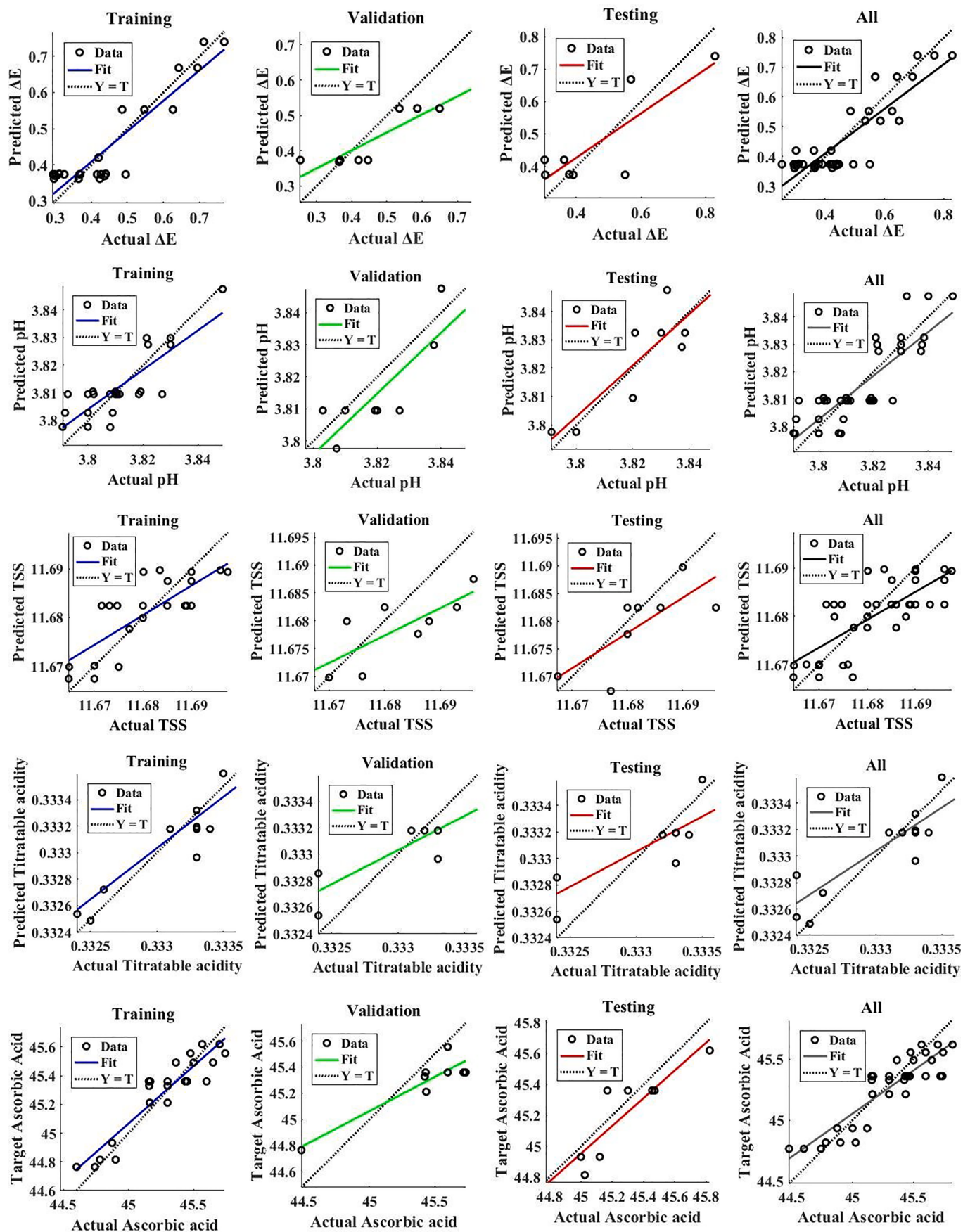


Fig. 7. Effect of process parameters on (a) Total colour change (b) pH (c) TSS (d) Titratable acidity (TA) (e) Vitamin C (f) TPC (g) TYC by Artificial Neural Network.

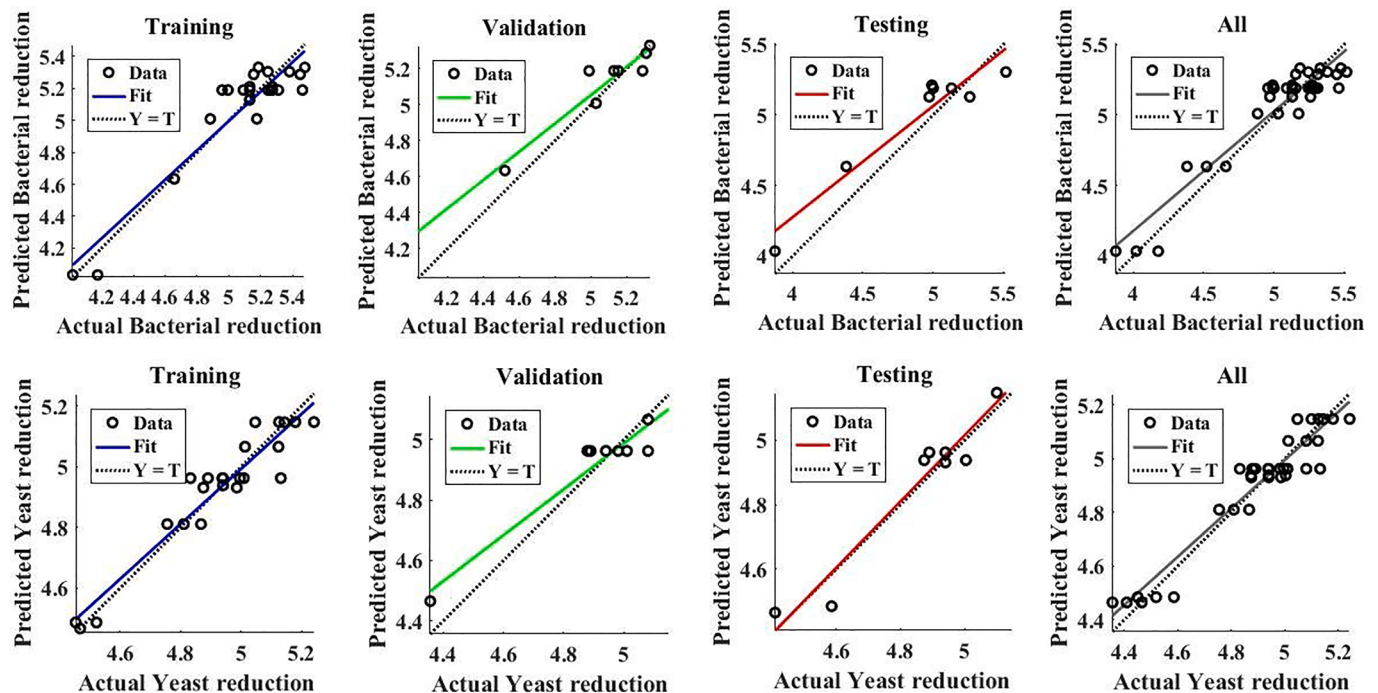


Fig. 7. (continued).

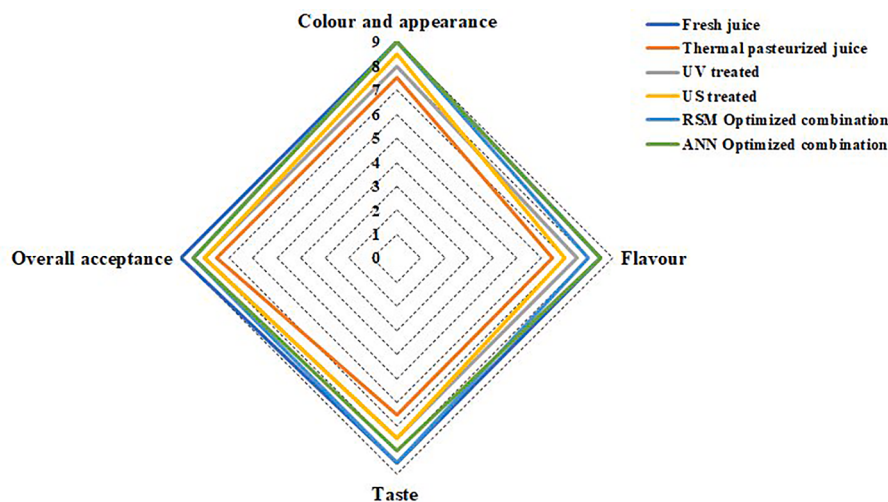


Fig. 8. Variation of organoleptic scores of sample.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ultsonch.2022.106166>.

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