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EXTRACTION OF PHENOLIC COMPOUNDS FROM FENUGREEK SEEDS: MODELLING AND ANALYSIS USING ARTIFICIAL NEURAL NETWORKS

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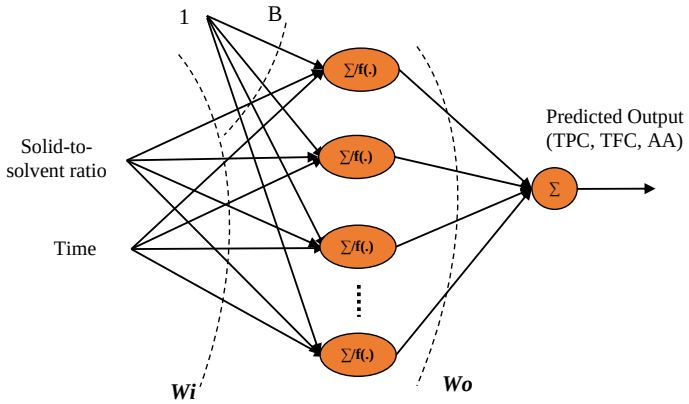
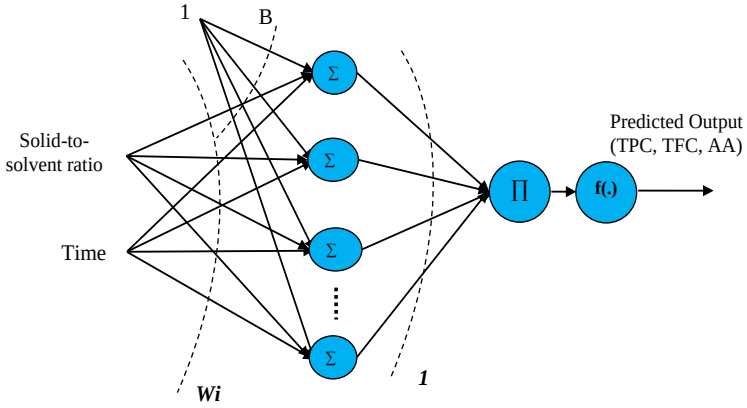
Highlights

- Modeling and analysis of the extraction process of bioactive compounds for fenugreek seeds.
- Experiments to collect the data of total phenolic compounds, total flavonoid content and antioxidant activity.
- High modelling performances with conventional artificial neural-networks and Pi-Sigma neural-networks.

Graphical Abstract



Modeling of bioactive compounds using conventional ANN model



Modeling of bioactive compounds using PSNN model

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ABSTRACT: This study introduces the modeling and analysis of the extraction process of bioactive compounds from fenugreek seeds in different solid-to-solvent ratios (0.5-60 g/L) and extraction times. Maceration was applied with agitation for the extraction processes and total phenolic compounds, total flavonoid content and antioxidant activity of the extracts were measured as experimental data. The amount of extractable phenolic compounds having antioxidant effect was increased by adjusting the solid-to-solvent ratio. According to obtained results, the highest values were determined as 12564.08±376.88 mg gallic acid/100 g dry sample, 7540.44±39.67 mg quercetin/100 g dry sample and 1904.80±17.43 mM Trolox/100 g dry sample for total phenolic compounds, total flavonoid content, and antioxidant activity, respectively. The extraction process was modeled using standard Artificial Neural Networks (ANN) and Pi-Sigma Neural-Networks (PSNN). The PSNN model had a higher prediction efficiency with lower RMSE (%) values varied between 0.94% and 1.30% for both training and testing.

Keywords: Fenugreek Seed, Phenolic Compounds, Antioxidant Activity, Modelling, Artificial Neural Network

1. INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L.) is a legume of the *Leguminosae* family that grows in the Mediterranean region, and its seeds are the most valuable plant part. Fenugreek seeds were used as medicine in ancient times, and today it is generally used as a spice with its unique flavor [1-2]. In addition to being preferred to give taste and flavor to curries, the seeds are used in the food industry to enhance flavor, prevent bacterial contamination, and extend shelf life. Fenugreek seeds are also added to many food products such as bakery and meat products, alcoholic beverages, candies, syrups, and sugary sauces, chewing gums, and sugar creams [3]. Also, fenugreek has been widely used as an important dietary supplement in several countries and most supplements are made from fenugreek seeds or seed extracts [2].

Fenugreek seed is a product with a very rich composition in terms of phenolic substances, dietary fiber, protein, oil, nitrogenous compounds, various minerals, and vitamins. Due to its rich composition, fenugreek seed is known to have important and positive effects on human health [4-5]. It is known that fenugreek seeds have antioxidant and antimicrobial properties, anti-inflammatory, hypoglycemic, antipyretic, hypolipidemic, anticholesterolemic, anthelmintic, antileprotic and antibronchitic effects [3, 6-7]. These effects of fenugreek seeds have been associated with flavonoids such as vitexin, tricine, narinjenin, quercetin, luteolin and molecules with antioxidant effects such as ascorbic acid, glutathione, β-carotene, alpha tocopherol in their extracts [8-10]. Therefore, the extraction of phenolic compounds having antioxidant activity from fenugreek seeds can potentially increase the economic value of this plant and create new usage areas for the product.

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Artificial neural networks (ANNs) are one of the well-known learning models that holds an important place in artificial intelligence and have been recently used in many applications of food processes [11]. So far, single-layered ANN models are used in many fields of science due to design and implementation advantages. The hidden layer and output layers of these ANN models are usually in the same standard form. Therefore, a numerous number of ANN synthesis studies can be found using this standard form in literature. However, single-layer ANN models are not uniform either. High-order neural networks (HONN) are also single-layered ANN models [12], and have likewise fast learning capacity, strong approximation, large storage capacity, high fault tolerance, and accurate mapping capability. On the other hand, multi-layered neural networks have disadvantages due to the large number of parameters, time-consuming training process for the complex and nonlinear processes [13]. The fundamental difference of conventional ANN and HONN models is based on the output construction that can be constructed with multiplicative and additive neuron models [14]. Pi-Sigma Neural-Networks (PSNN) are a class of HONN with a high-approximation capability that were introduced in [12]. PSNN is much more successful in prediction of time series or time-series-like data compared to conventional one hidden-layered ANN and other HONN models [13-15] and has fast convergence speed compared to the multi-layer perceptron networks [16]. Recently, convergence analysis of a new sigma-pi-sigma neural-network convergence analysis has been presented in [17]. In food processes, there are time series-like behaviors under unknown inputs of the environment and PSNN model has been known to be very successful in estimating time series.

In literature, ANN based modelling has been recently applied for simulation and optimization for the extractions of phenolic compounds from garlic [18], green and black tea [19], cocoa shell [20], sweet potato peel [21], peppermint [22], grape skin pomaces [23], and to predict the particle size of phenolic compounds in nano-systems [24]. However, there have been no studies modeling and analyzing the extraction of phenolic compounds from fenugreek seeds by ANN. Furthermore, with the best knowledge of authors, PSNN model has not been used in food processes of literature. Due to the importance of bioactive components, extraction must be achieved efficiently, so this study is conducted to analyze and optimize the extraction process. The aims of the present study are to propound the contents of phenolic compounds and antioxidant activity of fenugreek seeds, and to model the extraction process and simulate the effects of solid-to-solvent ratio on extraction efficiency.

2. MATERIALS AND METHODS

2.1. Material

Fenugreek seeds were purchased from a local market in Turkey. After removing the impurities, the seeds were granulated using a grinder (Sinbo SHB 3020, Turkey). The particles were sieved using a pore diameter of 630 μm sieve and the powder taken under the sieve was used in extraction processes. All chemicals (Folin-Ciocalteau reagent, sodium carbonate (Na_2CO_3), sodium nitrite ($NaNO_2$), aluminum chloride ($AlCl_3$), sodium acetate (CH_3COONa), 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, Trolox, quercetin) used were of analytical better/grade.

2.2. Extraction processes

Extraction of polyphenols from fenugreek seeds were carried out using classical extraction only with distilled water as a solvent. A magnetic stirrer was used at 400 rpm for agitation of the fenugreek powder and distilled water. The effect of extraction time and solid-to-solvent ratio on total phenolic compounds (TPC), total flavonoid content (TFC) and antioxidant activity (AA) of the extracts were investigated. Accordingly, 30-360 minutes of extraction times and solid-to-solvent ratio of 0.5-60 g/L were chosen as the extraction parameters. All the extraction processes were performed at 25°C. After extraction processes,

samples were centrifuged at 6000 rpm for five minutes, and supernatants were used for the analysis. All the experiments were duplicated.

2.3. Total phenolic compounds (TPC)

Folin-Ciocalteau method was used for determination of TPC present in fenugreek seed extracts [37]. The extract was mixed with diluted Folin-Ciocalteau at a ratio of 1:1 (v/v). A solution of Na_2CO_3 (210 g/L) was added to this mixture. The mixture was incubated at room temperature for 25 minutes and then the samples were centrifuged at 3800 rpm for 10 minutes. The absorbances of the supernatants were measured at 760 nm (PG Instruments T80, UK). TPC was presented as mg gallic acid/100 g dry sample.

2.4. Total flavonoid content (TFC)

TFC of the fenugreek seed extracts was determined by aluminum chloride method [6]. The extract diluted with distilled water was mixed with $NaNO_2$ and incubated for 5 minutes. Then 10% $AlCl_3$ was added, and incubation was continued for 6 minutes more. At the end of the incubation, 1 M NaOH solution was added and the absorbances of the samples were read at 510 nm wavelength. Total flavonoid compounds are expressed as mg quercetin/100 g dry sample.

2.5. Antioxidant activity (AA)

AA of the samples were measured with DPPH radical scavenging activity method [25]. 50 μL of sample was mixed with 0.1 mM (prepared in ethanol) of 1.95 ml DPPH. After incubation for 30 minutes, the absorbance of the samples was determined at 515 nm wavelength. The AA of the samples was explicated as mM Trolox/100 g dry sample.

2.6. Predictive modeling using artificial neural-networks

The extraction process is mathematically assumed as nonlinear, discrete-time, casual, two-input and single-output static mapping function which is modeled by artificial neural-network as:

$$\hat{y}_k = ANN\left(t_k, C_k, \widehat{W}\right) \tag{1}$$

where $\hat{y}(k)$ is the predicted output variable, ANN is the optimized artificial neural-network model, k is the experiment number or sample index, t_k is the time (duration of extraction process), C_k is the solid-to-solvent ratio and $\widehat{W}$ is the optimized parameters of the network. Designed model provides the predictions of there outputs such as TPC, TFC and AA at the same time. By doing that any of the input time and solid-to-solvent ratio input values the output predictions can be obtained by the designed artificial neural-networks. Figure 1 illustrates a one hidden layer standard ANN structure where its functional form is formulated as:

$$\hat{y} = W_o^T f(W_i x + B) \tag{2}$$

where $\hat{y}$ is the predicted output and x is the input values of the model. The W_i and W_o are input-layer and output-layer weighting parameters, respectively. The B vector is the bias parameter vector for hidden-layer neuron to prevent their sleepness. In equation (2), total parameter matrix can be rewritten as $\widehat{W} = [W_o\ W_i\ B]$ to be optimized for the construction of optimal model for input-output approximation. The $f(.)$ is the activation function of the neuron cells. Conventional logistic function is

$$f(x) = \frac{1}{1+e^{-x}} \tag{3}$$

here used as an activation function of the neurons [12, 15].

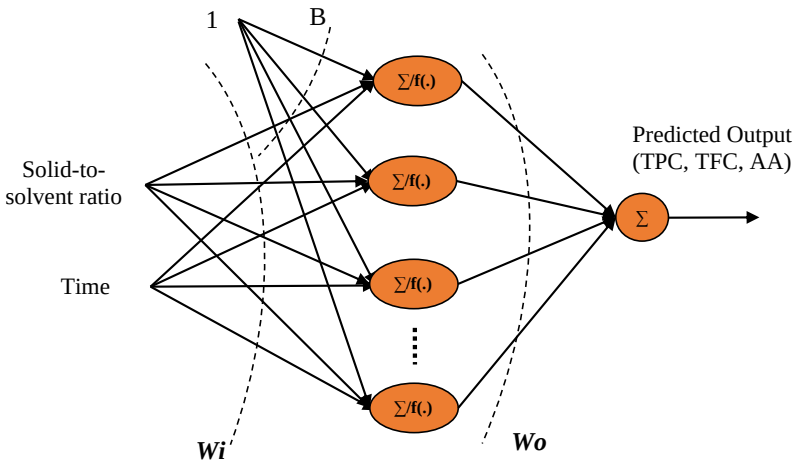


Figure 1. Standard ANN model.

2.7. Pi-Sigma Neural-Network (PSNN)

PSNN model was first introduced in Shin and Ghosh [12] where the multiplication of the linear combinations of the network inputs construct the output of the network. The linear combination of the network inputs shows the degree of the PSNN. When the problem definition is highly nonlinear and complex, in order to model the input-output behavior, there is need a relatively large degree. The large degree of PSNN provides better prediction results, but needs more computational time of training due to the overfitting. For standard regression data, small number of the linear combination might be enough for the output modeling. The PSNN model with n inputs and m outputs are illustrated in Figure 2. The linear combination of the inputs are calculated by tunable W_i parameter and biases parameter vector B is added to calculte the outputs of the hidden neuron cells. The j[th] neuron output of the hidden-layer is calculated as

$$h_k = f_h(\sum_{i=1}^{n} W_{ik} x_i + B_k) \quad k = 1 \dots H \tag{4}$$

where, f hidden activation function is selected as a linear activation function as $f_h(x) = x$. However, the outputs are calculated using logistic activation function $f(x) = \frac{1}{1+\exp(-x)}$ as

$$\hat{y}_r = f(\prod_{k=1}^{H}(h_k)) = \frac{1}{1+\exp(-\prod_{k=1}^{H}(h_k))} \quad r = 1 \dots m \tag{5}$$

In Figure 2, the neuron activation functions of hidden layer use linear combination of previous features then summation passes through a linear activation function. The outputs of the hidden layer neurons are multiplied to construct the output of the network where these outputs are passed through a nonlinear activation function such as logistic, tangent hyperbolic etc. The most important difference is that the W_o weight parameters of the PSNN are fixed constant and not trained. Input data of the designed models are normalized to [0,1] interval and LM optimization is used to optimize their parameters. The output prediction performance of the models is calculated by RMSE given as

$$RMSE = \sqrt{\frac{1}{N}\sum_{k=1}^{N} |\hat{e}(k)|^2} \tag{6}$$

where N is the number of the testing dataset and $\hat{e}(k) = y(k) - \hat{y}(k)$ is the prediction error of k[th] index [13].

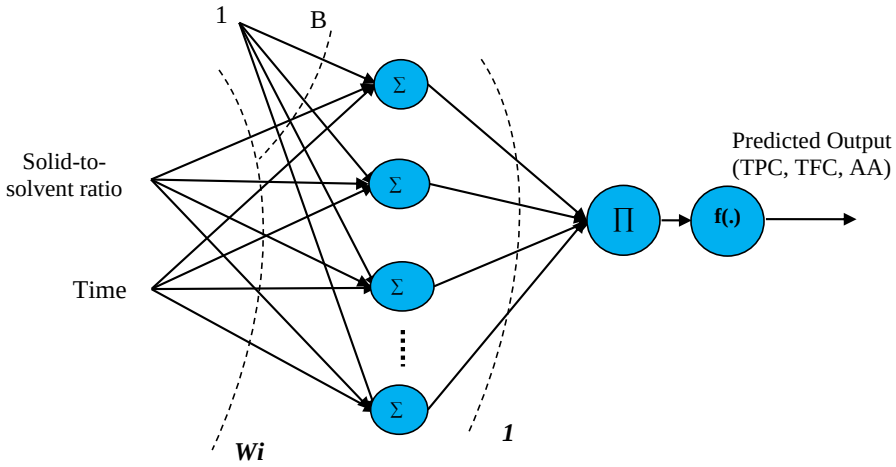


Figure 2. PSNN model.

3. RESULTS AND DISCUSSION

3.1. Effect of extraction parameters on bioactive extracts

Extraction is a process in the recovery of bioactive compounds from natural sources. The extraction conditions depend on factors such as the extraction method to be used, solvent type, pH, temperature, sample-to-solvent ratio, and extraction time as well as the nature of the bioactive components to be extracted [25]. Therefore, it is necessary to know how these parameters affect the extraction efficiency in obtaining fenugreek seed extracts rich in phenolic compounds. In this study ten different solid-to-solvent ratios varied from 0.5-60 g/L were used at different extraction times keeping all other parameters constant. According to obtained results, at 5 g/L and 180 min, the highest values were determined as 12564.08±376.88 mg gallic acid/100 g dry sample, 7540.44±39.67 mg quercetin/100 g dry sample and 1904.80±17.43 mM Trolox/100 g dry sample for total phenolic compounds, total flavonoid content, and antioxidant activity, respectively. When the extraction studies with fenugreek seeds were examined, it was seen that the TPC of the fenugreek seed extracts obtained using organic solvents such as methanol, ethyl acetate and hexane was 942-10631.6 mg gallic acid/100g dry [6, 8]. In addition, using pure water only, the TFC value was determined as 1749 mg quercetin/100 g dry sample, and it was reported that TFC comprised approximately 37% of the total phenolics in fenugreek seeds [4]. In a different study, it was stated that fenugreek seeds can contain flavonoids up to 100 mg/g dry sample level depending on the climatic conditions of the region [26]. From this point of view, higher values than the literature obtained in the current study showed that water can be used as a successful solvent that contains a solution to the problem of solvent toxicity as well as being inexpensive, highly accessible and environmentally friendly.

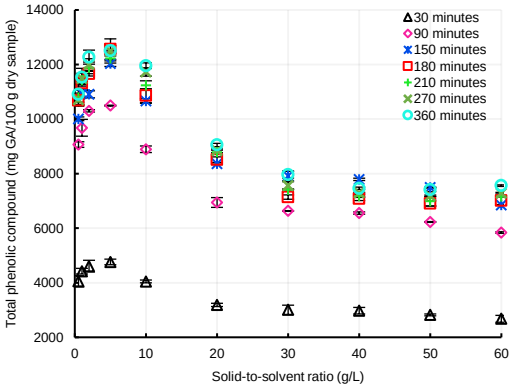
Fig 3a, Fig 3c and Fig 3e illustrate the effects of solid-to-solvent ratio on total phenolic compounds, total flavonoid contents, and antioxidant activity of fenugreek seeds. The amount of extractable phenolic compounds with antioxidant effect was increased by adjusting the solid-to-solvent ratio. It was seen that the amount of extracted phenolic compounds increased with increasing in the solid-to-solvent ratios up to a certain point (5 g/L), but then decreased and remained almost constant with the increase of the solid-to-solvent ratio (Fig 3). The concentration gradient between the vegetable tissue and the solvent is increased by increasing the amount of solvent for a fixed amount of solid matrix, resulting in a faster extraction rate [27]. Therefore, the increase in the amount of phenolic compounds in the extracts at increasing solid concentrations (up to 5 g/L) can be explained by the increase in concentration gradient. However, Wani et al. [36] stated that more gums could be extracted from the fenugreek samples rather than bioactive compounds if the distilled water is used as a solvent. Due to the increase in solid matter

content at high solid-to-solvent ratios (after 5 g/L), more gums were extracted, and highly viscous solutions were obtained during the process which may have affected the filtration process, resulting in lower TPC and TFC values. A similar trend of the TPC and TFC was observed for the AA of the samples which indicated that the phenolics and flavonoids were responsible for the antioxidant activity of the fenugreek seed extracts (Fig 3e). Hence, the literature studies [28-30] revealed that AA of fenugreek seed extract was highly correlated with TPC and TFC.

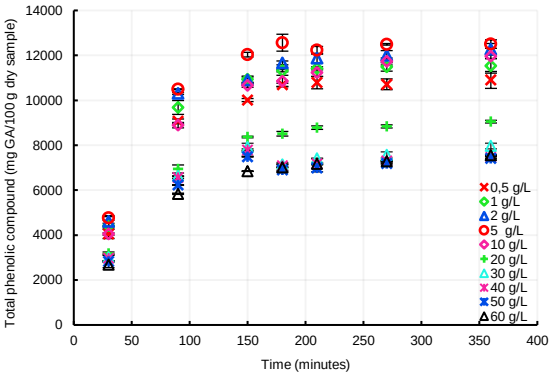
As can be seen from Figure 3b, Fig3d and Fig3f, the highest extraction rate was reached in the first 150 minutes. However, after this period, the extraction rate started to decrease and almost stabilized. As the concentration gradient decreases over time, the extraction yield starts to decrease with the increasing extraction times. Therefore, an excessive time is not needed to be able to extract more phenolic compounds from fenugreek seeds. Similar results observed in different extraction studies of phenolic compounds [31-33].

3.2. Predictive modeling of extraction process

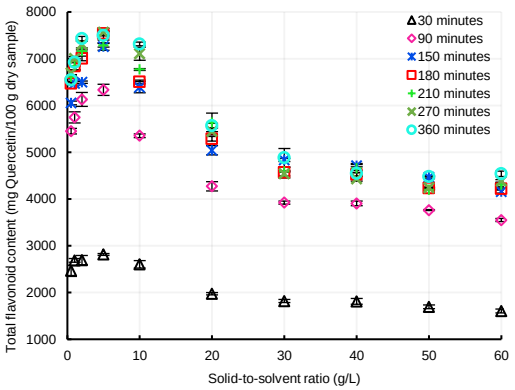
Artificial neural network (ANN) modeling is a challenging method in the estimation and prediction process parameters and food properties in a broad spectrum such as estimation of coumarin extraction yield from *Cuscuta reflexa* [34], prediction of particle size and polydispersity index of phenolic compound–loaded nanosystems [24], microwave-vacum drying characteristic of carrot [35], and etc. In this study, there are performed 70 experiments with different time periodes and concentration rates such that modeling of phenolic compounds for the extraction process is achieved based on all data sets or experiments instead of one-by-one time periods and solid-to-solvent ratios. It means that the designed models correspond to all cases of the extraction process. In the future, anyone can predict an estimate of phenolic compound output for a different combination of the solid-to-solvent ratio and time. Table 1 shows the RMSE and RMSE (%) performance results of the prediction errors for all modeling experiments. Remember that the ANN is the one hidden-layered conventional neural-network, PSNN is also one hidden layered higher-order neural-network with different functionalized outputs. RMSE results indicate a mean error per data calculated by Equation (6). However, $RMSE\ (\%) = \frac{RMSE}{\max(|y|)} \times 100$ shows how large the obtained RMSE is relative to the maximum value of the output. RMSE value can be considered relatively large for a neural-network prediction but, its RMSE (%) is very small which shows that very acceptable predictions are obtained for the modeling of the extraction experiments. In other words, designed models have a high learning and generalization capability for the extraction process. Although it has fewer parameters, PSNN was shown here with experimental results that it predicts much better than standard ANN, and it is predicted to be used in future food processes.



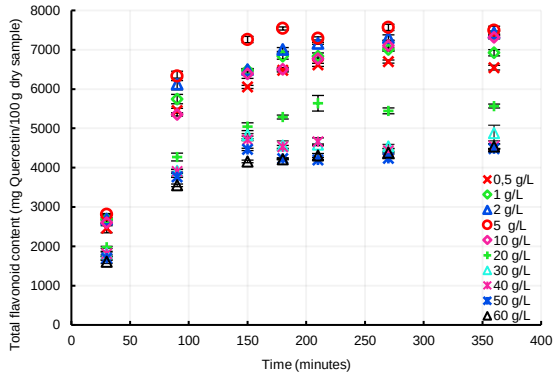
(a) solid-to-solvent ratio vs. TPC



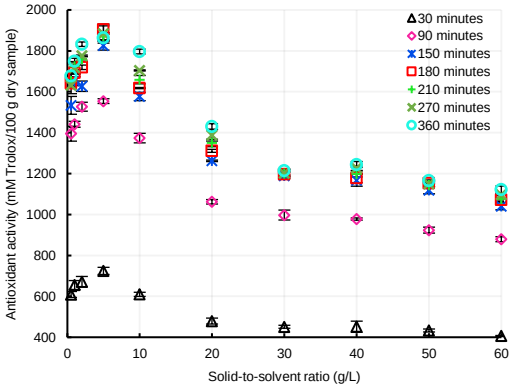
(b) time vs. TPC



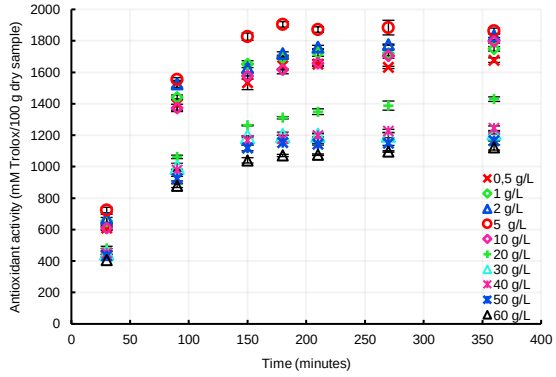
(c) solid-to-solvent ratio vs. TFC



(d) time vs. TFC



(e) solid-to-solvent ratio vs. AA



(f) time vs. AA

Figure 3. Experimental results (a-b) total phenolic compounds (TPC), (c-d) total flavonoid content (TFC), (e-f) antioxidant activity (AA).

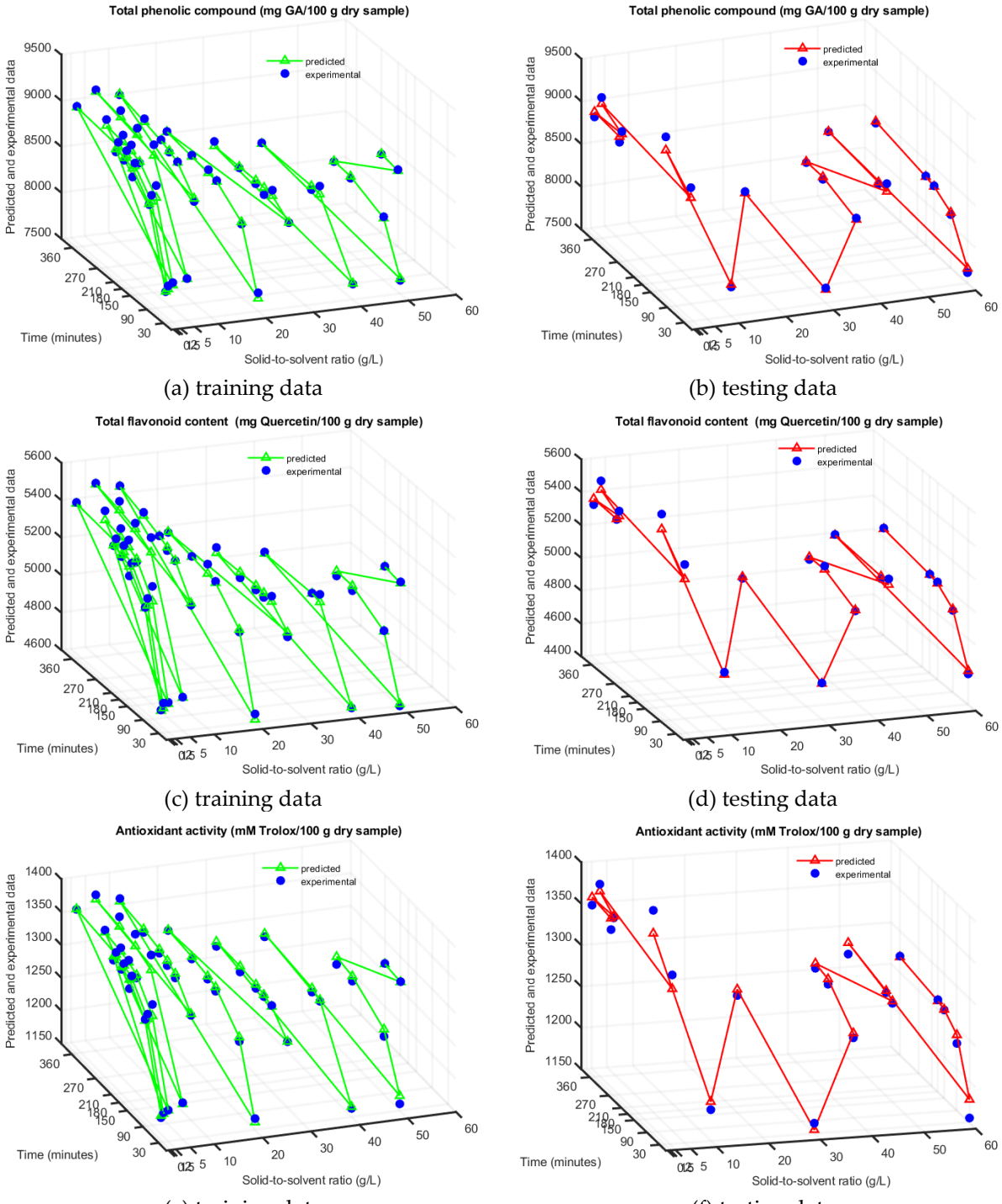
Table 1. RMSE performances of neural networks.

		For Training Data			*For Testing Data*		
		TPC	TFC	AA	TPC	TFC	AA
ANN	RMSE	142.02	212.12	16.28	178.95	201.87	24.20
	RMSE (%)	1.13	2.80	0.85	1.42	2.67	1.27
PSNN	RMSE	118.75	66.93	15.92	163.19	96.67	20.23
	RMSE (%)	0.94	0.87	0.83	1.30	0.27	1.06

Figure 4 illustrates modeling and prediction results where the collected data of the extraction experiments are divided into two parts as training and testing parts. The training dataset has 50 data points, testing dataset has 20 data points where both randomly chosen from the experiments. Then, modeling and prediction results are plotted according to randomly selected datasets. Notice that we did not use validation dataset for the structural design of the neural networks instead, we applied grid-search for the model design. According to the grid search of parameter space, optimal neural-networks models are obtained as follows: i) ANN has 1 hidden layer with 30 neurons and totally 90 weighting parameters, PSNN has 1 hidden layer with 10^{th} order output neuron then there exist totally 30 weighting parameters, respectively. Figure 4a and Figure 4b demonstrate the prediction results of TPC for training and testing samples. There is seen that 7 experiments exist with 10 data points where data points are taken in cascade form. In the extremum points of the TPC, there are relatively large prediction errors. Figure 4c and Figure 4d show the prediction results of TFC. Note that all plots are obtained by PSNN model with 10^{th} order. In the TFC modeling, the same random points are used for training and testing, but small RMSE errors are calculated. From Figure 3, it is seen that the changes on the TFC data are relatively small compared to the TPC and AA therefore ANN models can easily approximate and predict the future values. Figure 4e and Figure 4f give the prediction results of AA. Even though training data points are modeled with the smallest RMSE performances, the RMSE results of testing are large. In general, it is provided that all RMSE results are less than 1.3% which is very acceptable for future applications.

4. CONCLUSIONS

This study presents two important results. The first is the investigation of the effects of solid-to-solvent ratio and time on the extraction of phenolic compounds. Although the amount of phenolic compounds and antioxidant capacity obtained at different solid-to-solvent ratios and times vary, quite high amounts of phenolic compounds were obtained in the applied process. The experimental results showed that even if only water is used as a solvent, fenugreek seed extracts containing high amount of bioactive components can be obtained by adjusting the solid-to-solvent ratios and processing time. These extracts can be used for the development of functional foods and/or pharmaceutical industry for their health benefits if the extraction process can be scaled up. The second is modeling and estimation of the extraction processes with two different neural-network models such as standard ANN and PSNN. By simulating the extraction process with the created ANN models, bioactivity can be predicted for different conditions, thus facilitating the transfer of processes to a large scale. In addition, modeling and prediction are needed to obtain optimal phenolic compounds for automatic extraction processes.



(a) training data (b) testing data

(c) training data (d) testing data

(e) training data (f) testing data

Figure 4. Prediction of (a-b) total phenolic compounds (TPC), (c-d) total flavonoid content (TFC), (e-f) antioxidant activity (AA)

Declaration of Ethical Standards

Authors declare to comply with all ethical guidelines, including authorship, citation, data reporting, and original research publication.

Credit Authorship Contribution Statement

Selami BEYHAN: The author conducted modelling and analysis of data and edited the article.
Hilal İŞLEROĞLU: The author performed real-time experiments and edited the 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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Data Availability

Research data has not been made available in a repository.

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