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Estimating Hydroxymethyfurfural (HMF) Concentration Via Modified Seliwanoff Test Using Artificial Neural Network (ANN)

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HIGHLIGHTS

- A linear relationship was revealed between HMF concentration and red color resulting from the modified Seliwanoff test.
- Artificial intelligence interpretation of the concentration-color relationship were evaluated.
- The modified Seliwanoff test has been shown to be used for HMF determination.

Abstract: Hydroxymethylfurfural (HMF) is a quality indicator, especially in foods where changes in protein-carbohydrate interactions are observed during the applied process. In this study absorbance and L*, a*, b* values of red color emerged due to the relationship between hydroxymethylfurfural (HMF) and resorcinol during the modified Seliwanoff test were used as input data artificial neural network (ANN) to determine the HMF concentration for the first time. A linear relationship, between HMF concentration and absorbance of red color, can be represented by equation absorbance = 0.0020 + 0.0012* concentration of HMF (mg L^{-1}) with R^2 = 99.6%, Fisher ratio: 0.18, p value of lack of fit: 0.975, correlation coefficient: 0.9960. Intra-day and inter-day precision expressed as relative standard deviation (RSD) %, were 2.35 - 3.65% and 3.16 - 4.73%, respectively. Recovery rates and RSDs were in the range of 99.34 - 100.47% and 1.58 - 3.68%. It showed high correlation compared to HPLC method used as reference method (0.998). The R^2 values of ANN for estimation of HMF concentration were found 0.90 for training, 0.96 for validation, and 0.99 for testing and AARD was found 8.85%. Evaluation of the absorbance and L*, a*, b* values of the red color with artificial intelligence is a reliable way to determine the HMF concentration.

Keywords: hydroxymethylfurfural (HMF); seliwanoff test; artificial neural network (ANN).

INTRODUCTION

Hydroxymethylfurfural (5-hydroxymethyl-2-furaldehyde) or HMF is a furanic compound with a cyclic aldehyde structure. It consists of aromatic alcohol, aldehyde and furan ring [1, 2]. HMF is formed as a result of two main reactions known as non-enzymatic browning reaction: (1) as an intermediate product as a result of Maillard reaction, (2) by dehydration of hexoses in acidic environment (caramelization) [3].

While HMF can be used as one of the quality parameters that can be used in response to optimize process conditions [4], in some cases it is an important compound that must be monitored for the determination of adulteration in foods and in some cases for food safety [5]. Therefore, when HMF is considered as a quality and safety parameter, it appears as a chemical indicator that should be examined in food quality and food safety issues. In Codex Alimentarius, created by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO), HMF upper limit value for honey is specified as 40 mg/kg [6, 7]. The European Economic Community Fruits and Vegetables Association of the Fruit and Nectar Industry (AIJN) has defined the HMF amount among the absolute quality parameters of fruit and vegetable juices (maximum HMF amount is maximum 20 mg/L in fruit juices and 25 mg/kg in concentrates) [8]. Although some studies have found carcinogenic and mutagenic activity of HMF both in vitro and in animal assays [3, 9], others have detected positive effects like antioxidant, anti-allergen, anti-sickling and anti-inflammatory capacities [10, 11].

In addition to importance of HMF in the food industry, HMF is also thought as a typical biomass-derived platform compound to use to synthesize numerous high-quality fuels and high-value chemicals [12-14]. In environmental applications, positive properties of HMF come to the fore as it is a chemically active compound due to its functional group content [12].

Two main analytical methods, spectrophotometric and chromatographic, are commonly recommended for HMF determination [15]. Except from spectrophotometric and HPLC method, some of the proposed methods as new in the literature for HMF detection are: flow injection method [16], ATR-FTIR [17], DART/TOF-MS [18], NMR [19], Capillary-electrophoresis tandem mass spectrometry ($CE\text{-}MS^2$) [20], electrochemical biosensor chip [21], Micellar Electrokinetic Capillary Chromatography [22, 23] and ELISA [24].

The proposed spectrophotometric methods are known as White (1979) method in the UV region and Winkler (1955) method in the visible region. As the chromatographic method, it is the reverse phase HPLC method. While the White method is based on the reaction of HMF with sulfite ion (HSO3-) and detection at 284 nm [25], the Winkler method is based on the absorbance measurement at 550 nm of the colored complex formed as a result of the reaction between HMF and p-toluidine in the barbituric acid environment [26]. In some studies, the weak specificity of barbituric acid to HMF and the use of toxic chemicals such as p-toluidine are stated as disadvantages of these spectrophotometric methods [27]. Also chromatographic determination with expensive equipment such as HPLC causes this method to be inadequate in terms of easy applicability. Therefore, in order to be an alternative to other spectrophotometric methods, there is a need for new, short analysis time, environmentally friendly and easily applicable procedures for HMF detection.

In the previous study [28], a new spectrophotometric method (modified Seliwanoff test) was reported for the detection of HMF and performed validation studies in honey matrix. The method developed is based on the Seliwanoff test, a qualitative colour test developed by Russian chemist Theodor Seliwanoff, is well-known colour reaction for ketoses and occurs boiling aqueous hydrochloric acid (HCl) containing resorcinol. With that study HMF concentration was determined quantitatively obtained by spectrophotometer according to the novel method developed by modifying the Seliwanoff test, which is a qualitative method.

Artificial Neural Networks are one of the artificial intelligence techniques that imitate the working structure of the human brain [29]. Nowadays, artificial neural networks are mainly used in areas such as diagnosis, classification, prediction, control, data association, and data filtering [30]. There are many areas where artificial neural networks are used. Its main areas of use include macroeconomic forecasts, assessment of bank loans, exchange rate forecasting, risk analysis, separation and recognition of objects or images, optimization of production systems, product analysis and design, quality analysis and control of products, planning and management analysis, analysis of cancer cells, control of robot systems are nonlinear system modeling, image processing, character handwriting and signature recognition, data mining [31]. There are many studies, such as the followings, that facilitate the traceability of food quality control by using the change in the physical or chemical properties of foods as inputs in the ANN approach: using colorimetric system along with ANN approach for evaluation of fish freshness [32], obtaining colorimetric parameters of meat by ANN [33], determining process parameters by using neural network with changes in color occured while thermal heating [34], predicting fermentation index (FI) of fermented cocoa beans using color measurement

and artificial neural network (ANNs) [35], after different drying methods, tracing of changes (in colour parameters, vitamin C concentrations and β-carotene concentrations in root vegetables) and the predicting of the physical (total dissolved solids and extraction yield) and chemical (total polyphenolic content and antioxidant activity) characteristics of the root vegetable were carried out by ANN modelling [36], clasifying of raw cow milk samples with using ANN [37], predicting the egg quality with ANN [38], developing new method using ANN to characterise and to classify some teas [39], applying ANN to characterize of honey [40], monitoring some fermentation product via ANN [41], determining polyphenolic profiles of cider with ANN [42], determining of maltol, ethyl maltol, vanillin and ethyl vanillin amounts with ANN [43], clasifying food vegetable oils by fluorimetry and artificial neural network [44], assessing chemical hazard levels (benzopyrene, heavy metal and aflatoxin B) of edible vegetable oil by ANN [45]. Besides the food industry ANN has used in many different fields for different purposes [46-49]. There are also thousands of calculated and experimental descriptors/molecular properties that are able to describe the chemical behaviour of substances. In several experiments, many variables can influence the chemical desired response [50]. Some examples of computational packages employed in chemometrics and containing several statistical tools such as MATLAB, R-program, Statistica etc. Generally, the PLS method is used to analyse only linear problems. However, when a large number of phenomena are present in the calibration problem, the relationship becomes non-linear. Therefore, artificial neural network can provide accurate results for complex problems [51].

In this article, the instrument validation of the modified spectrophotometric method developed on the basis of the Seliwanoff test and the artificial intelligence interpretation of the concentration-color relationship were evaluated. Artificial neural network is one of the Artificial Intelligence techniques. The aim of the artificial neural network (ANN) is to learn to recognize patterns in data. Once the artificial neural network has been trained on samples of data, it can make predictions by detecting similar patterns in future data. The novelty of this study is that the results obtained from the newly developed modified Seliwanoff test are used to determine the HMF concentration by evaluating with ANN. It was aimed to use ANN to correlate the red color, emerged due to the relationship between HMF and resorcinol during the modified Seliwannoff test, with the HMF concentration by measuring the absorbance and L*, a*, b* values.

MATERIAL AND METHODS

Reagents and chemicals

5-Hydroxymethyl furfural (≥99%), hydrochloric acid (36.5-38%), acetic acid (99.8-100.5%), acetonitrile (≥99.9%) were acquired from Sigma-Aldrich. Resorcinol was purchased from Merck. Ultrapure water (0.05 µS cm-1) was produced by a UV Milli-Q system from Millipore and it is used for the solutions and mobil phase preparation.

Absorbance measurement with UV/VIS Spectrophotometer

The novel modified Seliwanoff (spectrophotometric) method, which we reported in the previous article [28] is clearly as follows: HCl concentration 12%, resorcinol concentration 0.1 %, reaction time 30 min, sample:reagent (HCL-resorcinol) volume 1:2. Absorbance values at 485 nm obtained by UV–Vis spectrophotometer (Agilent Technologies, Cary 60, Victoria, Australia).

Reference method

An HPLC-UV system (Agilent 1260, USA) equipped with a C18 column, 5 µm and 4.6 mm × 250 mm was used to apply HPLC method as reference method to determine HMF amount. Filtered samples (20 µl) through 0.22 µm syringe filter (Millipore, Bedford, MA, USA) injected to system via mobil phase (95% acetic acid solution (1%) plus 5% acetonitrile) at 1 ml/min flow rate. The wavelength of the UV detector was 284 nm [52].

Color measurements

The CIE L*a*b* values of the colour revealed due to the relationship between HMF and resorcinol during the modified Seliwanoff test were measured using a Minolta Spectrophotometer CM-5 (Minolta Camera Co., Japan). Resorcinol solution with HCl was used as a target for the calculation of total color difference (∆E). ∆E values were calculated from the L*a*b* values according to the following Equation (1):

$$\Delta E = \sqrt{(L^* - L^*_{target})^2 + (a^* - a^*_{target})^2 + (b^* - b^*_{target})^2} \quad (1)$$

Training and testing the neural network

Artificial neural networks are computer systems that perform the learning function, which is the most basic feature of the human brain. This system consists of interconnected with artificial neural cells of the networks and each link has a weight value. Artificial neural networks come together in 3 layers and form a network. These are input layers, hidden layers, and output layer. In our research, artificial neural network has four input, ten hidden layers and two outputs (Figure 1).

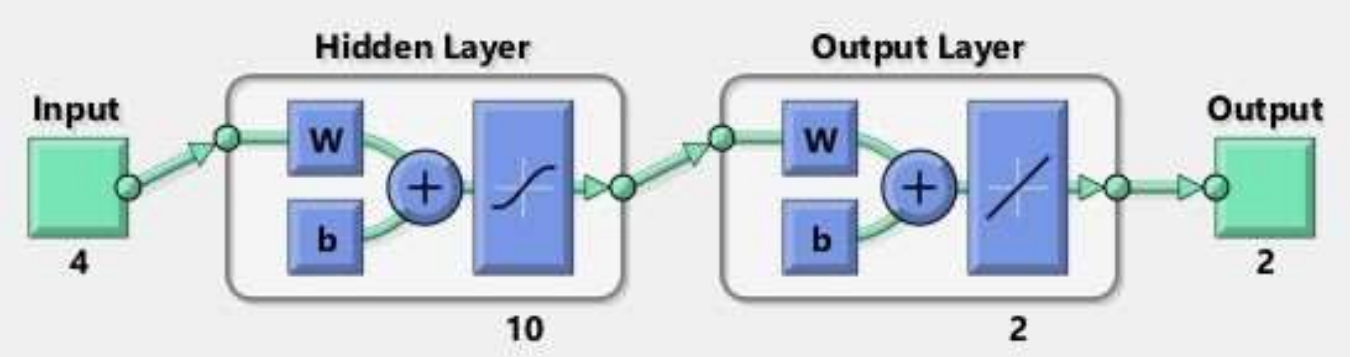


Figure 1. Schema of Artificial neural network

The information transmitted from the input layer to the network is processed in intermediate layers and sent to the output layer. The network can only produce correct outputs for the inputs when the weights have the correct values. In this process called the network training process, the values are randomly selected at the beginning. Then, during the training, each example is shown to the network and the weights are changed according to the learning rule of the network. Then another sample is presented to the network and weights are changed again and the most accurate values are tried to be found.

Method validation parameters

Linearity, limit of detection (LOD), limit of quantification (LOQ), precision and accuracy were evaluated as validation parameters of the proposed modified Seliwanoff spectrophotometric method. AOAC [53] and ICH [54] guidelines were used to validation study.

Linearity

The calibration curve was studied with aqueous standard solutions of HMF in the range of 1-100 mg L^{-1}. Regression analysis, lack-of-fit test and F-test was used to check the linearity of the calibration curve using Minitab® 17.1.0 software at 95% confidence level.

Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ were calculated according to ICH [54] using the following Eq. (2) and (3):

$$LOD = 3S_a/m \quad (2)$$
$$LOQ = 10S_a/m \quad (3)$$

Where S_a = standard deviation of the intercept of the regression line and m = slope of the calibration curve. To calculation of the S_a and m values, Linest function of Microsoft Excel 2013 was used.

Precision

The precision parameter has two components: intra-day precision (repeatability) and inter-day (intermediate) precision. Intra-day precision is the measure of the closeness of the measurement results obtained in the same laboratory, with the same device/method, under the same application conditions, by the same person in a short time interval, in the same or similar matrices. On the other hand, inter-day precision

means evaluating the variation in analysis when a method is used on different days in a laboratory [53, 55]. Relative standard deviation (%) was used to evaluating precision.

Accuracy

Accuracy indicates how close the average of a certain number of analysis results is to the true value. It was evaluated according to the recovery results. The water was spiked in three concentration levels (20, 40, 60 mg L^{-1}). Recovery percentages (%) were calculated as (the amount of HMF founded/the amount of HMF added) x 100 and used as accuracy estimates.

RESULTS AND DISCUSSION

Instrument method validation parameters of modified Seliwanoff test

Linearity

The relationship between the HMF concentration and the absorbance of the red complex released as a result of the modified Seliwanoff reaction was evaluated with standard HMF solutions in the concentration range of 1-100 mg L^{-1} where the Lambert-Beer law applies. Regression analysis was applied to the absorbance values obtained for each concentration. The linearity of the curve drawn using the absorbance values obtained against concentration was examined not only by considering at the correlation coefficient (r), but also by the lack of fit value and the F test (Fisher ratio). In order to evaluation about the linearity of the curve, $F_{table} > F_{calculated}$, lack of fit *p* value should be insignificant ($p > 0.05$) and correlation coefficient (r) should be close to 1 [55, 56].

The critical value of F and the value of F calculated by experimentally was compared at the 95% confidence level for 6 and 16 degrees of freedom. Results ($F_{calculated}$ (0.18) < $F_{tabulated}$ (2.74); *p* value of lack of fit = 0.975; correlation coefficient = 0.9960) showed that the curve presents linearity. It means that, by observing the linear relationship between HMF concentration and the absorbance of the color revealed by the modified Seliwanoff test which is used in the qualitative determination of carbohydrates can form the basis of new spectrophotometric methods to be proposed for quantitative determination of HMF. This proven quantitative relation can be expressed by the equation is absorbance = 0.0020 + 0.0012*concentration of HMF (mg L^{-1}) with R^2 = 99.6%. The linear curve with confidence and prediction intervals are presented in Figure 2a.

In order to calculate the linearity of the calibration curve, correlation coefficient, lack of fit test and F test parameters, the following equations Eq.(4-9) were used:

$$SS_{\mathrm{r}} = \sum_{i=1}^{I}\sum_{j=1}^{J_{\mathrm{i}}} (y_{\mathrm{ij}} - \hat{y}_{\mathrm{i}})^2 \tag{4}$$

$$SS_{\varepsilon} = \sum_{i=1}^{I}\sum_{j=1}^{J_{\mathrm{i}}} (y_{\mathrm{ij}} - \bar{y}_{\mathrm{i}})^2 \tag{5}$$

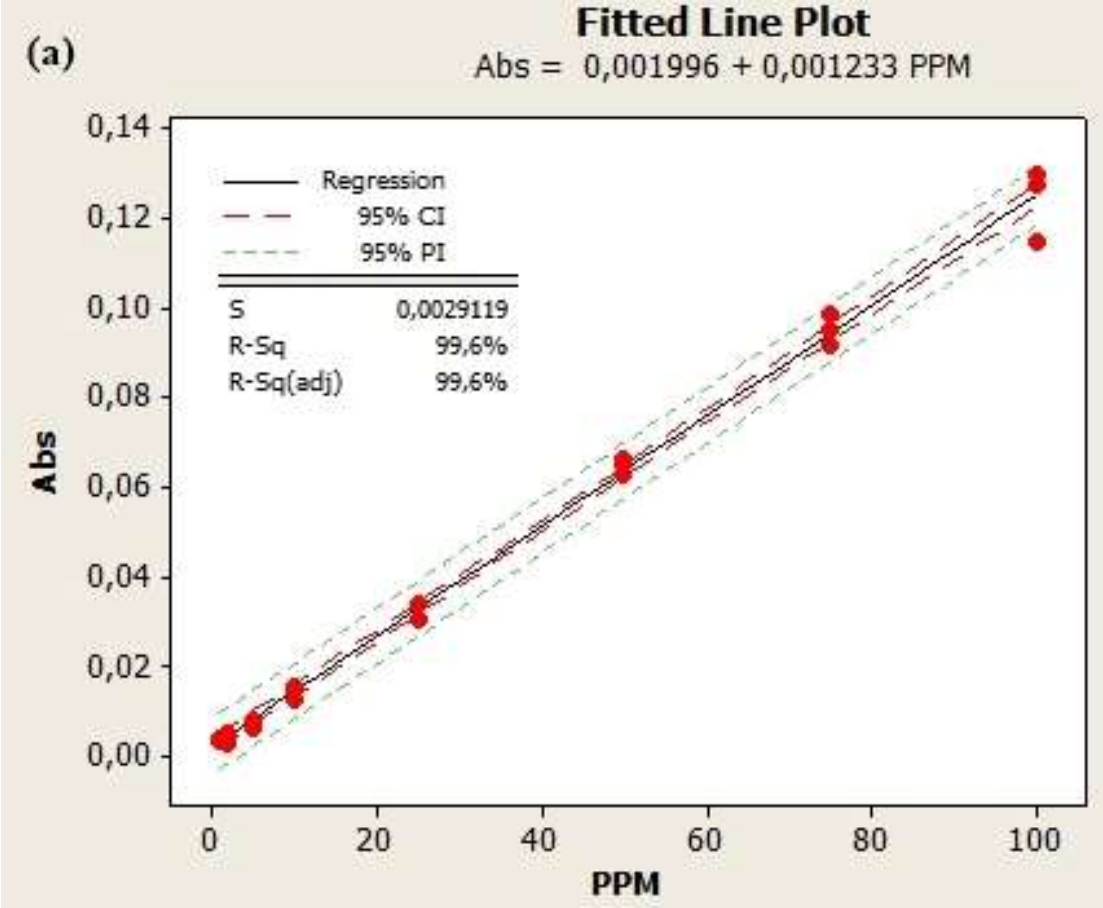
$$SS_{\mathrm{lof}} = SS_{\mathrm{r}} - SS_{\varepsilon} = \sum_{i=1}^{I} (\bar{y}_{\mathrm{i}} - \hat{y}i)^2 \tag{6}$$

$$\sigma^2{}_{\varepsilon} = \frac{SS_{\varepsilon}}{(IJ - I)} \tag{7}$$

$$\sigma^2{}_{\mathrm{lof}} = \frac{SS_{\mathrm{lof}}}{(I - 2)} \tag{8}$$

$$F_{(I-2)/(IJ-I)} = \frac{\sigma^{\prime}{}_{\mathrm{lof}}}{\sigma^2{}_{\varepsilon}} \tag{9}$$

Where SS_r: Residual error sum of squares; SS_ε: Pure experimental error sum of squares; SS_{lof}: Lack-of-fit error sum of squares; y_{ij}: Experimental response; $\bar{y}$: Average response at every concentration level; $\hat{y}$: Estimated response obtained by using calibration curve; F: Fisher ratio; I: Number of concentration level (8); J: Replicate number of each concentration (3).


(a)
Fitted Line Plot
Abs = 0,001996 + 0,001233 PPM
Regression
95% CI
95% PI
S 0,0029119
R-Sq 99,6%
R-Sq(adj) 99,6%
Abs
PPM
0,14
0,12
0,10
0,08
0,06
0,04
0,02
0,00
0
20
40
60
80
100

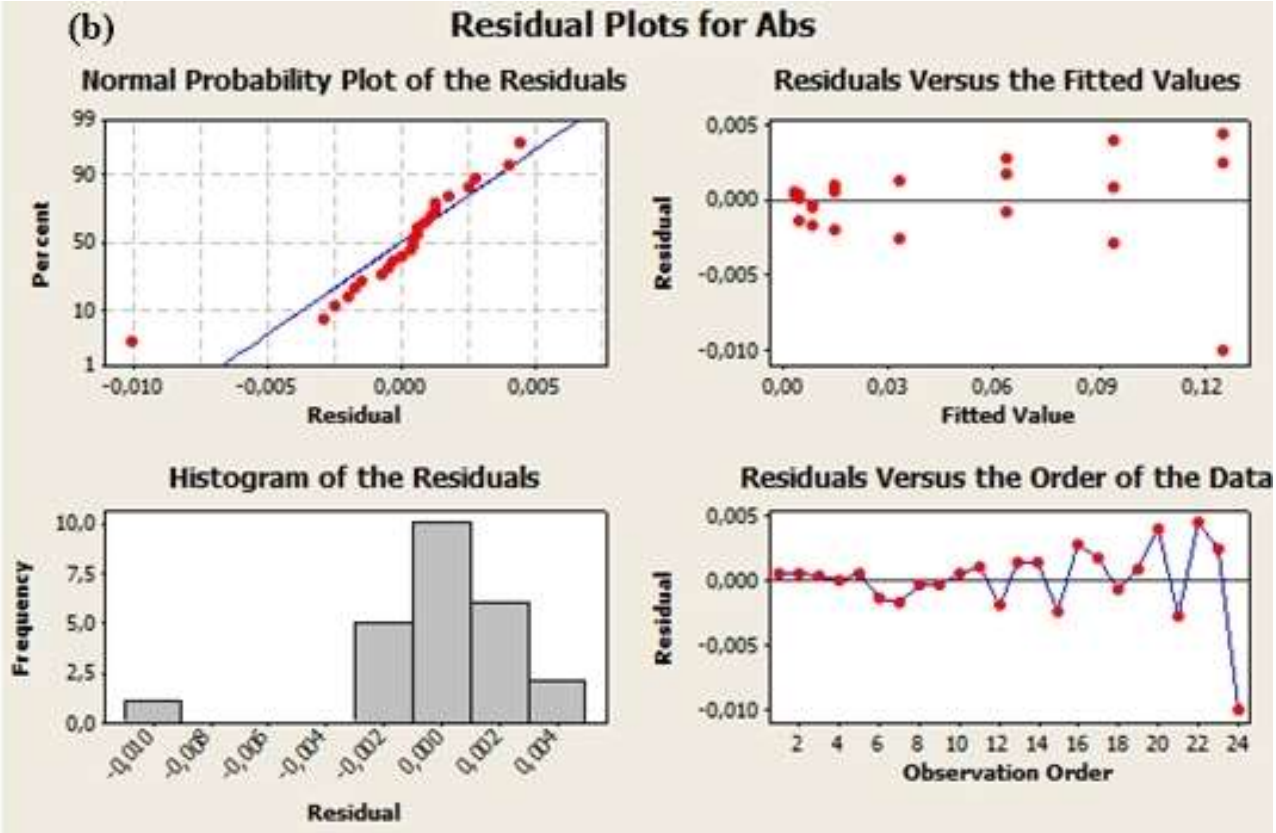

(b)
Residual Plots for Abs
Normal Probability Plot of the Residuals
Percent
Residual
Residuals Versus the Fitted Values
Residual
Fitted Value
Histogram of the Residuals
Frequency
Residual
Residuals Versus the Order of the Data
Residual
Observation Order

Figure 2. Regression line of calibration curve (a) and residual plots for absorbance (b).

The calibration curve characterization parameters are shown in the Table 1. Residual analysis, which is a mathematical method, helps to evaluate the fit of the model. Figure 2b shows that the residual values obtained for absorbance are normal (which can be understood from the probability and histogram graphs shown on the left) and randomly distributed (it can be understood from the graphs plotted against the observation and fit values shown on the right). All these results confirm that the calibration curve fitted the linear model.

Table 1. Calibration curve and characterization

Calibration curve	Abs= 0.00200 + 0.00123ppm
SS_{ε}	0.000174
SS_{lof}	0.000012
σ^2_{lof}	$2x10^{-6}$
σ^2_{ε}	$1.09x10^{-5}$
Fisher ratio, F	0.18
Correlation coefficient	0.998
p value of lack-of-fit	0.975

SS_{ε}: Pure experimental error sum of squares; SS_{lof}: Lack-of-fit error sum of squares; σ^2_{lof}: lack-of-fit variance; σ^2_{ε}: purely experimental variance.

Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ values of 2.19 ppm and 6.65 ppm were obtained, respectively. These values can also be called instrument LOD and LOQ because they were calculated from the calibration curve obtained in the water matrix of the standard HMF.

Precision and accuracy

The relative standard deviation (RSD, %) from the intra- and inter-day data was calculated to investigate the precision of the proposed method. RSD % value range of intra-day (repeatability) data (six replicates analyzed on the same day) was found as 2.35–3.65% (Table 2).

Table 2. Precision and accuracy parameters

Precision, RSD %		
Concentration	Intra-day (n=6)	Inter-day (n=18)
20 ppm	3.65	4.73
40 ppm	2.35	3.16
60 ppm	2.89	3.38
Accuracy		
Concentration	Recovery %	RSD %
20 ppm	100.47	3.68
40 ppm	99.34	2.62
60 ppm	99.38	1.58

RSD %: Relative standard deviation

The inter-day precision was evaluated as the RSD % (3.16–4.73%) calculated from analysis result of three consecutive days with six replicates in each day (Table 2). Since the RSD values calculated for the

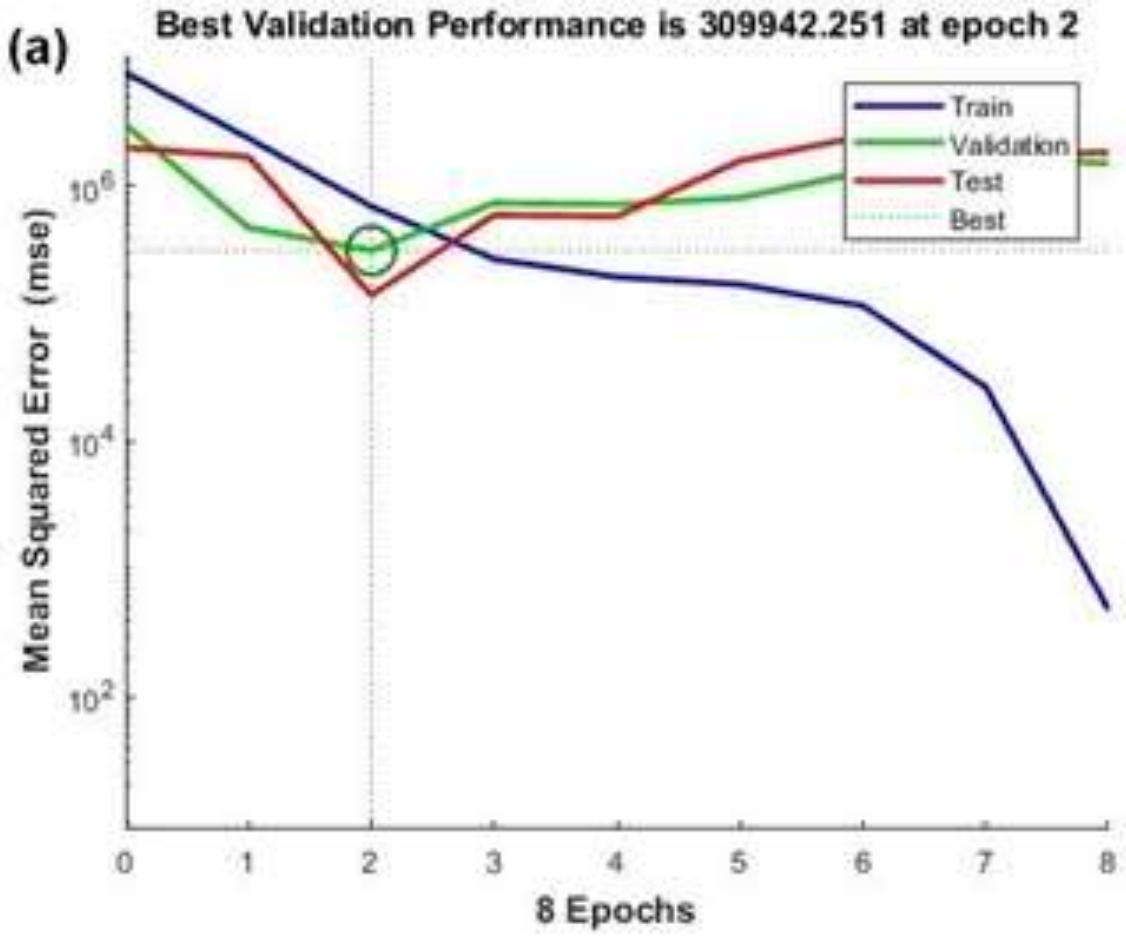
evaluation of the intra/inter-day precision are lower than the values specified in the limits (RSD, %:5.3–7.3%), it complied with the AOAC guide [53].

Recovery rates and RSDs at three different concentration HMF levels was used to evaluation of the method accuracy. 99.34-100.47% and 1.58–3.68% were calculated as recovery and RSDs range, respectively (Table 2). The fact that these results are within acceptable limits of AOAC (80–110%) indicates the method has a good accuracy [53].

Artificial Neural Network results

The vast majority of engineering practices use supervised learning. The neural network is trained by giving the artificial neural network a set of sample information based on the task it is intended to perform. The goal is to get a target output for a particular input. Target output is provided by the auditor. If the target is not reached when the target is compared with the output obtained, the weights of the connections are adjusted according to the learning approach adopted and the process is repeated. In this study, MLP (multilayer perceptron) algorithm is used. MLP is the most popular, effective, and easy to learn model for complex and multilayered networks [57].

In this research, the number of hidden layer is two. The performance plot shows the value of the performance function versus the iteration number. Each iteration of the complete training set is called an epoch. In each epoch the network adjusts the weights in the direction that reduces the error. Many epochs are usually required before training is completed. Training automatically stops when generalization stops as indicated by an increase in the MSE (Mean Square Error) of the validation samples. Lower values are better while zero means no error. Regression analysis was performed to measure the correlation between outputs and targets. When the training were perfect, the network outputs and the targets would be exactly equal, but the relationship is rarely perfect in practice (Figure 3a).



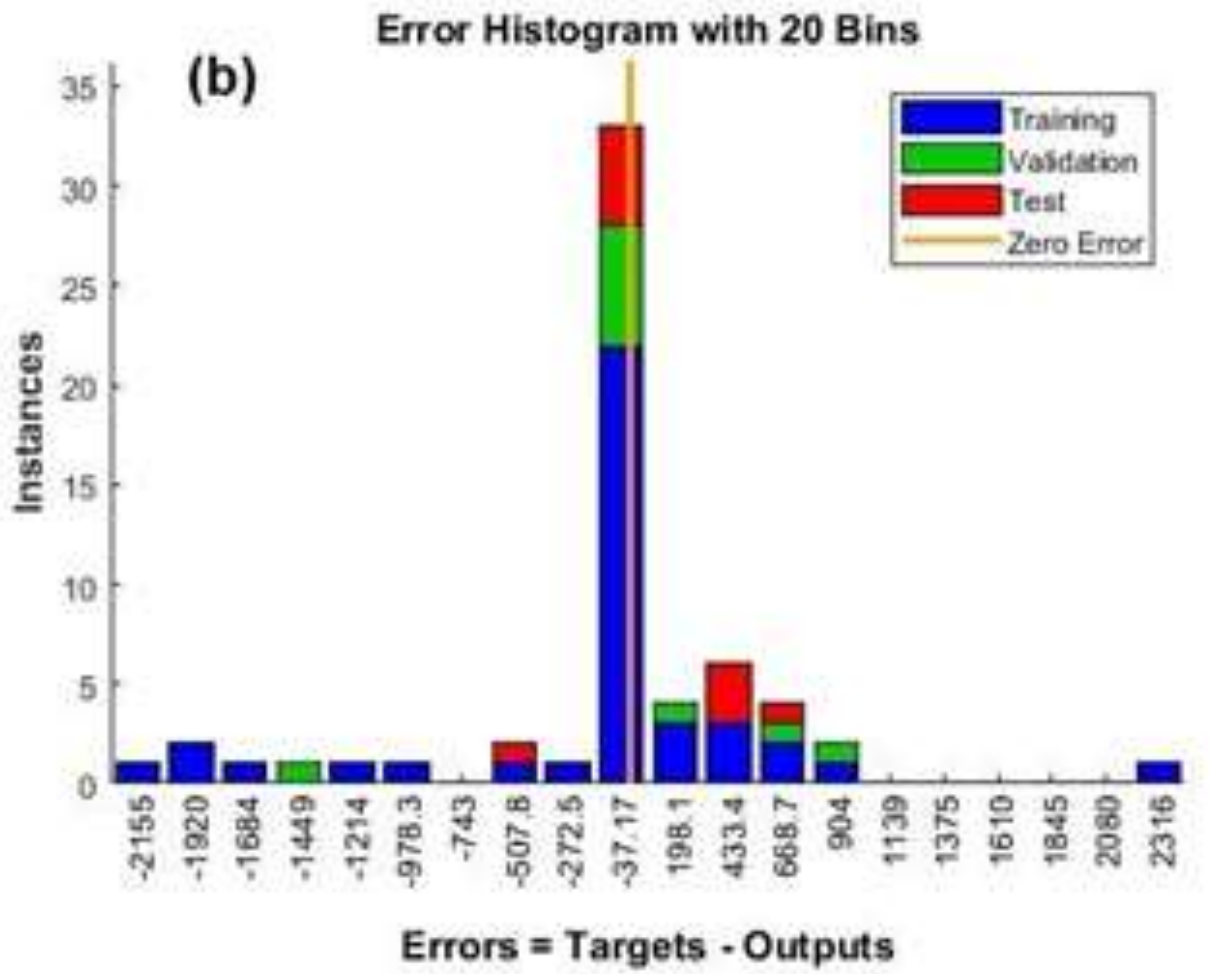


Figure 3. The learning curve (a); The error histogram for the training of the data using the Levenberg-Marquardt (b)

The error histogram (Figure 3b) is the histogram of the errors between target values and predicted values after training a feed forward neural network [58]. As these error values indicate how predicted values are differing from the target values, hence these can be negative. Bins are the number of vertical bars you are observing on the graph.

The total error range is divided into 20 smaller bins here. Y-axis represents the number of samples obtained from the dataset which lies in a particular bin. For example, at the mid of the plot, we have a bin corresponding to the error of 37.17 and the height of that bin for training dataset lies below but near to 20 and validation and test dataset lies between 30 and 35. It means that many samples from the different datasets have an error lies in that following range. Zero error line corresponding to the zero error value on the error axis (i.e. X-axis). In this case zero error point falls under the bin with centre 37.17.

Performance of neural networks can be shown by the validation graphs obtained by using the validation/test data (Figure 4a).

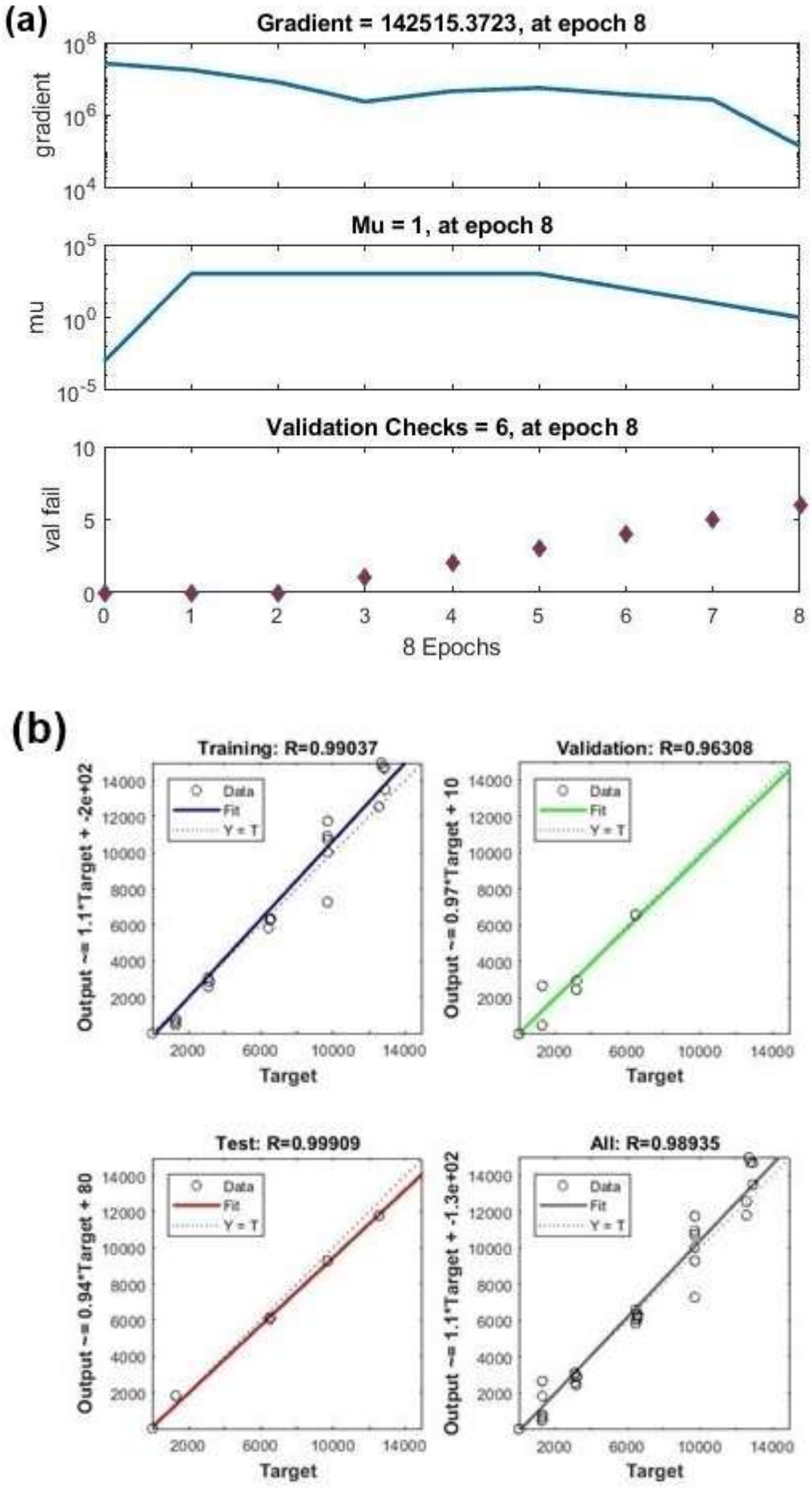


Figure 4. The plot for the training state parameters (a); the results for the regression between the output data and the targets for the LM (b).

To find the best model for the result, the generated code was run in MATLAB and a plot of the best resulting network based on the average performance of training and test errors with the number of training epochs is shown in Figure 4a shows that the large values for network decrease to a smaller value as the weights are improved, that is, network training.

The results for the regression between the output data and the targets for the LM algorithm for the hidden layer. The regression analysis shows that the results were 98.935% accurate with the most accurate prediction. The accuracy of the results demonstrates that the collected data was significant for predicting. The regression results in this research was used for comparison of the performance of the ANN showing that this approach is using for the best predicting (Figure 4b). The testing was the evaluation of the performance ANN for estimating the best result. The first process was based on statistical evaluation for mean, coefficient variation and variables [59]. The error indicated the deviation of the real estimates from the real data. The error standard deviation measurements which revealed the optimal setting necessary for better predictability [60]. The mean square error (MSE) was used.

The ANN has been demonstrated as an efficient model for the complex data computation was calculated after training and testing of the model. According to the R values, it is seen that the network makes high accuracy predictions (Table 3). Some statistical criteria such as the mean-squared-error (MSE), the coefficient of determination (R^2) and the absolute average relative deviation (AARD) is defined as follows equations [61,62].

$$\mathrm{MSE} = \frac{1}{n}\sum_{i=1}^{n}\left(\mathrm{Y}^{\mathrm{i,mod.}} - \mathrm{Y}^{\mathrm{i,exp.}}\right)^2 \tag{10}$$

$$\mathrm{R}^2 = 1 - \frac{\sum_{\mathrm{i=1}}^{\mathrm{n}}\left(\mathrm{Y}^{\mathrm{i,mod.}} - \mathrm{Y}^{\mathrm{i,exp.}}\right)^2}{\sum_{\mathrm{i=1}}^{\mathrm{n}}\left(\mathrm{Y}^{\mathrm{i,mod.}} - \mathrm{Y}\right)^2} \tag{11}$$

$$AARD\ (\%) = \frac{1}{n}\sum_{i=1}^{n}\left(\left|\frac{\left(\mathrm{Y}^{\mathrm{i,mod.}} - \mathrm{Y}^{\mathrm{i,exp.}}\right)}{\left(\mathrm{Y}^{\mathrm{i,exp.}}\right)}\right|\right) \mathrm{x}\ 100 \tag{12}$$

Table 3. The mean square error (MSE) and R values

	MSE	R
Training	688193.80738e^{-0}	9.90371e^{-1}
Validation	3099042.25100e^{-0}	9.63080e^{-1}
Testing	132281.40603e^{-0}	9.99089e^{-1}

Comparison of methods

HMF concentrations (10, 25, 50, 75 and 100 ppm) were calculated by modified Seliwanoff method as well as by HPLC as a reference method. Also HMF values predicted by ANN. The values found are shown in Table 4.

Table 4. HMF amount obtained by modified Seliwanoff test, HPLC and ANN methods

Actual HMF concentration (ppm)	Determined HMF values (ppm) by methods		
	Novel spectrophotometric method	Reference HPLC method	ANN predicted
10	9.21 ± 0.40	10.28 ± 0.04	9.93 ± 0.26
25	23.42 ± 0.44	25.02 ± 0.31	25.15 ± 0.77
50	50.53 ± 3.56	51.63 ± 0.41	50.00 ± 0.26
75	74.90 ± 2.56	77.06 ± 0.02	75.33 ± 1.20
100	94.51 ± 2.84	101.06 ± 1.11	99.86 ± 1.12

Each value is the mean ± SD (n=3)

Linear regression results at 95% confidence level demonstrated that there was a linear relationship between the actual HMF concentrations and the HMF values determined by different methods (modified Seliwanoff, reference HPLC method and ANN). When the actual concentration values are compared with the values determined by methods or estimated with ANN, the correlation coefficient is 0.996 for modified Seliwanoff test and 1.0 for both HPLC and ANN. Also F_{reg} = 3202,59; 47825,37 and 41768,59, respectively. These results show that the HMF content can be determined with higher accuracy applying ANN to data compared to the calibration curve method based on linear statistics. Erbakan and coauthors [63] determined the HMF content with 0.987 correlation coefficient in the honey by spectrophotometry and image processing. HMF content was determined by Chua [64] using statistical techniques including multivariate data analysis and neural network modelling with correlation coefficient, R^2 0.9163. The developed a GA-ANN model by Xu and coauthors [65] was found to be a more accurate prediction method for the F and HMF contents of fermented lotus root than linear regression-based models. In a study made by Mandić and coauthors [66] the mathematical model in the form of an artificial neural network was developed to predict the behavior of physicochemical changes of cookie samples. According to goodness of fit tests applied, HMF content of cookies determined with 0.84 R^2 value in that study.

CONCLUSION

The instrumental method parameters of the modified Seliwanoff test developed for quantitative HMF determination were studied. The linear relationship between HMF concentration and red color released by chemical reaction of HMF and resorcinol during modified Seliwanoff test was defined. Absorbance values obtained by novel modified Seliwanoff method and colorimetric data (L*, a*, b*) were used with artificial neural network to determine HMF concentration. HMF amounts determined by modified Seliwanoff method, HPLC method and ANN were compared. ANN can accurately recognize the relationship between any set of inputs and outputs without a physical model. The ability of ANN is essentially independent of the complexity of the underlying relationship such as nonlinearity, multiple variables, and parameters. According the result, estimation accuracy of HMF concentration was found 98%. The estimated relationship was obtained from a simulation of the ANN model. All the data were obtained from the calculation of the responses of the HMF concentration as affected by colorimetric data. It can be seen that the estimated relationship was closely related to the actual data. These results suggest that a reliable computational model could be obtained for predicting the HMF concentration with any colorimetric data.

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