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Original Paper

Heat-reflux processing of black peppercorn into bioactive antioxidant oleoresins: a three-functioned Taguchi-based grey relational grading

Olusegun Abayomi Olalere
Email : oao94@bath.ac.uk
Affiliationids : Aff1 Aff2, Correspondingaffiliationid : Aff1

Chee-Yuen Gan DisplayOrder:Western
Email : cygan@usm.my
Affiliationids : Aff2

Abiola Ezekiel Taiwo Affiliationids : Aff3

Abdurahman Hamid Nour Affiliationids : Aff4

Bernardo Castro Dominguez
Email : bcd28@bath.ac.uk
Affiliationids : Aff1

Oladayo Adeyi Affiliationids : Aff5

Gbemisola J. Fadimu Affiliationids : Aff6

~~Fawale~~Samson Olumide ~~Samson~~Fawale Affiliationids : Aff7

Oladipupo Q. Adiamo Affiliationids : Aff7

Aff1 Department of Chemical Engineering, University of Bath, Claverton Down, Bath, BA2 7AY, ~~UK~~United Kingdom, ~~Somerset~~

Aff2 Analytical Biochemistry Research Centre (ABrC), Universiti Sains Malaysia, University Innovation Incubator Building, Sains@USM, Lebuh Bukit Jambul, 11900, Bayan Lepas, Penang, Malaysia

Aff3 Faculty of Engineering, Mangosuthu University of Technology, Durban, South Africa

Aff4 Department of Chemical Engineering, Universiti Malaysia Pahang, Lebuhraya Tun Razak, 26300, Gambang, Pahang, Malaysia

Aff5 Department of Chemical Engineering, Michael Okpara University of Agriculture, Umudike, Nigeria

Aff6 School of Science, RMIT University, GPO Box 2476, Melbourne, VIC, 3001, Australia

Aff7 ARC Industrial Transformation Training Centre for Uniquely Australian Foods, Centre for Nutrition and Food Sciences, Queensland Alliance for Agriculture and Food Innovation (QAAFI), The University of Queensland, Indooroopilly, QLD, Australia

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Abstract

The focus of this research is to identify the best set of factors that influence the heat-reflux recovery of total phenolic content and antioxidant activities under multiple quality characteristics. **AQ1** Parametric Taguchi L_9 orthogonal design and grey relational analysis technique were used to investigate the effect of three variables—reflux duration, particle size, and feed-to-solvent ratio on the multiple responses of total phenolic contents, DPPH, and H_2O_2 activities. According to the grey relational grades response table, the ideal number of criteria for the heat reflux results were 120 min of reflux duration, 0.2 mm of particle size, and a feed-solvent ratio of 1:16. The total phenolic content, DPPH, and H_2O_2 scavenging activities were measured as 35.23 ± 0.004 mgGAE/g d.w, 107.57 ± 0.04 g/mL, and 87.78 ± 0.32 g/mL, respectively. Moreover, with the Levenberg–Marquardt (LM) neural network architecture, the trained network has a mean square error (MSE) of 3.7646E−07 and an R^2 of 0.9500 as the training function outcome, indicating a significant predicted endpoint. The confirmatory experimental results show a 41.9 per cent improvement in relation to the predicted values. The results of this study indicated that, optimising the heat reflux process would be an innovative and beneficial approach for preparing bioactive compounds from functional plants, resulting in cost **AQ2** savings while increasing antioxidant capacity and overall phenolic recovery.

Keywords

Antioxidants
Black pepper
Grey relational analysis (GRA)
Artificial neural network
Optimization
Total phenolic content

Introduction

Black peppercorns are powerful, hot-tasting, concentrated spice that is usually dried and powdered and is used to spice meats, seafood, veggies, condiments, stews, marinade, noodles, and other foods. Due to their nutraceutical potential, its by-products have been reported useful as food additives in form of oleoresin paste with many numerous health and nutritional **AQ3** benefits [1]. The growing demand for pepper offers a remarkable business opportunity for new marketers to enter the industry. Owning to this marginal interest and surge in the market value of black pepper there is an urgent need for its nutraceutical's diversification into other value-added by-products. One of such product diversifications is the extraction of spice oleoresins from black pepper consisting of both resin and essential oils with high potential use as feedstocks in food and pharmaceutical industries. Black pepper contains carbohydrates (37.4%), proteins (25.5%), fibre (23.6%), moisture (4.7%), and fat (5.3%), in addition to minerals such as potassium (0.66%), calcium (0.20%), phosphorus (0.16%), and magnesium (0.16%). Terpenes are the most significant volatile flavour and aromatic components in black pepper containing nitrogen-containing limonene, linalool, methyl-propanal, butyric acid, and 3-methylbutyric acid. Moreover, bioactive compounds such as 2,3-diethyl-5-methylpyrazine and 2-isopropyl-3-methoxypyrazine are the main component for black pepper's pungency and flavoury capacity.

Furthermore, the presence of antioxidants in the oleoresin extracts (fixed oil) offered a remarkable advancement for the treatment of some life-threatening free radical disorder such as cancer, diabetes, cardiovascular and neurological diseases [1]. The inherent antioxidant bioactive compounds in black pepper have also been reported to prevent chemical carcinogenesis via activation of xenobiotic enzymes [2]. Hence the dietary intake of black pepper extracts could **AQ4** provide natural antioxidants for boosting human immunity and prevent degenerative diseases [3]. The substantial amount of antioxidants in black pepper is largely associated with their total phenolic contents as reported by Poompavai and Gowri [4].

To adequately harness these functional properties, the hydro-distillation techniques has been previously used for recovering natural antioxidants from tough nuts, wood, seeds and hard surface powdered natural products [5]. The demerit from hydro-distillation method has provoked recent research focus into its heat-refluxing re-configuration. Take for instance, spice oils were extracted from white pepper and black pepper and tested for antioxidant activity by Abd El Mageed et al. [6]. The antioxidant properties of the spice oil were shown to diminished throughout the hydro distillation process. However, Khajeh and Ghanbari [7] reported that the difficulties in heat control are the major drawback in the use of hydro distillation which could result in extraction rate variation and hence a low extract quality. In heat reflux, the liquid is boiled, but the vapour is allowed to condense and flow back into the original flask. Allowing the condensed vapour to flow back into the original reaction flask, helps in the solvation of high molecular compounds. The continual recycling of the solvent helps reduce overheating by providing a cooling effects and drive the extraction to completion [8].

Furthermore, many researchers had studied the effect of extraction factors on the total phenolic content and antioxidants in black peppercorns. However, there has been little effort on the quality interaction between them at the response setting level [6]. It's also important to know that there is a correlation between the total phenolic and antioxidant activity with its combined effects determining its functional and therapeutic properties [9]. Previous studies conducted on the black pepper only investigated the antioxidant and total phenolic as a separate entity without recourse to how extraction parameters affects them on a combined response entity [10]. Determining the effects of extraction parameters on the combined quality characteristics of total phenolic content and antioxidant activity is typically a challenging endeavour due to the multiple response features involved. The grey relational analysis is therefore a veritable tool frequently utilized to quantify the degree of correlation between two or more responses via the grey relational grades (GRG) [11]. Grey relational analysis is a technique for estimating grey relational degree and identifying the impact criterion of the system is the key characteristic or the impact degree across process variables. Grey correlation grade is an indicator of correlation between two response settings [12]. The pattern, magnitude, and pace of change in grey correlation degree indicate the corresponding variation of variables in the process of system development. When the variability of two variables or systems follow the same trajectory of change, the two variables have a higher level of grey correlation; conversely, they have a lesser level of grey correlation [13]. Numerous studies have used grey relational analysis to improve control settings with multiple responses using grey relational grading [14, 15]. In optimization problems, the grey relational analysis is frequently used to aggregate various performance indicators (responses) into a single function called the grey relational grade [12]. Although many researchers had employed this method in construction operation, mechanical process, there has been no reported use for the intensification of heat reflux extraction [10].

Artificial neural network (ANN) is an intelligent modelling tool that deals with non-linear behaviour of inputs and desired outputs data [16]. The relationship between several input and output variables can be easily reached by optimization [17]. It has been reported that Grey-Taguchi combined with ANN enhances process performance [11]. The optimized ANN output is usually developed by using a backward feed propagation method [18]. Thus, combining Grey-Taguchi with artificial neural network will help improve the optimization of heat-reflux recovery of bioactive Oleoresins from Black Peppercorn. In this study, the heat reflux recovery of antioxidants and total phenolic content were optimized and intensified using an integrated orthogonal optimization, Deng grey relational analysis and supported by artificial neural network modelling. These were used to evaluate the best total phenolic and antioxidant activity as a combined criterion to create an optimum output using minimal sample size and inputs.

Materials and methods

Sample collection and extraction

The standard grade dried black and white pepper were identified and purchased from the Malaysian Pepper Board (MPB) located in Sarawak, Malaysia. The specified qualities of the pepper procured from the MPB are presented in Table 1. Grinder Knife Mill Grindomix (GM-200 model, Germany) was directly used to grind up the purchased samples to a particle size of 0.50 mm and then stored in an airtight black container against a ray of natural light. Reflux condenser modifications were made to the procedure employed by Rmili et al. [19]. Twenty-five grams of powdered black pepper samples was weighed into a one-litre volumetric flask with distilled water. In heat reflux, the liquid is boiled, but the vapour is allowed to condense and flow back into the original flask. Allowing the condensed vapour to flow back into the original reaction flask, allow you to dissolve hard compounds by reflux. The reaction progresses more quickly when the solvent is recycled again and over again. A rotary evaporator was used to filter and concentrate the sample at 200 rpm (BUCHI, R-200 model, Germany). Before further analysis, the extract was stored at 4 °C in a dark container at that temperature.

Table 1

Specified quality of the standard black pepper procured from MPB

Physical characteristics	Black pepper
Colour	Dark brown
Berry size (mm)	4.5
Extraneous matter (% w/w, max)	1.0
Moisture content (% w/w, max)	12.0
Source Malaysian Pepper Board, (2021)	

Determination of total phenolic content (TPC)

The Folin-Ciocalteu technique described by Saravanan and Parimelazhagan, (2014) was modified to quantify the TPC in black pepper extracts. Oleoresin black pepper extracts (20 L) and Folin-Ciocalteu, 2N (Sigma Aldrich®, Germany) were dissolved in 1.58 mL of de-ionized water. Afterward, the mixture was kept in the dark for 5 min. 300 L of sodium carbonate was added to the mixture, which was kept in the dark for another 120 min at 25 °C before being discarded. 765 nm was measured using a UV–VIS Spectrophotometer (Hitachi U-1800, Japan), the absorbance of plant extracts, standard and blank, was then determined. The total phenolic content of the oleoresin extracts was quantified in mg/L gallic acid equivalents by using gallic acid as a calibration curve reference. Using Eq. (1), the total phenolic content was determined in three replicates.

$$TPC = \frac{c\,(mg_{GAE}/L) * V\,(L_{Solvent})}{m\,(g_{dw\ dry\ weight})} \tag{1}$$

For instance, c is the concentration of a test sample in mg/L from the calibration curve, V is the volume (mL) of solvent injected, and m is the black pepper dried sample mass.

DPPH-free radical scavenging assay

The extracts' radical-scavenging activity was evaluated using a spectrophotometer [UV–Vis spectrophotometer (U-1800 model, USA)] calibrated to 517 nm. Zhang et al. [20], DPPH radical scavenging technique was utilised in this study. In order to prepare a DPPH solution of 0.10 mM, a dark purple crystalline solid was mixed with a 95% absolute ethanol. To prepare the negative control (A_0), ethanol and 2.5 mL of the DPPH solution were mixed together and incubated at room temperature in the dark for 30 min, and the absorbance was measured using a UV–Vis spectrophotometer. An absorbance denoted by A_2 was measured, and this includes a mixture of black pepper extracts (at different concentrations) and 2.5% ethanol. The A_2 was measured in order to minimize the extract's colour combination. The percentage of inhibition was thereafter calculated by averaging the results of all three tests. DPPH free radical scavenging activity was tested at five different concentrations of 50, 100, 150, 200, and 250 g/mL with the test solution. There were 2.5 mL of DPPPH solution and 0.5% spice extracts in this experiment, which yielded A_1 as the absorbance. The formula in Eq. (2) was used to compute the % inhibition.

$$\text{Scavenging activity (DPPH)} = \left(1 - \left[\frac{A_1 - A_2}{A_0}\right]\right) * 100\% \quad (2)$$

The antioxidant activity was determined by the IC_{50} value in a free radical—scavenging assay. The percentage of inhibition was plotted against the five extracts concentrations to generate the inhibition curve. Linear regression absorbance data points were used to establish the implications.

Hydrogen peroxide free-radical scavenging assay

The H_2O_2-scavenging abilities of the spice extracts were determined in line with Odeja et al. [21], approach. The peroxide stock solution was prepared by diluting 4.53 mL in 1 L of distilled water to a concentration of 30 percent. There is an equivalent of 40 nm in each 1 mL of the solution. Phosphate dibasic and monobasic powders, weighing 6.8 g and 2 g, respectively, were dissolved in 40 mL of distilled water to produce a 50 nM phosphate buffer (pH 7.4). For each 50-mL solution, a conical flask was filled and inverted many times to enable the components to mix. In order to achieve a 7.4 pH, the sodium hydrogen phosphate monobasic solution was gently mixed with the potassium hydrogen phosphate dibasic solutions. A UV–Vis spectrophotometer was used to measure the concentration of hydrogen peroxide at 285 nm (U-1800 model, USA).

An anti-oxidative assay was performed using a solution of black pepper extracts in hydrogen peroxide to see how well they neutralised the free radicals, which absorbed light at 285 nm (nm) over this time period (without H_2O_2). Five spice concentrations (50–250 g/mL) were used to measure the test solution's capacity to scavenge free radicals in the H2O2 solution. An absorbance reading of A1 was recorded after 10 min of incubation using 3.6 mL of H_2O_2 solution with 0.6 mL of spice extracts. All experiments were performed in sets of three. The fraction of hydrogen peroxide scavenged by black pepper extracts were determined using Eq. 3.

$$\text{Scavenging activity } (H_2O_2) = \left(\left[\frac{A_0 - A_1}{A_0}\right]\right) * 100\% \quad (3)$$

There were many different concentrations tested to see how well the black pepper extracts could reduce the original H_2O_2 solution's oxidative damage by half at steady state (IC_{50}). The smaller the IC_{50}, the more powerful the extracts are in scavenging.

Optimization via Taguchi-grey relational model

Taguchi's technique is sufficient for determining the best operational parameter settings for a single response attribute. Multi-response optimization using the grey relational analysis is the proven approach when there are two or more responses with distinct quality attributes. Grey analysis may also be used to discover if two apparently unrelated finite sets of data are comparable [12, 22]. As a result, in this study, the multi-response optimization of total phenolic content and antioxidant activity was achieved by combining the two responses into a single grey relational grade (GRG).

Normalizing the response values

In grey relational analysis, when the response value range are significantly large, the function of the factors is ignored. In such instances the analysis may yield erroneous outputs; hence, the need to perform a data pre-processing to normalize the real response values within the range of 0 to unity [12, 22]. In order to pre-process data by utilizing the grey relational analysis, the responses of the modified sequences can be categorized into either larger-the-better or smaller-the-better for maximization or minimization problems, respectively. In this study, since the tasks is to maximize both the total phenolic contents and the antioxidant activity, hence the larger-the-better normalization applies as presented in Eq. (4).

$$x_i^*(k) = \frac{y_i(k) - min y_i(k)}{max_{y_i(k)} - min_{y_i(k)}} \quad (4)$$

where $x_i^*(k)$ is the baseline sequences following pre-processing for the ith trial and $y_i(k)$ is the starting sequence of the average of the responses

Estimation of deviation sequence, grey relational coefficient (GRC) and grade (GRG)

The deviation sequence is the difference between the absolute response values of each pre-processed trials with Δ_{min} and Δ_{max} respectively, being the minimum and maximum values as presented in Eqs. (5) and (6). The grey relational coefficient was estimated using the Eq. (6). The identification coefficient is represented by φ and it's usually set as 0.5. The grey relational grade (GRG) was thereafter computed as the mean of GRC for all experimental trials as presented in Eq. (7) where $\Delta_{min} = 0$ and $\varphi\Delta_{max} = 0.5 * 1$.

$$\Delta_{0i}(k) = |x_0^*(k) - x_i^*(k)| \quad (5)$$

$$GRC = \frac{\Delta_{min} + \varphi\Delta_{max}}{\Delta_{0i}(k) + \varphi\Delta_{max}} \quad (6)$$

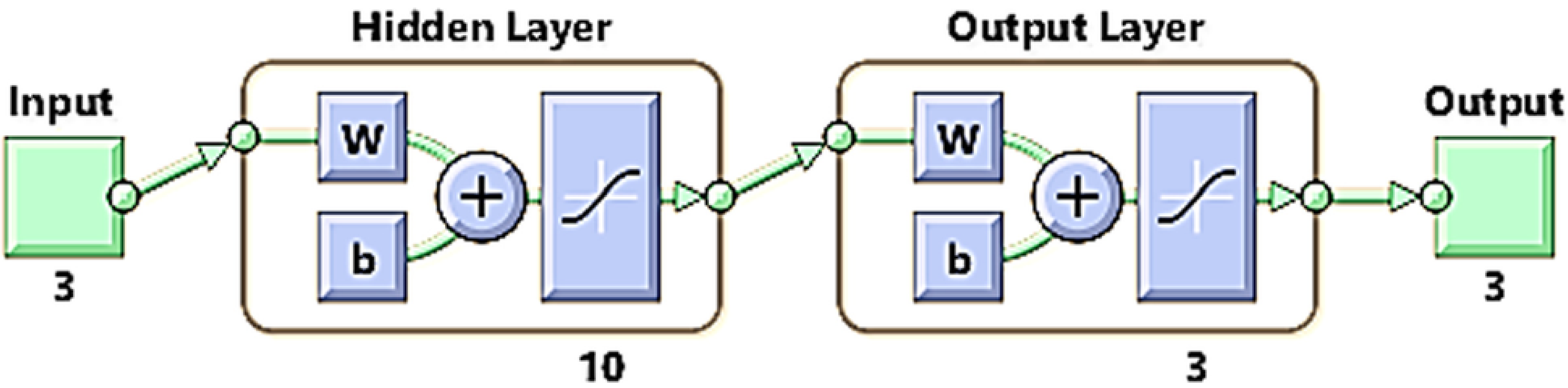
$$\mathrm{GRG} = \frac{1}{n}\sum_{i=1}^{n} GRC(k) \quad (7)$$

Modelling of artificial neural networks

The experimental data sets used in the ANN analysis were divided into three categories: training, testing, and validation. The training set comprised 70% of the entire data sets, with the other 30% dispersed as testing and validation data sets for network performance evaluation. The investigation was carried out using the MATLAB program 2017® neural network tool box. A feed forward back propagation network connection type was used. Reflux time (h), particle size (mm), and feed-solvent ratio were used as network inputs, with total phenolic content, DPPH, and H_2O_2 scavenging activity as outputs. In the MATLAB Software (2017a) graphical user interface, the input/output variables data sets were loaded into the neural network tool box. The Trainlm function was used to train the network. To fit the data, the Levenberg–Marquardt technique was utilized, and the network training function modifies the weight and bias values based on the chosen algorithm. Learngdm was used for the adaptive learning function. The Tanh function is used to transmit a neuron's input signal to its output signal, as shown in Fig. 1 neural network architecture. After a series of trials, the network was trained with ten hidden neurons, resulting in a 3–10–3 structure.

Fig. 1

Artificial neural network structure



Results and discussion

Optimized conditions and main effects of extraction factors on GRG

An L_9 (2^4) orthogonal matrix was designed using three operational levels with extraction variables listed as: reflux time (h_1:120, 180, and 240 min), particle size (h_2: 0.1, 0.2 and 0.3 mm), and feed-solvent ratio (h_3: 1:14, 1:16 and 1:18). The heat reflux extraction results indicated that 120 min of reflux duration, 0.2 mm of particle size, and a feed-solvent ratio of 1:16 were the best extraction conditions. The total phenolic content, DPPH, and H_2O_2 scavenging activity were 35.230.004 mgGAE/g d.w, 107.570.04 µg/mL, and 87.780.32 µg/mL, respectively, with an aggregated grey relational grade of 1.00 and SN ratio of zero under the optimum conditions. Table 2 showed the condition at which the extraction parameters jointly optimized the grey relational grade. Hence, by using the above conditions, an improved total phenolic content and antioxidant recovery was achieved.

Table 2

Experimental layout of L_9 orthogonal array and grey relational grades

Independent variables			TPC ($mg_{GAE/g\ d.w}$)	Antioxidant activities		Normalization of response data			Grey relational coefficient (GRC)			GRG	SNR
				DPPH activity	H_2O_2 activity	$(TPC)_n$	$(DPPH)_n$	$(H_2O_2)_n$	$(TPC)_{GRC}$	$(DPPH)_{GRC}$	$(H_2O_2)_{GRC}$		
h_1	h_2	h_3		(µg/mL)	(µg/mL)								
1	1	1	25.55 ± 0.46	85.40 ± 0.230	55.03 ± 0.34	0.51	0.69	0.49	0.50	0.50	0.50	0.50	−6.02
1	2	2	35.23 ± 0.004	107.57 ± 0.04	87.78 ± 0.32	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00
1	3	3	20.77 ± 0.002	64.22 ± 0.005	41.69 ± 0.11	0.26	0.40	0.28	0.40	0.40	0.40	0.40	−7.96
2	1	2	29.02 ± 0.01	97.06 ± 0.120	58.22 ± 0.04	0.68	0.85	0.54	0.61	0.61	0.61	0.61	−4.29

[a]Values are means ± SD of triplicate run, Subscript ‘n’ is the pre-processed/normalized response, GRC and GRG represents the grey relational coefficient and grade respectively

Independent variables			TPC ($mg_{GAE/g\ d.w}$)	Antioxidant activities		Normalization of response data			Grey relational coefficient (GRC)			GRG	SNR
				DPPH activity	H_2O_2 activity	$(TPC)_n$	$(DPPH)_n$	$(H_2O_2)_n$	$(TPC)_{GRC}$	$(DPPH)_{GRC}$	$(H_2O_2)_{GRC}$		
h_1	h_2	h_3		(µg/mL)	(µg/mL)								
2	2	3	15.65 ± 0.53	35.19 ± 0.02	24.08 ± 0.03	0.00	0.00	0.00	0.33	0.33	0.33	0.33	− 9.63
2	3	1	22.69 ± 0.31	77.69 ± 0.004	46.98 ± 0.09	0.36	0.59	0.36	0.44	0.44	0.44	0.44	− 7.13
3	1	3	27.54 ± 0.37	86.43 ± 0.01	54.66 ± 0.12	0.61	0.71	0.48	0.56	0.56	0.56	0.56	− 5.04
3	2	1	23.04 ± 0.001	79.23 ± 0.022	48.08 ± 0.32	0.38	0.61	0.38	0.45	0.45	0.45	0.45	− 6.94
3	3	2	19.53 ± 0.29	45.87 ± 0.011	34.65 ± 0.45	0.20	0.15	0.17	0.38	0.38	0.38	0.38	− 8.40

[a]Values are means ± SD of triplicate run, Subscript 'n' is the pre-processed/normalized response, GRC and GRG represents the grey relational coefficient and grade respectively

Figure 2 showed the individualistic effects of each process variables on the grey relational grade (GRG). The maximum average GRG from the signal-to-noise ratio response prediction function were obtained at level-1 of reflux duration (120 min), at level-1 of particle size (1:14), and at level-2 of feed-solvent ratio (1:16) with corresponding SNR of − 4.66, 5.12 and − 4.23, respectively. The decreasing order of significance of each process parameters to the average grey relational grade (GRG) in accordance with the delta ranking with corresponding percentage follow the order: feed-solvent ratio > particle size > reflux duration. The feed-solvent ratio has the most significant contribution to the total phenolic and antioxidant capacity of black pepper extracts, while the reflux duration has the least effects on the combined quality characteristics of the bio-products from heat reflux extraction [10]. An optimal feed-solvent ratio guarantees uniform and efficient heating. Much solvent promotes poor reflux heating because the reflux heat energy is absorbed by the solvent, requiring more reflux duration. A low feed-to-solvent ratio induces a heat transfer resistance because the distribution of active compounds is concentrated in certain locations, thereby limiting the flow of the compounds bioactive constituents from the black pepper cellulosic cell wall [23].

Fig. 2

Main effects plots for S/N ratio

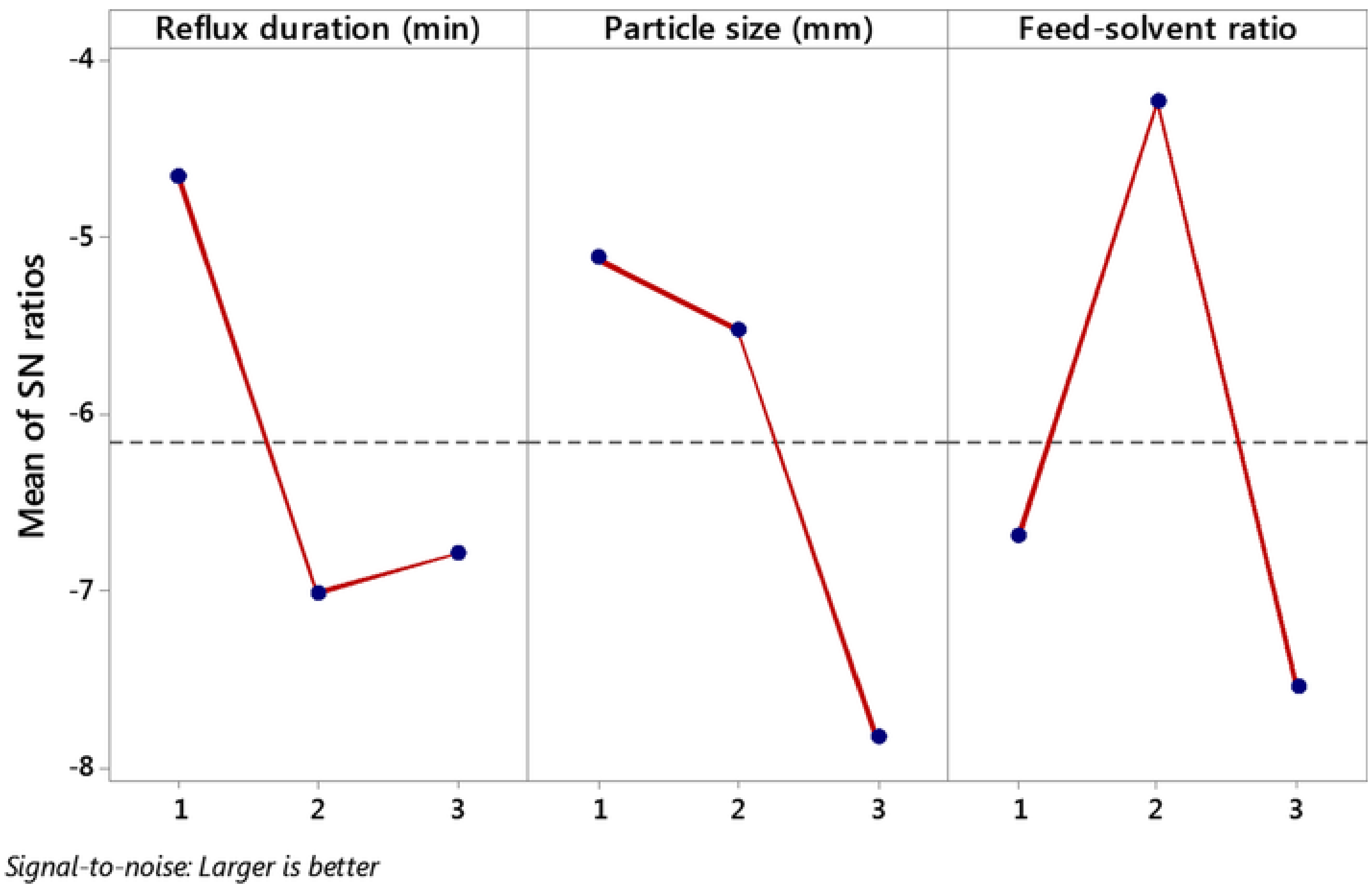


Table 3 highlights the key system parameters revealed from delta statistics for the mean GRG. The delta statistics were determined by subtracting the greatest and lowest average value of each variable. The differential value was then used to determine rankings. The largest delta value was awarded the top rank and indicates the most important factor influencing GRG. According to Table 3, the most relevant parameter is the feed-solvent ratio, which has a delta value of 0.6633. The particle size is the second most significant parameter, with a delta value of 0.5933, closely by the reflux time of 0.6333.

Table 3

Response table for GRG means

Level	Reflux duration (min)	Particle size (mm)	Feed-solvent ratio

Level	Reflux duration (min)	Particle size (mm)	Feed-solvent ratio
β1	0.6333[a]	0.5567	0.4633
β2	0.4600	0.5933[a]	0.6633[a]
β3	0.4633	0.4067	0.4300
Delta	0.1733	0.1867	0.2333
Rank	3	2	1

[a]Represents the desirable level

Confirmatory test

The final phase in grey relational analysis is to predict and validate the improved results of the responses once the best parameters have already been established. After establishing the ideal factor level by GRG the quality characteristics was predicted and subsequently validated. From Eq. (8), the ideal heat-reflux aided extraction process parameters were determined by comparing them against the point prediction feature (i.e., GRA) of difference between total means of GRG and average means from the grey relational degree.

$$GRG_{predicted} = GRG_m + \sum_{i=1}^{m}(GRG_0 - GRG_m) \tag{8}$$

where GRG_0 the maximum of mean GRG at optimal operational level, GRG_m is the mean GRG.

Based on an average mean GRD and the overall mean GRG, optimum process parameters for heat refluxing were determined. The mean GRD (i.e. GRG_m) was estimated to be 0.5189, by adding all the mean GRG in Table 3 and finding the average by dividing by m (= 9). Moreover, GRG_0 is the maximum mean GRG for each factors as indicated by asterisk sign (i.e. 0.6333, 0.5933 and 0.6633). The predicted GRG (i.e. $GRG_{predicted}$) was estimated to be 0.8936 using Eq. (8). By comparing the predicted GRG ($GRG_{predicted} = 0.8936$) and the experimental GRG (i.e.$GRG_m = 0.5189$), the validation experimental findings are in satisfactory correlation with the predicted values with 41.9% improvement. The improvement in experimental results over the preliminary designed initial parameters validates the Taguchi approach in combination with grey relational analysis for significantly maximizing the total phenolic content and antioxidant recovery from black peppercorn using the heat reflux technology.

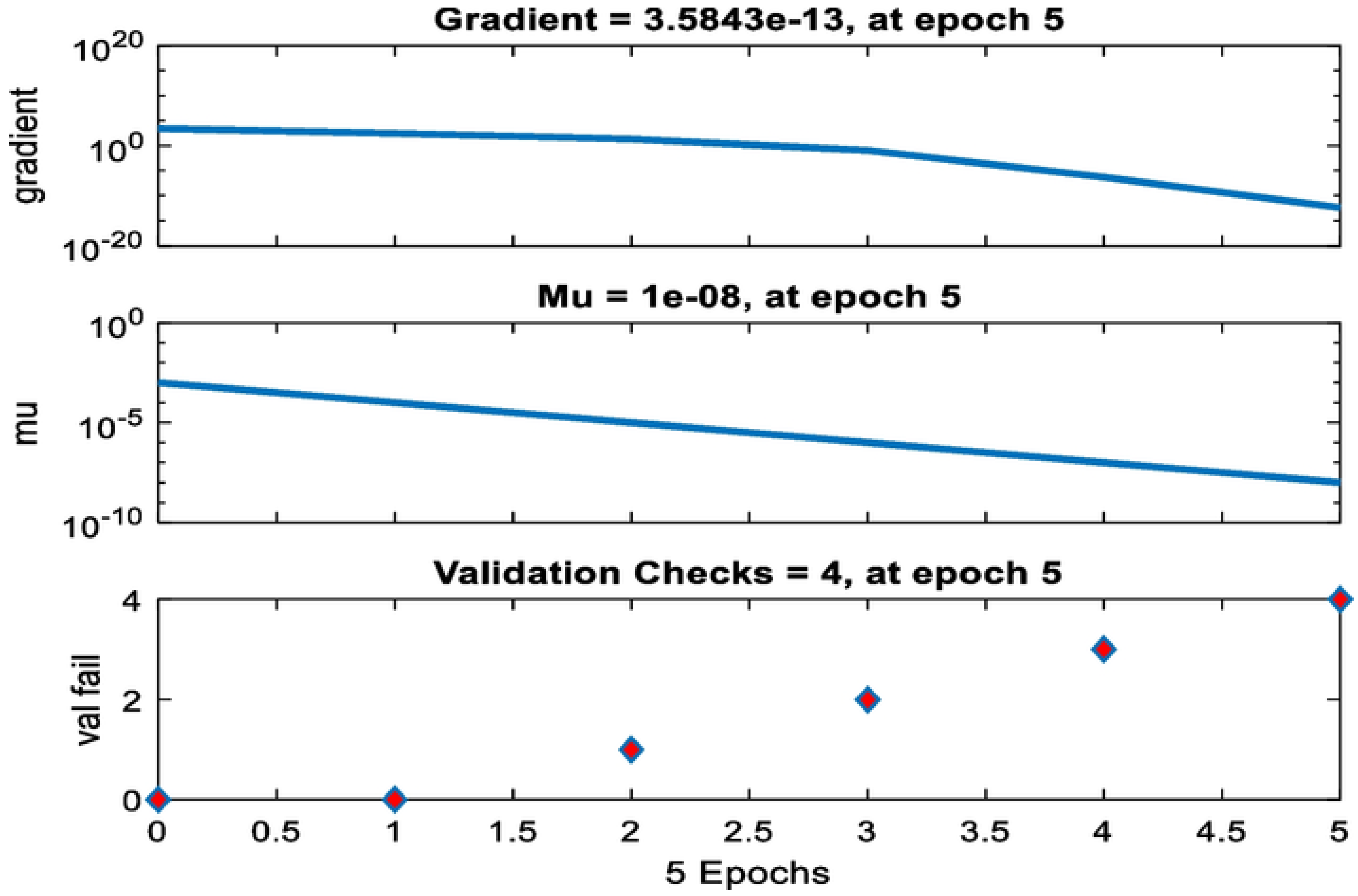
Training and neural network analysis

The neural fitting technique was used in selecting data, design a network, and train it. Its evaluation was done using the mean square error (MSE) and regression analysis coefficient (R^2). The back-propagation technique was used as training algorithm and Levenberg–Marquardt approach was used to minimize errors in MLP networks. Supervised training is performed on the dependent and independent variables.

For the ANN modelling, a total of 9 samples were used for training, testing, and validation. Errors are propagated back across the neurons throughout the training process, and the weights of the neurons are modified as a result. This training procedure is repeated until the desired outcome is achieved. The network was trained for 9 iterations after trial and error was used to identify the best number of neurons for the hidden layer. The trained network has a mean square error (MSE) of 3.7646E−07 and R^2 of 0.9500 as training function outcome. Thus, once the network's performance approaches optimal fit, it endures training for another three epochs before terminating the training process, preventing the network from over-fitting. The best fit point is checked in the neural network performance parameter at 3.5843E−13 as shown in Fig. 3.

Fig. 3

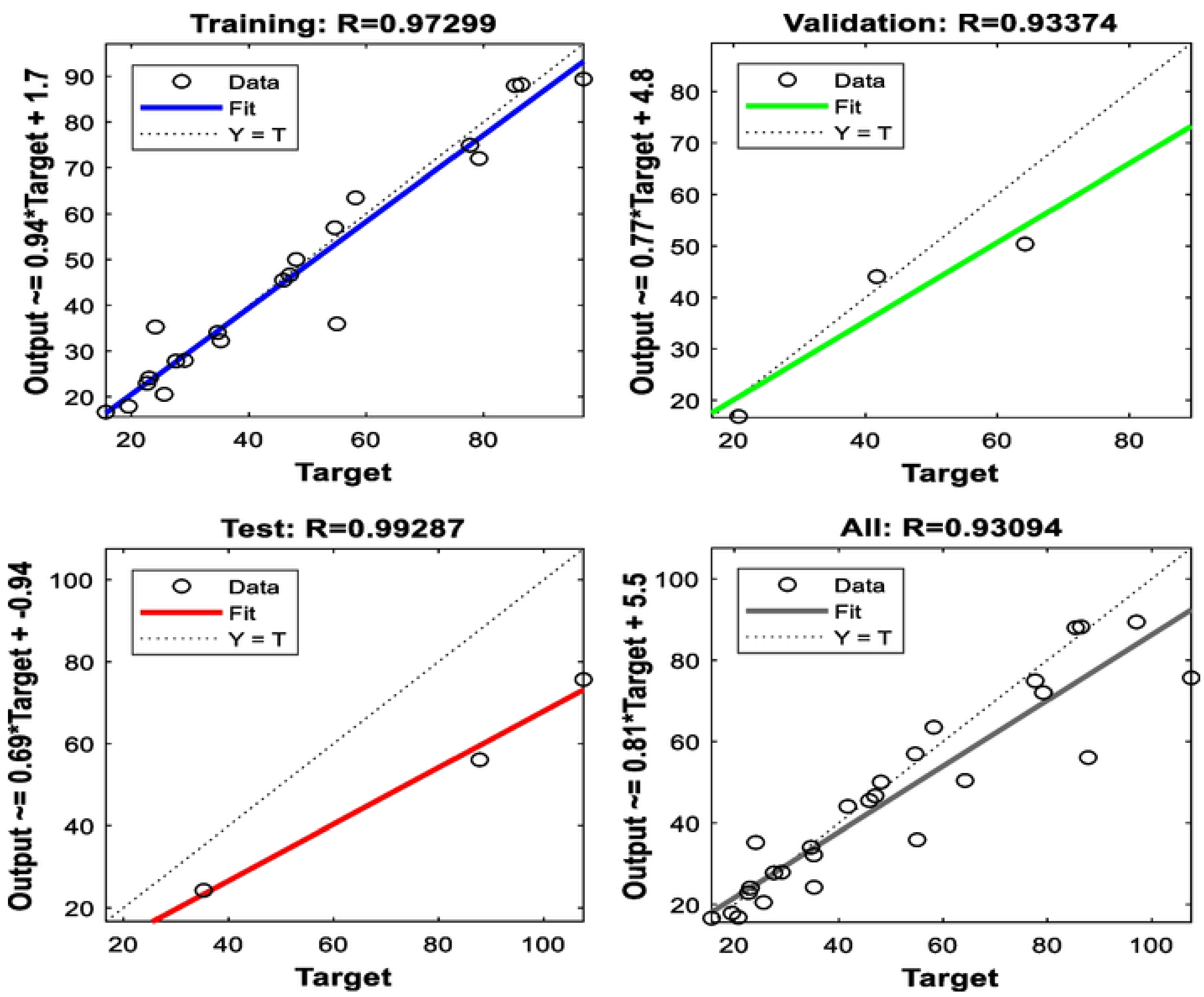
Neural network training states plots



In order for the training process to be successful, the regression value for the training process must be high, and larger regression coefficients acquired between network outputs are a sign of successful training in the supervised neural learning process. Furthermore, when the number of iterations in the training process was increased, the mean square error produced from the training process showed a minor decrease in magnitude. The lower RMSE values further supported the validity of a solid training programme and improved prediction [17]. As indicated in the graph, the best fit point occurs at epoch number five. The parity plot of the training, testing, and total data sets is shown in Fig. 4. The regression coefficient quantifies the relationship between predicted response (output) and experimental data sets [18].

Fig. 4

Training, testing, validation and whole data parity plots



Conclusion

Taguchi technique and grey **AQ5** relational analysis were used to effectively optimise heat reflux-assisted extraction (HRAE) of black pepper seeds in this investigation. The total recovery of phenolic and antioxidant capacity of black pepper seeds were determined under the optimal reflux conditions, with a feed-solvent ratio of 1:16 black pepper seeds, 0.2 mm particle size, and 120 min of reflux. Under this condition, the total phenolic content, DPPH, and H_2O_2 scavenging activity were measured to be 35.23 ± 0.004 mgGAE/g d.w, 107.57 ±

0.04 g/mL, and 87.78 ± 0.32 g/mL, respectively. Moreover, using the Levenberg Marquardt (LM) neural network approach with 10 hidden neurons and a 3–5–10–1 model architecture, the experimental data effectively trained and with better prediction. The trained network has an R^2 of 0.9500 and a mean square error (MSE) of 3.7646E−07, which indicated a good, predicted output. The relative redesigned heat-refluxing experimental set-up therefore shows a good potential for an optimised and intensified process scale-up in biopharmaceutical industry.

Publisher's Note

Declarations

Conflict of interest Authors declare there is no conflict of interest whatsoever.

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