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Central composite design, Pareto analysis, and artificial neural network for modeling of microwave processing parameters for tender coconut water

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ABSTRACT

Polyphenol oxidases (PPO) and peroxidases (POD) are the major enzymes that affect the quality of tender coconut water (TCW). Advanced thermal treatment such as microwave treatment has the potential for the inactivation of food enzymes. The experiments were conducted at three different microwave power levels (450, 600, and 900 W) and five different exposure times (70, 80, 90, 100, 110, and 120 s). The modeling and optimization of process parameters were done using a central composite design and artificial neural network. The microwave power level of 600 W for 120 s exposure time was suitable for enzyme inactivation with minimal quality loss. Optimized treatment has pH=5.02, total soluble solids (TSS)=5.68 °Brix, turbidity=12.51 NTU, titratable acid (TA)=0.07% of malic acid, PPO=0, POD=0, phenolic content=37.238 mg GAE/L and overall acceptability (OA)=7.5. These results confirmed that microwave treatment could be the potential alternative to conventional thermal treatment for processing tender coconut water.

1. Introduction

Tender coconut water (TCW) is a natural drink that has immense domestic and international market potential. Several studies have found that TCW is rich in minerals (Ca, K, Na, P, Mg, Fe, Zn, Cu) and vitamins (C, B1, B2, B3, B5, B6) [1] and accords numerous health benefits [2,3]. Consumers have been more health-conscious in recent years and prefer natural beverages over artificial or carbonated beverages [4,5]. Furthermore, worldwide coconut production has witnessed an increase to 61,865,423 metric tonnes in 2018-19 [6].

TCW has enormous potential to emerge as a significant health drink in the markets. The TCW is enclosed within three outer layers of the fruit, botanically termed exocarp, mesocarp, and endocarp [7]. The bulky nature of tender coconut increases the transportation cost. Therefore, the bottled, ready-to-drink TCW is popular among sellers and consumers. A significant problem associated with the processing and bottling of TCW is enzymatic reactions caused due to the activities of polyphenol oxidases (PPO) and peroxidases (POD). These enzymatic reactions affect the color (changes the almost transparent nut water to pink) and flavor profile of the TCW. The pink color formation could be arrested by devising suitable post-harvest conservation and thermal processing technique [8].

In this context, the current preservation technologies followed by TCW processing industries involve conventional thermal treatment (80 °C for a few seconds) and preservatives (citric acid and nisin), and/or carbonation. Loss of flavor, color, taste, and nutrients are the demerits of the thermal treatment. Nasution et al. [9] found that the heat treatment at 95 °C and 10 min affect the volatile, aroma compounds of TCW, thereby decreasing the overall acceptability of the product. Since the PPO and POD are thermo-stable enzymes, eliminating these enzymes by utilizing non-thermal technologies recorded little success. Also, the economic feasibility and commercial aspects of non-thermal technologies remain significant challenges for their large-scale adoption.

The advanced thermal technology of volumetric heating, such as microwave treatment, could be a viable alternative solution for the TCW processing industries. Earlier studies have proven that strawberry puree's microwave treatment has better retention during long-term storage than the conventional heat-treated samples [10,11]. Similarly, the

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inactivation of enzymes could be achieved with a lesser time using microwave treatment than the conventional heat treatment method [12]. Valadez-Carmona et al. [13] found that microwave drying has effectively controlled the PPO enzyme activity (66%) in cacao pod husks than hot air (27%) and freeze-drying (52%) methodologies. Microwave processing under low power (390 W) and long processing time effectively activated the peroxidase enzyme in tomato puree [14]. Microwave processing has also been conducted for deciphering the PPO and POD enzyme inactivation kinetics in a simulated solution of coconut water [15]. However, the simulated solutions have different physical and functional properties compared to the original tender coconut water. However, they have not optimized the processing conditions. To design an efficient microwave heating system for preserving TCW, optimizing the process parameters such as power level and exposure time is indispensable. Based on our knowledge, there is no study on optimizing microwave processing parameters for TCW using advanced statistical tools.

Therefore, the current study studied the effects of microwave treatment parameters (power level and exposure time) on physicochemical parameters, enzyme inactivation rate, and overall acceptability of TCW. The process parameters using a central composite design and artificial neural network technique were optimized.

2. Materials and methods

2.1. Sample collection

Tender coconuts (variety: West Coast Tall) of 6,7 months maturity were harvested from ICAR-Central Plantation Crops Research Institute (ICAR-CPCRI) Farm, Kasaragod, India. Collected nuts were immediately transported to Agro-Processing Complex, and the TCW was extracted using in-house developed Punch and Cutter [16]. The extracted water was filtered through a coarse strainer (700 μm) to remove the impurities and stored under a deep freezer (-18 °C) until further use. Before microwave treatment, the TCW was placed in the room temperature condition to equilibrate with ambient temperature (29 ± 2 °C and 62% RH). Then, TCW was filled with 75 ml in polypropylene (PP) bottles (97.35 mm height and 49.03 mm diameter, Tarsons Pvt. Ltd., Kolkata, India). The bottles containing TCW were individually subjected to microwave treatment.

2.2. Microwave treatment

A microwave oven (Samsung CE1041DFB1/XTL, India) working in the frequency range of 2450 MHz was used for the experiment. It consists of a seven-segment three-digit display for setting up various parameters viz. power level, exposure time, and heating mode. The microwave bottom base has a rotating platform which allows for an even distribution of the microwave heating. Bottled TCW was kept at the center of the rotating base and left uncovered to avoid excess pressure build-up. The experiments were conducted at different power levels (450, 600, and 900 W) and exposure times (70, 80, 90, 100, 110, and 120 s). Based on the preliminary studies, we have selected 900, 600, and 450 W power levels and 70 to 120 s exposure times with 10 s intervals. Less than 70 s treatment time renders a relatively low enzyme inactivation rate, whereas when the treatment time exceeds 120 s, it severely affects the flavor profile of the TCW. Beside, the temperature of TCW was measured using an infrared thermometer (Metravi MT-3) during microwave processing.

2.3. Determination of quality parameters

The following quality parameters were analyzed after the microwave treatment. All the experiments were replicated three times.

2.3.1. pH and total soluble solids (TSS)

pH was measured using a handheld pH meter (Tester35-Eutech Instruments; Accuracy±0.1). TSS, expressed as °Brix, was determined using a pocket refractometer (PAL-BX/RI; ±0.1% accuracy).

2.3.2. Turbidity

Turbidity was determined using Turbidimeter (Eutech TN-100) in NTU. The measurement range of the equipment varied from 0 to 2000 NTU with ±1 accuracy.

2.3.3. Titratable acidity (TA)

TA measurement was conducted by following the protocol suggested by Thimmaiah [17]. Malic acid is predominant in TCW [1]; therefore, the TA was expressed as malic acid (equivalent weight = 67.05 g) in 100 ml.

2.3.4. Phenolic content

The phenolic content determination was carried out using Folin-Ciocalteu's method [18] with slight modifications. Gallic acid was used as a standard solution (1 μg/mL). The diluted solution of gallic acid was taken in 10, 20, 30, 40, and 50 ppm concentrations in test tubes to prepare a standard curve of absorbance vs. concentration. The TCW samples (0.1 mL) in replications were taken in test tubes. All the test tubes were then made up to 1 ml using distilled water, and a blank sample was kept with distilled water alone. The test tubes were then added with 0.2 ml of 50% FCR (Folic-Ciocalteu's Reagent) and 2 ml of freshly prepared 7% Na_2CO_3. These samples were vortexed and kept in the dark for about an hour at room temperature. Later, absorbance at 750 nm was recorded using the Shimadzu UV-160 spectrophotometer. The blank value was deducted from all the other samples, and the standard graph was plotted. The quantity of phenol was expressed as mg gallic acid equivalents (GAE) in /100 mL.

2.3.5. Polyphenol oxidases (PPO) activity

PPO was determined using the method described by Porto et al. [19] with slight modifications. Briefly, one mL of treated tender coconut water sample was added with 1 ml potassium phosphate buffer (pH=6) containing catechol (0.1 M) as the substrate. The reaction was carried out at room temperature, and absorbance readings were measured using a spectrophotometer (Shimadzu UV-160) at 425 nm at every 15 s intervals until it reached a constant value. The enzymatic activity was calculated using Eq. (1), where the slope of the absorbance vs. time plot was denoted by 'k.' One unit of enzyme activity is defined as the amount of enzyme that causes a change of 0.001 in the absorbance per minute.

$$PPO\ Activity = \frac{k}{0.001} \tag{1}$$

2.3.6. Peroxidases (POD) activity

POD activity was determined according to the method described by Porto et al. [19] with slight modifications. Phosphate-Citrate buffer (pH=5) with 1% pyrogallol substrate was prepared. One mL of treated tender coconut water sample was added with 950 μL of buffer solution and 50 μL of 3% H_2O_2 as substrate. The reaction was carried out at room temperature, and absorbance readings were measured by spectrophotometer (Shimadzu UV-160) at 470 nm, and the measurements were taken at every 15 s for 5 min. The enzymatic activity was calculated using Eq. (2), where the slope of the absorbance vs. time plot was denoted by 'k.'

$$POD\ Activity = \frac{k}{0.001} \tag{2}$$

2.3.7. Sensory evaluation

A trained sensory panel of eight members was selected from ICAR-CPCRI, India, to conduct a sensory evaluation of treated samples. The panel was assigned scores in the 9-point Hedonic Scale based on aroma, taste, color, and overall acceptability (OA). The untreated sample was considered a control, and it has the characteristics of pleasant flavor and sweet taste. All the 18 samples were given numbers starting from S1 to S18 and represented in a radar plot with color-coding. The sample S1 corresponds to 900 W power level and 120 s exposure time, S2 corresponds to 900 W power level, and 110 s exposure time, S3 corresponds to 900 W power level, and 100 s exposure time, S4 corresponds to 900 W power level, and 90 s exposure time, S5 corresponds to 900 W power level, and 80 s exposure time, S6 corresponds to 900 W power level and 70 s exposure time. Similarly, 600 W power levels with different exposure times coded as S7-S12 and 450 W treatments with other exposure times coded as S13-S18.

2.4. Response surface methodology (RSM) modeling

A custom-made response surface methodology was designed to determine the optimal power level and exposure time. The custom response was suitable to fit the discrete microwave conditions in a response surface study. The analysis was carried out using Minitab® 19.1.1 (64-bit) software. The present study used a central composite design (CCD) with two independent variables such as power level (450, 600, and 900 W) and exposure time (70, 80, 90, 100, 110, and 120 s).

Quality attributes of each run were measured using dependent variables viz. pH, TSS, TA, turbidity, phenolic content, overall acceptability (OA), relative PPO, and relative POD activity. These responses are taken since they are found to represent fundamental qualities of tender coconut water. The relationship between independent variables and response was calculated using a second-order polynomial equation. The coefficients were calculated using Minitab® 19.1.1 (64-bit) during response surface regression. The model generated from the microwave treatment study results is depicted in the analysis of variance (ANOVA) Table 1. The models and constituent terms are evaluated using ANOVA. Linear, square, and 2-way interactions terms were evaluated using R^2, F-value, and P-value [20]. Values of P less than 0.05 were considered significant at a chosen confidence interval of 95%. Responses (pH, TSS, Turbidity, TA, Phenolic content, OA, and relative PPO and POD) were modelled by associating coefficients and constants to various terms represented in the equation (Table 2). The relative PPO and POD measurements are the ratio of enzyme activity after treatment to fresh TCW.

2.5. Artificial neural network (ANN) model

The ANN or multi-layered neural network was created with supervised learning. The process parameters such as power level and exposure time were assigned as input parameters, and the ANN model was developed for responses. Apart from the RSM data, additional replication points were used in the ANN model. The neural network was created using the "nnstart" toolbox in MATLAB online R2020b software. 'nftool' tool was selected from the "nnstart" option for creating an artificial neural network. The total data set was divided into 65% training, 20% validation, and 15% testing using the toolbox wizard. The variables were normalized to attain the same importance in the learning process [21]. The data fit was carried out using various neurons in the hidden layer ranging from 1 to 20. Performances functions and mean squared error (MSE) were calculated for each instance. To find the weight and bias values for neurons, a backpropagation algorithm "trainlm" was used since it was the fastest and first choice for supervised learning algorithms [22].

Similarly, the learning function was "learngdm," a decent gradient function with momentum weight and bias learning. A feed-forward network with one hidden layer was chosen, connecting all the neurons through a forward pass. This system is found to predict the input-output

Table 1
ANOVA table showing linear, quadratic and interaction effect of microwave processing parameters.

	pH			TSS		TA		Turbidity		Phenolic Content		PPO		POD		Overall Acceptability	
Source	DF	*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	*F*-Value	*P*-Value
Model	5	1.15	0.389	11.86	<0.001	26.47	<0.001	4.23	0.019	42.03	<0.001	35.64	<0.001	106.96	<0.001	45.28	<0.001
Linear	2	0.49	0.627	21.86	<0.001	54.96	<0.001	9.63	0.003	99.80	<0.001	72.27	<0.001	228.00	<0.001	52.92	<0.001
Power Level	1	0.18	0.677	6.60	0.025	34.93	<0.001	14.45	0.003	32.41	<0.001	135.36	<0.001	397.88	<0.001	105.84	<0.001
Exposure Time	1	0.79	0.392	37.12	<0.001	74.99	0.000	4.81	0.049	167.19	<0.001	9.18	0.010	58.12	<0.001	0.00	0.999
Square	2	2.15	0.159	8.97	0.004	7.08	0.009	0.63	0.552	0.25	0.786	26.31	<0.001	41.94	<0.001	33.34	<0.001
Power Level*Power Level	1	2.60	0.133	14.15	0.003	1.25	0.286	0.17	0.689	0.49	0.498	49.91	<0.001	83.85	<0.001	63.79	<0.001
Exposure Time*Exposure Time	1	1.70	0.217	3.78	0.076	12.91	0.004	1.08	0.319	0.00	0.962	2.72	0.125	0.03	0.859	2.89	0.115
2-Way Interaction	1	0.03	0.873	2.66	0.129	17.70	0.001	1.24	0.288	24.09	<0.001	4.43	0.057	34.86	<0.001	15.15	0.002
Power Level*Exposure Time	1	0.03	0.873	2.66	0.129	17.70	0.001	1.24	0.288	24.09	<0.001	4.43	0.057	34.86	<0.001	15.15	0.002
Lack of Fit			0.809		0.018		0.142		0.027		0.043		0.001		0.003		0.007
R^2		32.30		83.18		91.69		63.78		94.6		93.69		96.89		92.87	
Adj-R^2		4.10		76.17		88.22		48.68		92.35		91.06		96.89		92.87	
Adj-SS Total		0.04		1.12		0.0002		199.427		4.37		1.42		0.26		28.98	

TA-Titratable acidity; PPO-Polyphenol oxidase; POD- Peroxidas.

Table 2
Linear regression equations developed using central composite design.

Property	Model	R^2	RMSE
pH	$Y_1 = 4.358 + 0.001035\ A + 0.00963\ B - 0.000001A^2 - 0.000056B^2 + 0.000001\ A \times B$	54.26	0.045
Total soluble solids(TSS)	$Y_2 = 4.80 + 0.00591\ A - 0.0414\ B - 0.000005\ A^2 + 0.000220\ B^2 + 0.000014\ AB$	84.22	0.1198
Titratable acidity (TA)	$Y_3 = 0.0249 + 0.000007\ A + 0.001004\ B + 0.000000\ A^2 - 0.000005\ B^2 - 0.000000\ AB$	95.47	0.0013
Turbidity	$Y_4 = -14.3 + 0.0079\ A + 0.380\ B - 0.000011\ A^2 - 0.00231\ B^2 + 0.000192\ AB$	74.8	2.345
Phenolic Content	$Y_5 = 36.67 - 0.00226\ A - 0.0077\ B - 0.000001\ A^2 - 0.000006\ B^2 + 0.000050\ AB$	95.77	0.1374
Relative PPO	$Y_6 = 6.170 - 0.01157\ A - 0.0372\ B + 0.000007\ A^2 + 0.000131\ B^2 + 0.000013\ AB$	94.47	0.0840
Relative POD	$Y_7 = 2.214 - 0.004381\ A - 0.00917\ B + 0.000002\ A^2 + 0.000004\ B^2 + 0.000009\ AB$	98.48	0.0211
Overall Acceptance	$Y_8 = -14.55 + 0.04597\ A + 0.1685\ B - 0.000031\ A^2 - 0.000542\ B^2 - 0.000097\ AB$	97.27	0.3378

(Y_x=Response value, A= Power level, B= Exposure time, TSS-Total soluble solids, TA-Titratable acidity, R^2=Coefficient of determination, RMSE=Root Mean Square Error).

of any finite mapping problems [23]. They created a neural network assigned a "purelin" transfer function that converted the weighted input values to output. Later the network was trained using the trainlm function that updates according to Levenberg-Marquardt optimization.

3. Results and discussion

3.1. Effect of microwave processing parameters on pH

The effect of power level and exposure time on pH of TCW is presented in Fig. 1a. The pH values ranged from 5.03 to 5.20 for different combinations of treatments. The initial pH of fresh TCW was 5.23, and an insignificant decreasing pattern was observed after microwave treatment. The pH of fresh TCW was in the average values of other tender coconut varieties [24,25]. According to Food and Agriculture Organization (FAO) regulations, the pH range of bottled coconut water has to be in the range of 5–5.4 [26]. The lowest pH of 5.03 was recorded for TCW that had undergone 900 W for 120 s, 600 W for 120 s, 450 W for 100 s, and 450 W for 80 s. Also, the highest pH value of 5.2 was observed for 600 W for 100 s treatment. Very high-temperature treatments might have caused a drop in the pH of TCW [27]. The conversion of sugars to acids during microwave heating may decrease the pH [25]. Moreover, similar observations were made during microwave treatment of juices in which sugar is present [28]. Statistical analysis results reveal that the model is insignificant, leading to the interpretation that there is no significant influence of various treatments on the pH of TCW. The histogram of the data shows that the model tends to predict the pH values, which are more than the actual values (Fig. 2a), and a standard error of 0.04. The verses fit (residual vs. fitted plot) shows scattered data points indicating a significant number of residuals in fitted value (Fig. 2b). Thus, the trend causes a severe dip in R^2 value to 32.30%, thereby diminishing the predicting ability of the model.

3.2. Effect of microwave processing parameters on total soluble solids (TSS)

The TSS values ranged from 5.10 to 5.96 °Brix for various microwave treatments. Variation of TSS concerning microwave processing parameters is shown in Fig. 1b. The TSS of fresh TCW was found to be 5.45 °Brix. FAO regulations of TCW warrant TSS between 5 and 6.5 [26]. The results of TSS of TCW in our experiment comply with that. The highest value of TSS (5.96 °Brix) was observed for TCW subjected to 600 W power level and 120 s exposure time, whereas the lowest value (5.10 °Brix) was found for 450 W and 80 s. A similar TSS content was found while processing the TCW with conventional and non-thermal methods [29–31]. A slight increase of TSS at a high power level and the exposure time was accompanied by weight loss of the sample. The high power level and exposure time cause evaporation of the water. At the same time, the 900 and 600 W power levels with ≥100 s exposure time were found to increase the temperature of TCW to greater than 98 °C. Thus, the TSS of TCW tends to increase, possibly due to the evaporation effect. An investigation conducted by Gunathunga et al. [32] reported that TCW exhibited an increase in TSS following heat treatment at 85 °C and 60 s due to water evaporation. Microwave treatment for 90 °C (60 s) was sufficient to cause evaporation of water and increase the TSS of sugarcane juice [33]. The ANOVA (Table 1) shows significant values for the model and its terms. A Pareto chart of standardized effects ($\alpha = 0.05$) suggests that linear and square term of power level is the only factor that affects the TSS model. The histogram for the model had only slight residuals, which are close to zero as shown in Fig. 2c and a standard error of 0.11. Versus fit obtained for the data indicates that after 5.2 °Brix, the model starts accumulating residuals, as shown in Fig. 2d. R^2 indicates the model's ability to predict beyond the experimental range $R^2_{(pred)}$, estimated by predicting each observation from the remaining values. A scattered versus plot thus shows the reason for the lower $R^2_{(pred)}$ (51.69) value. The overall R^2 value of 83.18% suggests that the model could predict well within the range of the experiment.

3.3. Effect of microwave processing parameters on titratable acidity (TA)

Fig. 1c depicts the effect of power level and exposure time on the TA value of TCW. The TA values ranged from 0.053 to 0.067% of malic acid. Raw coconut water sample had TA 0.07% of malic acid, but increased power and exposure time decreased the TA. The 5% decrease in TA at 900 W power level and 120 s exposure time could be due to the absence of involvement of sugar in hydrolysis reaction and formation of acids [34]. A decrease in TA during pasteurization (temperature increase from 70 to 80 °C) of radish juice was observed [34]. Terms involved in the model were analyzed based on p-values, implying that all the other terms show high significance (95% confidence level). The ANOVA results (Table 1) also depict a high F-value coupled with a small p-value underscoring the significance of the model. The histogram obtained for the model had residuals values between -0.002 and 0.002, but the frequency of deviated values is less (Fig. 2e). The versus fit for the data indicated that from the TA values of 0.060 onwards, the residuals were following a declining pattern compared to the starting point (Fig. 2f) and standard error was 0.001. The model exhibits good estimation or predictability in the experimental range due to a high R^2 value of 91.69%. Similarly, $R^2_{(pred)}$=77.26% within 20% difference with $R^2_{(adj)}$=88.22% were proposed, suggesting that the model can predict beyond the experimental range.

3.4. Effect of microwave processing parameters on turbidity

The turbidity values range from 8.55 to 17.79 NTU for various microwave treatments. An increase in power level and exposure time increases the turbidity (Fig. 1d). The fresh coconut water had turbidity of 7.15 NTU, which increased to 8.55 for 450 W and 90 s treatment and 17.79 NTU for 900 W and 120 s exposure time. The high oxidation rate changed the transparent TCW to creamy white during the heating process, increasing the turbidity. This could be attributed to the Maillard reaction, phenolic oxidation, and caramelization effects [35]. The significant changes in color value were also observed during sterilization of TCW at 121 °C for 20 min [27]. The combination of maximum power

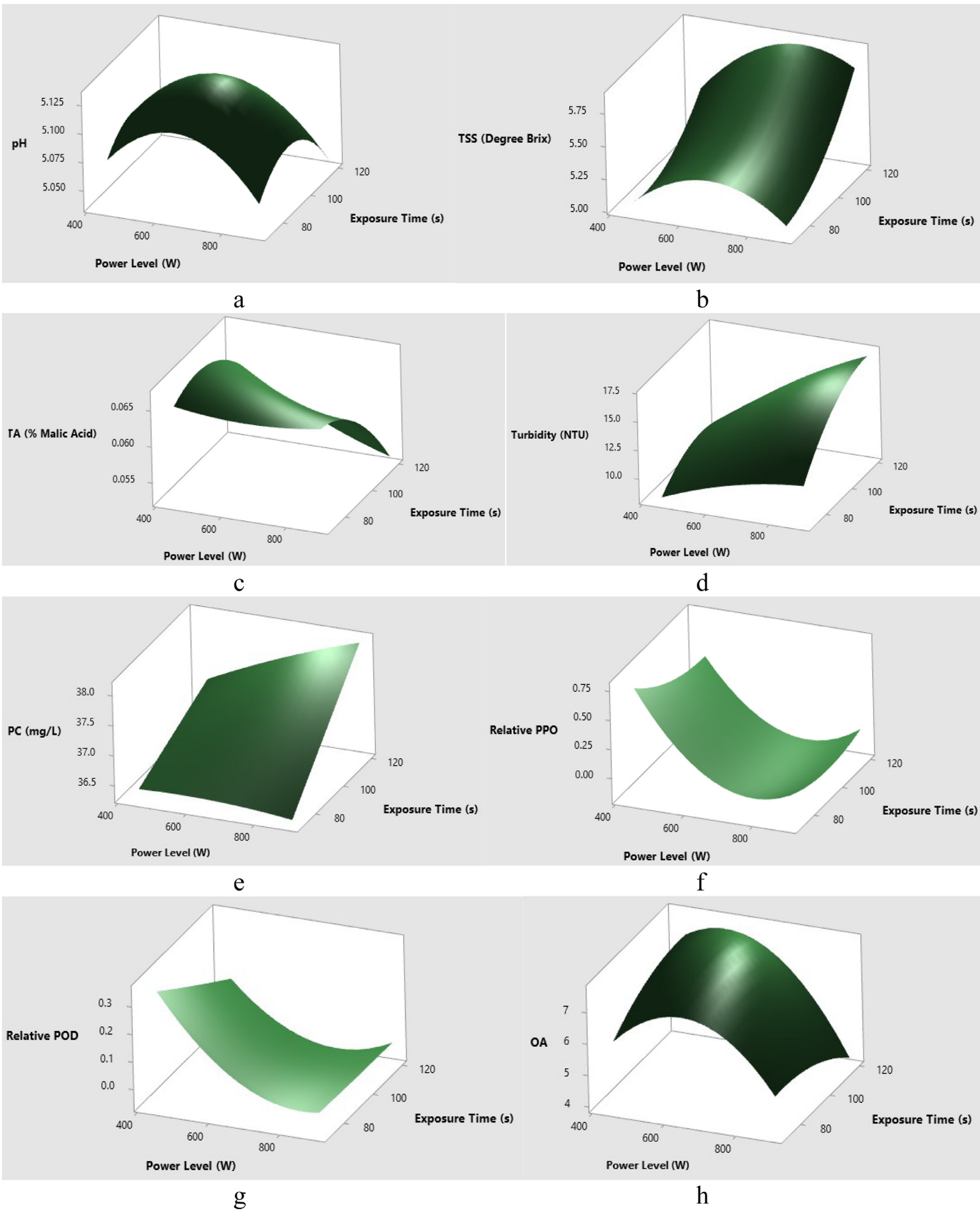

Fig. 1. Response surface plots depicting effect of power level and exposure time on responses (a) pH, (b) TSS, (c) TA, (d) Turbidity, (e) Phenolic Content (PC), (f) Relative PPO inactivation, (g) Relative POD inactivation, (h) Overall acceptability.

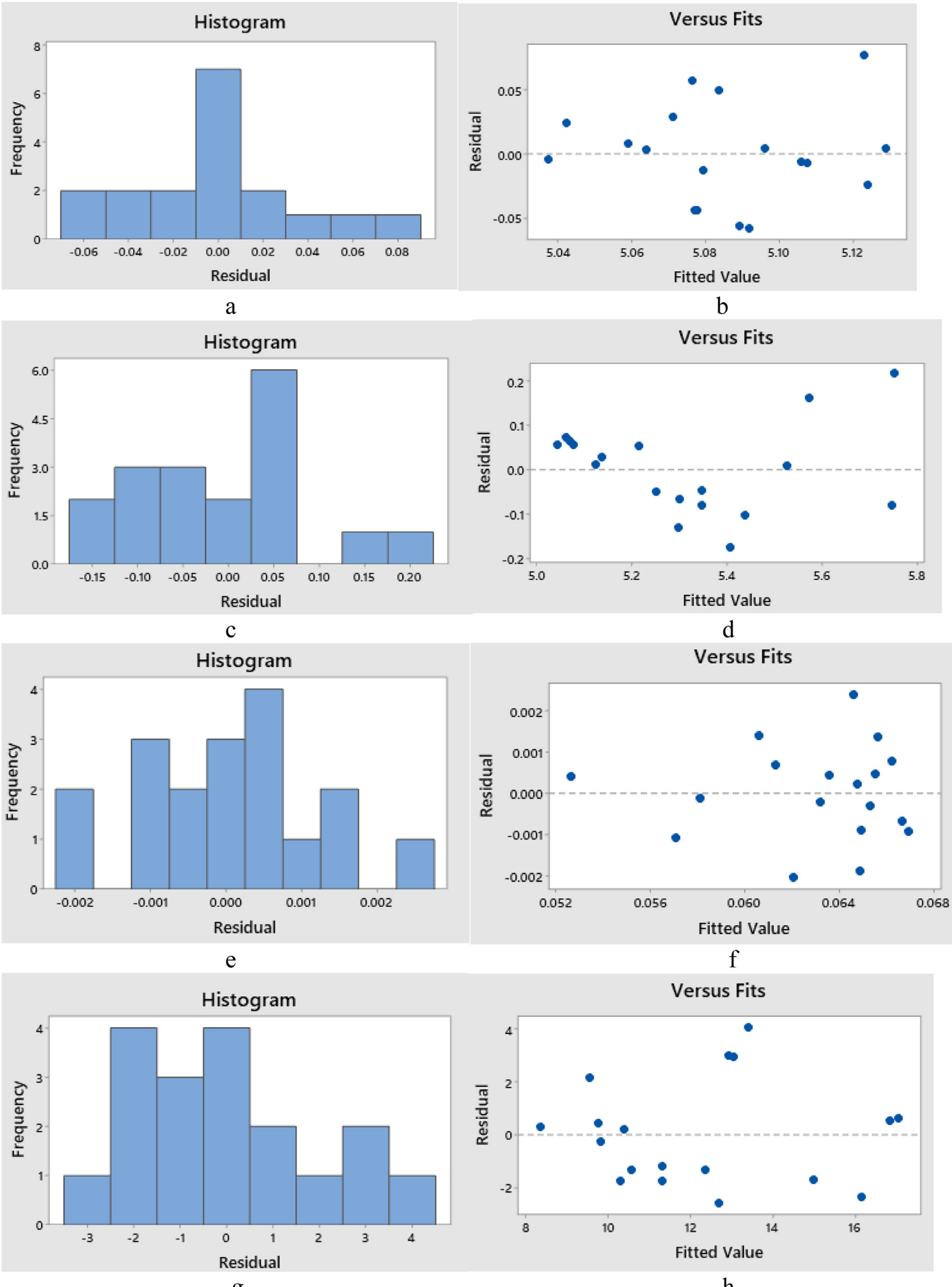

Fig. 2. I. Histogram of different responses (a) pH, (c) TSS, (e) TA, (g) Turbidity, (i) Phenolic Content (PC), (k) Relative PPO inactivation, (m) Relative POD inactivation, (o) Overall acceptability. II. Versus fit of different responses (b) pH, (d) TSS, (f) TA, (h) Turbidity, (j) Phenolic Content (PC), (l) Relative PPO inactivation, (n) Relative POD inactivation, (p) Overall acceptability.

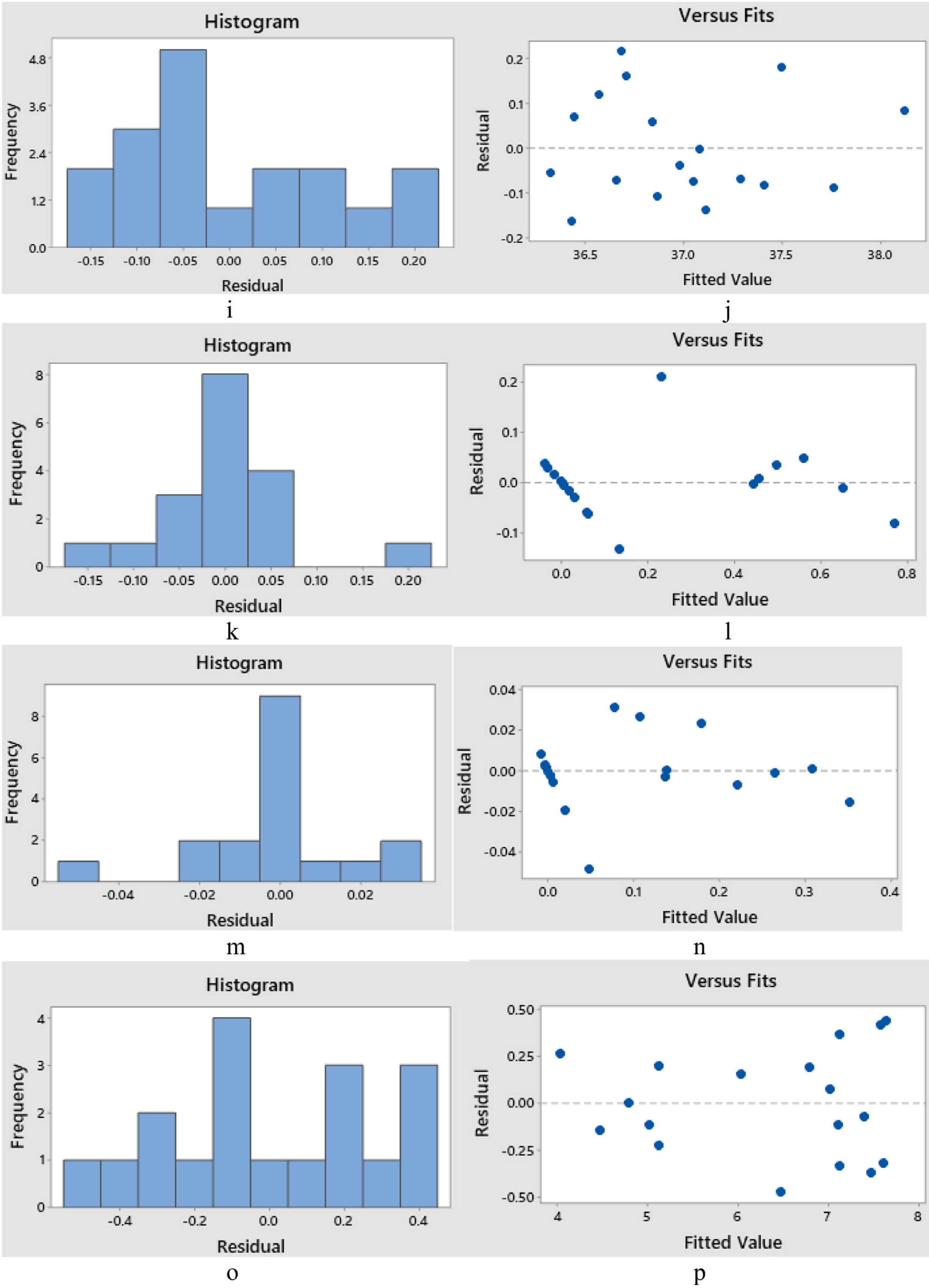

Fig. 2. Continued

level (900 W) and exposure time (120 s) increased the treatment temperature up to 120 °C, which in turn caused an increase in the turbidity over two folds compared to the fresh untreated sample. The terms (linear, quadratic, and interaction) analyzed by ANOVA suggest that only power level significantly influences turbidity. Comparison of F and p values makes the model barely significant with the current data points. The analysis also proposed a linear model; therefore, the lack of quadratic terms develops a planar response surface. The histogram indicated that many residuals (as shown in Fig. 2g.) are a reason for lower R^2 values, and the standard error was 2.34.

Similarly, the verses fit provides a highly scattered plot where only four observations have negligible residuals (Fig. 2h). This results in lower R^2 (63.78%) value which may not yield sufficient accuracy on prediction, especially if the values are greater than 12 NTU. This effect was translated to low $R_{(pred)}$ (26.99) and $R_{(adj)}$ (48.28%) values, indicating an insufficient data fit.

3.5. Effect of microwave processing parameters on phenolic content (PC)

The phenolic content of TCW ranges from 36.2 to 40.02 mg GAE/L for different microwave treatments. The variation of phenolic content concerning microwave processing parameters is shown in Fig. 1e. The higher phenolic content (40.02 mg GAE/L) of TCW was observed at 900 W power level and 120 s exposure time, whereas the lower phenolic content (36.2 mg GAE/L) was recorded at 450 W and 70 s. Phenolics increased 4,5% compared to fresh TCW, common when subjected to high-temperature treatments [36,37]. The increase in phenolics may be associated with the release of free flavonoids [38] and higher thermal stability of quercetin derivatives [39]. The terms were analyzed by ANOVA, obtaining p-values less than 0.05, resulting in significant terms in the model. The model possesses a high F-value associated with a lower p-value (almost equal to zero), ensuring a less noisy data set. The analysis also proposed a 2-factor interaction model; therefore, lack of quadratic term develops planar response surface. The histogram depicts a significant number of residuals, but the model still becomes stable due to small values of residuals, as shown in Fig. 2i. The verses fit has an interesting result converging in the middle with zero residuals and diverges at both ends, as shown in Fig. 2j, and the standard error was 0.13. The model was suitable for the present study since the convergence was on par with the optimized range later on in the results. Therefore, the response has a high R^2 (94.60%), suggesting a successful prediction in the experimental range. The adjusted and predicted R^2 (92.35%, 85.5%) are sufficiently high to predict the experimental range.

3.6. Effect of microwave processing parameters on relative polyphenol oxidases (PPO) levels

The relative PPO activity levels compared to the control samples ranged between 0 and 69%, as shown in Fig. 1f. Treatment conditions above 600 W for 80 s treatment reduced the PPO levels to untraceable amounts. The corresponding temperature for the lower microwave treatment (450 W for 70 s) was found to be 87 °C. The enzyme inactivation could be attained at a higher temperature (>97 °C) and short exposure time, but a significant deterioration of sensory attributes was observed at higher temperature treatment [15]. The heat treatment modifies the secondary structure and disruption of the tertiary structure of the enzymes along with the deformation and aggregation of enzymes [40]. Similar structural changes of α and β structures were observed in enzymes of juices while inactivating PPO at the temperature range of 55–70 °C [41,42]. Benlloch-Tinoco et al. [43] highlighted that microwave energy might reduce the PPO activity due to polar and charged protein moieties. Chourio et al. [37] also observed a temperature of 90 °C and 300 s exposure is required for pressure-assisted PPO inactivation in TCW. The model was found to be significant with lower p values in the ANOVA (Table 1). Sufficiently high F-values associated with the data suggest that significantly less noise distortion. The histogram for the

Table 3
Validation of predicted results.

Statistical parameters	Microwave processing parameters		Tender coconut water quality parameters and enzyme activity								
	Power Level (W)	Time (s)	pH	Total soluble solids (°Brix)	Turbidity (NTU)	TA (% Malic acid)	Relative PPO	Relative POD	Phenolic content (mg GAE/L)	Overall acceptability	Desirability
Predicted Result	604.54	120	5.23	5.6	12.7561	0.079	0	0	37.506	7.46	0.90
Rounded Result	600	120	5.08	5.75	12.91	0.077	0.0165	.0482	37.078	7.65	0.8530
Experimental	600	120	5.02 ± 0.1	5.63 ± 0.06	12.51 ± 0.18	0.070 ± 0.002	0 ± 0.00	0 ± 0.00	37.238 ± 0.81	7.5 ± 0.3	–

PPO-Polyphenol oxidase; POD- Peroxidase.

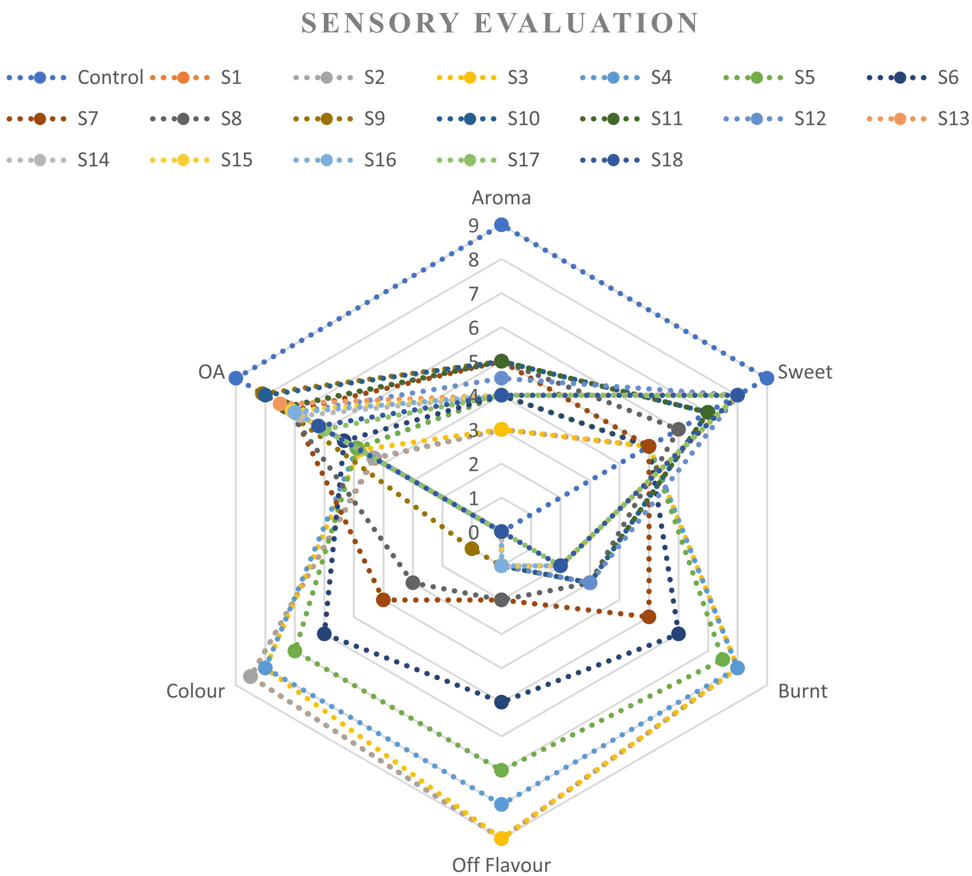

Fig. 3. Radar plot of sensory evaluation.

data depicts the highest frequency for zero residuals, indicating a good data set as shown in Fig. 2k, and the standard error was 0.08. However, the verses fit in Fig. 2l indicates a significant increase in residuals at 600 W for 70 s treatment due to a sudden increase in relative PPO content. The transition from 80 s to 70 s at 600 W power level had a temperature drop from 80 °C to 70 °C, which could be the reason for sudden enzymatic presence at a lower temperature where PPO is active [37]. Overall, the data had an R^2 value of 93.69%, indicating a good estimation of the experimental results. This response also possesses good $R^2_{(adj)}$ and $R^2_{(pred)}$ (91.06% and 83.13%), which indicates that the model has sufficient capability to estimate beyond the experimental range.

3.7. Effect of microwave processing parameters on relative peroxidases (POD)levels

The effect of microwave power level and exposure time on relative POD activity is shown in Fig 1g. The relative POD levels range from 0 to 33.82% for various microwave treatments. It was observed that POD was more heat resistant than PPO. Kubo et al. [44] highlighted that the peroxidase inactivation in fruit juices is dependent on the spatial distribution of temperature throughout the microwave chamber. Veitch [45] proposed that the POD inactivation occurs when the calcium atom is lost or when the distance between the heme group and tryptophan residue increases. The dielectric plasma-based treatment of tender coconut water reported that the heat resistance was more excellent for POD [46]. Siguemoto et al. [47] observed that the POD exhibited more thermostability than PPO in a cloudy apple juice under microwave heating. The POD enzyme structure gets destroyed during high-temperature exposure than PPO inactivation. The thermal energy generated may cause the Van der Waals force and hydrogen bonds to get disrupted, leading to the destruction of secondary and tertiary structures of the enzyme. Ultrasonic energy inactivation of POD enzyme used micro-steaming and shear force against Van der Waals force and hydrogen bonds [48].

The ANOVA results (Table 1) and *p*-values suggest that the model has significant linear and quadratic terms except for the quadratic term of exposure time. The histogram of the data has the highest frequency for zero residuals, implying a consistent dataset (Fig. 2m) and a standard error of 0.02. However, the verses fit (Fig. 2n.) exhibits few peaks when the relative enzymatic activity starts, but comparatively, residual levels are very low. The R^2 value of 97.81% indicates consistent data which can estimate accurately within the experimental range. Since the quadratic model had been omitted, the adjusted R^2 = 96.89% has a sufficiently large value and a close $R^2_{(pred)}$=95.90% allowing an accurate prediction of the experimental result.

3.8. Effect of microwave processing parameters on overall acceptability (OA)

The overall acceptability (OA) ranges vary from 4.3 to 8.1 for various microwave treatments, which varied in quadratic nature with power level and exposure time (Fig. 1h). The highest OA of 8.1 was obtained for 600 W and 100 s treatment whereas, the lowest value of 4.3 was found for 900 W and 120 s treatment. The primary reason for the decrease in OA for 900 W treatments is the development of yellow color in TCW, which could be due to Maillard reactions that convert the initially transparent TCW. Also, cooked flavor and change in taste are reasons for decreased OA of TCW. The model was significant for all terms with very low *p*-values except the linear and squared terms of exposure time (Table 1). *F*-value in the respective study is associated with a very less *p*-value indicating that noise influence on higher *F*-value is very low. The histogram conveys that the model often overestimates or underestimates the value and causes residuals, as shown in Fig. 2o. The verses fit data spread even though most of the data is close to zero residual lines as shown in Fig. 2p and standard error was 0.33. This data, when modeled, corresponds to $R^2$94.97%, which has a good prediction capability with the experimental range. The $R^2_{(adj)}$ and $R^2_{(pred)}$ (92.87% and 87.21%) are also on par, allowing a prediction of a parameter beyond the experimental range.

The sensory evaluation depicts a considerable loss of quality with increased microwave power level and exposure time. This is due to the off-flavor and off-color development due to the Maillard reaction. The top parts of the pentagon represent desired properties, and the bottom half depicts undesired properties (Fig. 3). The properties of fresh coconut water lose the desirable properties at 900 W power level with 120 s exposure time (S9). The sensory panel also mentioned a considerable development of cooked flavor at temperatures above 90 °C, indicating the overheating of the product. A similar result of burning was documented in microwave extraction of fruit juice [49].

3.9. Optimization and validation

The optimization process was carried out per the set conditions, such as overall acceptability and phenolic content fixed as maximum and relative POD activity, relative PPO activity, and turbidity set as a minimum. The pH and TSS parameters have lower R^2 values and fewer significant model terms. Since all the responses of pH and TSS were within FAO guidelines limit of bottled tender coconut water, these responses were not considered for optimization. Since titratable acidity during different treatments did not have significant variations, it was not optimized for the process. Higher importance was given to maximizing overall acceptability and minimizing enzyme activity since these are the most critical factors influencing the quality of TCW. Higher phenolic content and lesser turbidity were considered added responses, given the importance of 5. The target values were automatically populated according to the goal condition.

According to the goal mentioned above conditions, the obtained solution was 604.545 W and 120 s. The microwave apparatus changes the power level and exposure time as discrete set values; therefore, the optimized result was shifted to the nearest possible power level. Other response parameters were estimated for the corresponding point. The desirability of the solution obtained was 90% but later decreased to 85.3% due to the shift of power level.

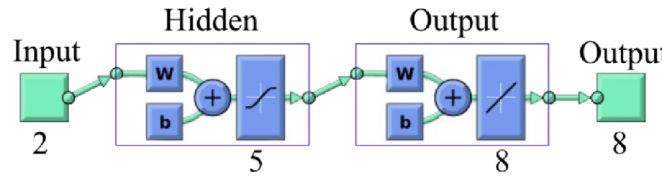

Fig. 4. Simulink diagram of ANN network developed for present study.

The result obtained in the post-optimization analysis found that predicted values are close to experimental values (Table 3). This confirms the prediction ability of the developed models.

3.9.1. Comparison of ANN and RSM models

Multilayer neural network model performance depends on the number of neurons in the hidden layer [50]. The optimal number of neurons was found as 5 with the lowest MSE (Mean squared error) value. The created network consisted of 2 inputs, 5 hidden and 8 output neurons, as shown in the Simulink diagram (Fig. 4). The training was continued until the validation and training error stop to decrease and attain a constant rate indicating the best epoch (epoch is the number of times the training vector is used for initializing weights and bias) was reached. Several neurons and a neural network training performance plot were used to ensure the "overfitting" of the model. The regression plot shown in Fig. 5 indicated that training, validation, and testing data attained a good fit (R = 0.99). The test result was exported to the MATLAB workspace and plotted against experimental values. The plot of predicted vs. experimental data was given trend line and R^2 values. Trend lines having R^2 ≈1 indicate an accurate prediction.

Compared to the RSM model, ANN was able to predict the data more accurately. The predicted vs. experimental plots of ANN show a higher generalization capacity of the ANN model. This was due to the capability of ANN to account for the nonlinearity of the system and the limitation of RSM to quadratic polynomials [51]. Also, a single ANN model could predict all the responses, whereas RSM needs different models for each response.

4. Conclusion

In this study, central composite design (CCD) was successfully employed to optimize microwave processing conditions of tender coconut water. The model was able to evaluate various quality parameters and

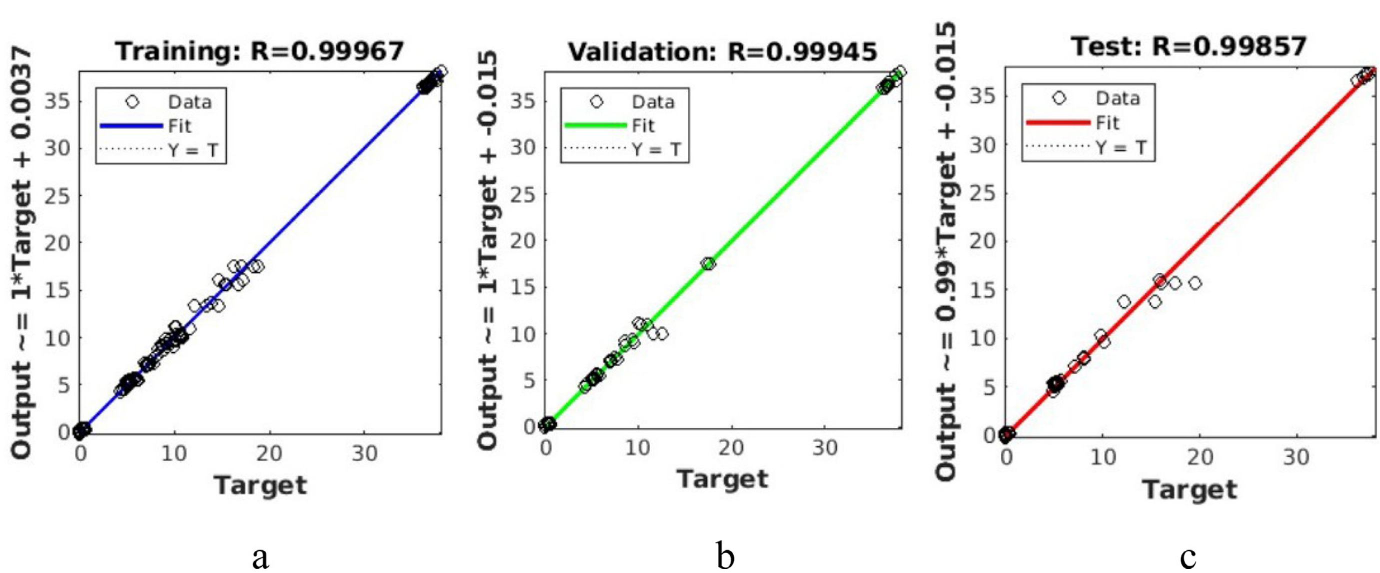

Fig. 5. Regression plots for training (a), validation (b) and testing (c) for ANN model.

their impact during microwave treatment. Moreover, among the various responses except for pH, all the other factors significantly affected the processing parameters. A power level of 600 W and exposure time of 120 s was optimal for microwave processing of tender coconut water. Also, the data modeled using supervised ANN learning was a better fit than the RSM model. A higher R^2 value in the range of 0.99 for all the factors in the ANN model depicts its best fit compared to the RSM models. Tender coconut water has huge research and commercial potential, and the current investigation provides valuable information about microwave processing of tender coconut water.

Data availability statement

All data generated or analyzed during this study are included in this published article.

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.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.meafoo.2021.100015.

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