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Development of a chemometric method for the analysis of Sudan III-IV dyes adulteration in chili powder using UV-visible spectroscopy data

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Abstract

Chili powder is a globally traded commodity and one of the most important parts of regular diet of the people of Bangladesh. It is reported that chili power has been adulterated by Sudan III-IV dyes since 2003. A simple, fast and cost effective method for the identification of Sudan dyes (III and IV) present in chili powder was proposed here and the method was based on the characterization of UV-visible spectral data using artificial neural network (ANN). Artificial neural network (ANN) was developed for the simultaneous assay of chili powder adulterated with Sudan III-IV. 47 standard mixture solutions were prepared using orthogonal experimental design (OED) to build a calibration data set. UV-visible spectra of these mixtures were obtained between 200 and 800 nm at 1 nm interval. The results of the artificial neural network were compared with that of other two calibration techniques namely, principal component regression (PCR) and partial least square regression (PLSR). ANN shows better prediction efficiencies comparing with PCR and PLSR. Prediction by ANN on the basis of spectroscopic data is 85% for chili powder, 70% for Sudan III and 60% for Sudan IV in terms of coefficient of determination (R^2). Six different branded chili powders collected from the local market, and were measured by using the proposed method. It was found that no samples contained Sudan III-IV. So, the proposed method can be easily used in the quality control of any chili powder adulterated with Sudan III-IV dyes as an alternative analysis tool.

Keywords: *Artificial neural network, Sudan III-IV, UV-visible spectroscopy, Orthogonal experimental design.*

INTRODUCTION

Chili is one type of vegetable that belongs to the family of *Solanaceae*. It is grown in all countries of Asia, major parts of Africa, United States and southern part of Europe. After drying chili is ground to a powder and then it is stored for a long period of time. The most important properties of chili are color and its pungency. Chili contains large amounts of vitamins A, B, C, and small amounts of carotene, which make it a good source of vitamins [1]. Chili powder is blended to a variety of processed foods such as curries, sauce and pickles and dried foods like chips and nuts as well. Most of the people of Bangladesh prefer chili with other spices in their regular diet.

Chili is not an esoteric species and is not very costly either. Even then, it attracts its own moderate share of adulteration. In Bangladesh, chili powder is adulterated mainly with brick powder and saw dust. Moreover in some cases, salt powder, talc powder, red oxide (Fe_2O_3), Sudan I-II-III-IV dyes; stones (to increase weight) are also found on chili powder [2].

Sudan dyes (Sudan I -IV dyes) are familiar compounds in the class of azo dyes. Among them the most available dyes used in Bangladesh are Sudan III and Sudan IV. These dyes are intentionally used as food adulterants especially in red chili and other foods like curry, processed meats and spice blends as they have extreme red-orange color and are in low price. But Sudan III and IV dyes as food additives are not allowed internationally because they cause carcinogenicity. Therefore, the authentication of chili powder must be addressed in order to assure its quality and safety Many analytical methods have been proposed for identification and quantification of Sudan dyes in chili powders, the majority of them based on chromatographic and spectroscopic procedures. Moreover, methods based on amperometric detection[3]

Areca catechu is commonly called betel nut, belongs to the family using Ag-NPs-GO-GCE, electro-analytical technique using activated glassy carbon electrode [4], random amplified polymorphic DNA procedure [5] and capillary electrophoresis [6] are also found in the literature. Among the chromatographic methods, UPLC-MS/MS [7], HPLC-PAD [8], microLC-electrospray ionization (ESI)-Q-TOF MS [9], LC-MS/MS [10], ESI-MS-MS [11] and molecularly imprinted SPE (MISPE)-HPLC [12] have also been proposed and these methods were satisfyingly used for the analysis of Sudan dyes in chili powders. Many workers have reported on the determinatoin, using FTIR [13], spectrophometric [14] and mass spectrocopic [15] methods, of sudan dyes in various food stuffs especially chili containging products. Apart from electrophoresis, chromatography and spectroscopic methods, few fluorimetric methods have been used to determine dyes in chili powder. They include flow injection chemiluminescence (FI-CL) [16], Mn–ZnS QDs luminescence [17] and intrinsic fluorescence [18]. Most of the methods involve complex separation techniques and need the use of different chemicals that are harmful for the environment. These limitations can be overcome by utilizing chemometric methods which can be considered promising, faster, direct, less time consuming and relatively less expensive alternatives for the identification of Sudan dyes in chili powders. Some papers make reference to the quantitative analysis of Sudan I-IV dyes in commercial spices by UV–visible spectroscopy using multivariate classifications such as PLS/DA, K-NN and SIMCA [19] and ^{1}H NMR-PLS– DA[20].

This study is the first report on the application of artificial neural network (ANN) using spectrophotometric data for prediction of sudan dye III and IV in chili powder. Here the prediction efficiency of ANN is also compared with other two chemometric calibration techniques namely, PCR and PLSR. We can describe ANN as a mathematical model which has a specific structure, made of a number of the signal processing elements such as nodes and neurons and configured in inter connecting layers. Each input vector is multiplied by its weight and the active neuron sums the products and proceed the sum via a transfer function to yield output [21]. ANN has a group of inter-connected artificial neurons and consists of input, hidden and output layers. Every layer also made of neurons and each neuron transforms the input. Each neuron sends outputs to other neurons to which it is connected. Receiving neuron determines weights and bias. Network is trained with a dataset of observations and it is optimized depending on its ability as to predict a set of known outcomes [22].

Because of various brands and trademarks of chili powders available in Bangladeshi market, it is very usual not to understand that the product we are going to buy is really a product without being adulterated. Chili powders have quite similar characteristics to Sudan III and Sudan IV. They are not easily distinguished from the dyes by only seeing the color because adulteration with the dyes is used to hide the products in which original color has already disappeared because of erroneous drying, storage and insects. In this work we propose spectrophotometric method combined with ANN for the prediction of Sudan III-IV in chili powder. Regression plots are obtained for individual descriptors (Chili powder, Sudan III, Sudan IV) which provide information about the performance of the model. ANN meets the requirement of sophisticated analysis methods to uncover the hidden causal relationships between single or multiple responses and a large set of properties. Finally, six samples of chili power of different commercial brands have been measure by the ANN with UV-spectroscopic data and got satisfactory result.

Experimental

Reagents, chemicals and samples

Sudan III, Sudan IV and acetonitrile (HPLC-grade) were collected from SF scientific Ltd (Dhaka, Bangladesh) and were preserved at room temperature. Six different commercial samples were purchased from the local markets in Dhaka, Bangladesh.

Instrumentation

A double beam spectrophotometer (Shimadzu UV-1700) with a 1.00 cm quartz cell was used for obtaining absorption spectra. In this work UV absorptions of mixture solutions were obtained in the wavelengths between 200-800 nm against a solvent blank and at 1 nm interval, digitized absorbance was sampled. Data obtained were processed by neural network toolbox of MATLAB (Ver. R2015a) and a licenced copy of CAMO the Unscrambler (Ver. 10.5) on Pentium IV personal computer.

Preparation of standard solutions for multivariate analysis

To develop the chemometric calibration, forty seven preparations (calibration set) of standard solutions containing mixture of chili powder, Sudan III and Sudan IV were prepared. In this procedure, at first, approximately one gram pure chili powder was weighed and 50 mL of acetonitrile solvent was added to it. Mixture was stirred in a magnetic stirrer for 1 hour. Glass filter and syringe filter were used for filtering the extract twice. Then it was diluted with appropriate amount of acetonitrile to obtain a final concentration of 100 ppm [19]. Sudan III and IV were dissolved in appropriate amount of acetonitrile individually. Then they were mixed with pure chili powder solution in different amounts according to the experimental design and then diluted with acetonitrile in order to obtain a final concentration of 100 ppm.

Preparation of real sample

One gram chili powder obtained from the market was weighed and 50 mL of acetonitrile solvent was added to it. The content was stirred in a magnetic stirrer for 1 hour. The extract was obtained by filtering with Whatman no.1 filter paper. Then it was diluted with appropriate amount of acetonitrile to obtain a final concentration of 100 ppm. [19]

Design of mixture

It can be shown that dyes under the investigation have similar spectra as per their chemical structures and there is considerable overlap (Figure not shown). Sudan III and IV show λ_{max} at 508 nm and 520 nm respectively. Furthermore, absorbance spectra of pure chili solution and together with a mixture of chili, Sudan III and Sudan IV are depicted in the following Fig.1. It can be seen from the figure that; the spectra of pure chili and the mixture have overlapped significantly. Thus, the overlapping spectra interrupt the quantification of the dyes in the mixture by traditional univariate calibration method. To minimize the overlapping, multivariate calibration technique using ANN was carried out.

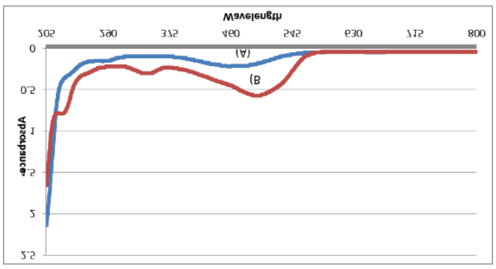

Fig 1. Absorption spectra of (A) pure chili powder and (B) mixture of chili powder and Sudan III-IV dyes.

Multivariate calibration needs a useful experimental design which provides a calibration set of standard mixtures for the prediction of best results. OED was used to prepare the standard solutions for calibration set. Using the experimental design ensures the construction and optimization of ANN. 47 mixture samples (Table 1) with different concentration of pure chili powder; Sudan III and Sudan IV were made. UV spectra of 47 observations were obtained in the spectral region between 205 and 800 nm as shown in the Fig. 2. The spectra were stored using UVPC software. Out of 47 mixture solutions and their respective concentrations, 75% mixture solutions were used for development of the model. Remaining 25% mixture solutions were used to validate the developed model. The design was performed by using the software SPSS (version 22.0).

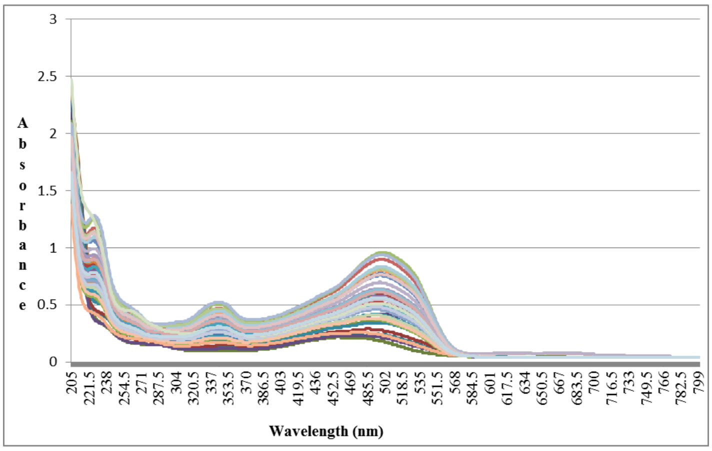

Fig 2. Spectral presentation of absorbance against wavelength for the standard solution data.

Table 1: Composition of the calibration samples through experimental design

Sample ID	Concentration (mg/L)			Sample ID	Concentration (mg/L)		
	Chili	Sudan III	Sudan IV		Chili	Sudan III	Sudan IV
1	100	0	0	S25	94.7	3.0	2.4
2	98.8	1.2	0.0	26	93.3	3.3	3.3
3	97.9	2.1	0.0	27	93.3	4.0	2.7
4	99.4	0.6	0.0	28	96.2	0.6	3.2
5	96.2	3.8	0.0	29	97.1	1.7	1.1
6	97.1	2.9	0.0	30	94.7	3.6	1.8
7	96.2	0.0	3.8	31	97.9	1.4	0.7
8	98.8	0.0	1.2	32	95.7	1.6	2.7
9	97.1	0.0	2.9	33	93.4	3.3	3.3
10	99.4	0.0	0.6	34	96.6	2.9	0.5
11	97.9	0.0	2.1	35	96.2	3.2	0.6
12	96.2	1.9	1.9	36	98.8	0.6	0.6
13	94.7	2.4	3.0	37	93.3	2.7	4.0
14	95.7	1.1	3.2	38	96.2	2.6	1.3
15	95.7	2.1	2.1	39	97.9	1.0	1.0
16	96.6	1.0	2.4	40	95.7	3.2	1.1
17	97.9	1.5	0.5	41	94.5	3.0	2.5
18	95.7	2.7	1.6	42	97.9	0.7	1.4
19	97.1	0.6	2.3	43	97.1	1.1	1.7
20	97.1	2.3	0.6	44	94.5	2.5	3.0
21	97.9	0.5	1.5	45	96.6	1.4	1.9
22	96.6	2.4	1.0	46	96.2	1.3	2.6
23	94.7	1.8	3.6	47	96.6	1.9	1.4
24	96.6	0.5	2.9				

Results and Discussion

Spectrum of spectral values and concentrations of Sudan III and IV obtained from the experimental design are considered as data matrix for the study. It has 1191 input neurons, 10 hidden layer neurons, and 1 output layer neuron as shown in the Fig.3. Model learns through training the weights to produce the correct output.

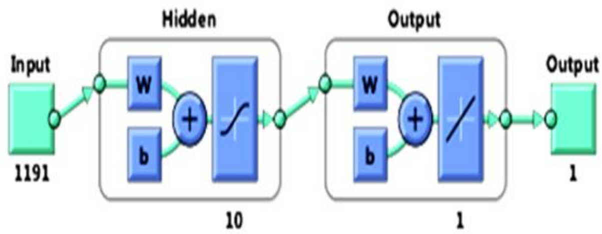

Fig 3. Network architecture. W: weights and b: bias units, which are part of individual neurons.

Regression of chili, Sudan III and IV

The prediction plots represent outputs and targets of training, validation, test, and overall data performed by ANN. Dashed line presenting in every plot in the following figure represents the ideal result where the value of outputs equals to targets while the solid line has the meaning of best fitting linear regression line among output and target values. R has an indication of the relationship between outputs and targets. R equal to 1 (one) indicates an exact linear relationship between output and target values whereas R close to zero indicates there is no linear relation among the output and target.

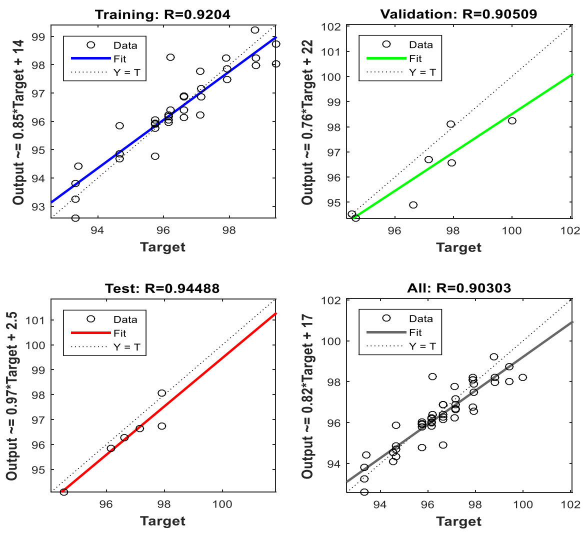

Fig 4. Regression plots of percentage of chili powder on the basis of UV spectroscopic data

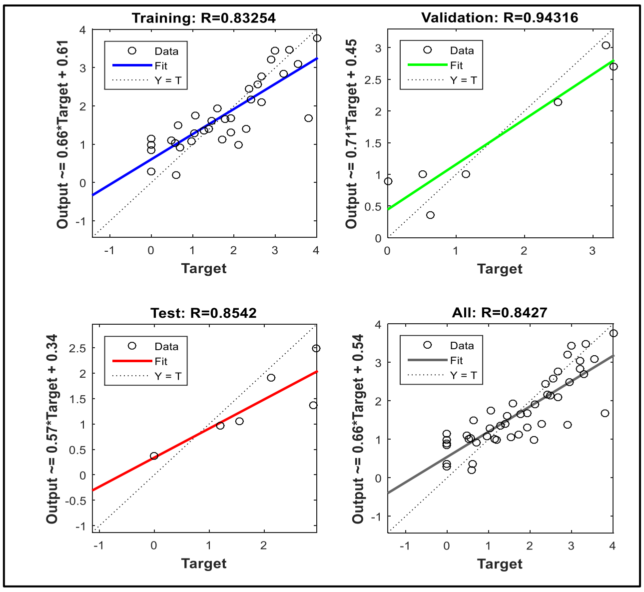

Fig 5. Regression plots of percentage of Sudan III on the basis if UV spectroscopic data

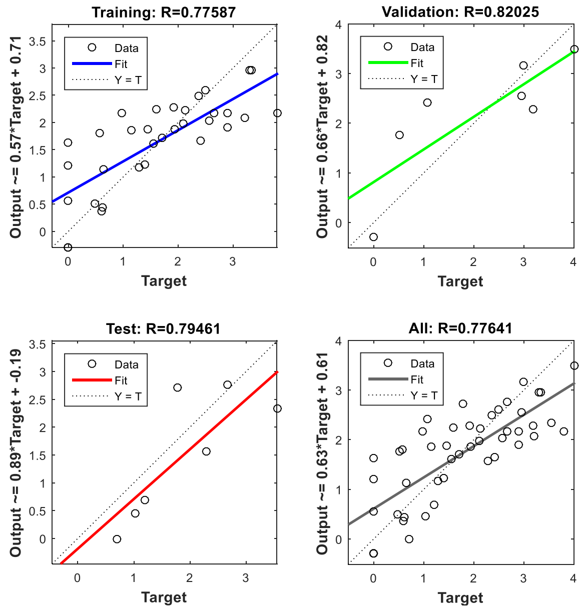

Fig 6. Regression plots of percentage of Sudan dye IV on the basis if UV spectroscopic data

In the modeling for the prediction of chili powder, Sudan III and Sudan IV, ANN was used. Feed forward back propagation was used to establish the model. The inputs were the original data set. Along with the input and output data there were ten hidden layers in the process. The values of R obtained for the chili, Sudan III and IV prediction models in training sets were 0.92, 0.83 and 0.78 respectively as shown in the **Figs. 4-6**, therefore weights were determined from the training sets on the other hand, concentration of samples in another set which is known as test sets was simultaneously predicted, and the R values of the test sets were 0.94, 0.85 and 0.79 for the compounds. ANN models were verified using an external prediction sets which were validation sets that describes the samples which do not belong to the training and test sets and the values of R obtained for chili, Sudan III and IV were 0.90, 0.94 and 0.82 respectively. Predicting abilities of training, validation and test sets can also be compared by the use of MSE values. The MSE values for the models to predict chili powder, Sudan III and IV were (0.41, 0.40, 0.49), (1.19, 0.23, 0.68) and (0.34, 0.50, 0.57) for calibration, validation and test sets respectively.

From the above prediction plots it is evident that training set express a good fit, validation and test results show R values which are close to one indicating a linear relation among output and target values

Mean square errors (MSE) against epochs during the training process of the optimum network were plotted in which the best results for validating data set were achieved at various epochs as shown in **Figs. 7-9** for chili powder, Sudan III and Sudan IV respectively. For chili, the best validation performance was obtained at the mean square error value of 0.331 at epoch 10 as shown in **Fig. 7** while for Sudan III the best validation performance was obtained at the mean square error of 0.535 at epoch 17 as shown in **Fig. 8**. Similarly, for Sudan IV, the best validation performance was obtained at the mean square error of 0.679 at epoch 22 as shown in **Fig. 9**.

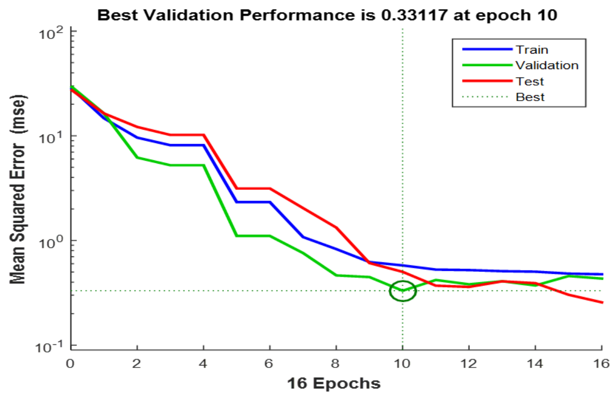

Fig 7. MSE plot of validation performance for chili powder.

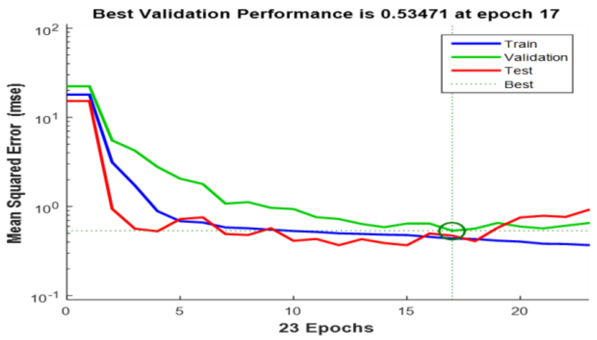

Fig 8. MSE plot of validation performance for Sudan III.

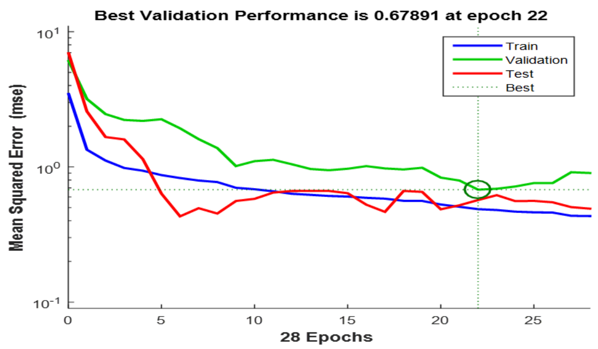

Fig 9. MSE plot of validation performance for Sudan IV.

Comparison

The proposed method was compared with the PCR, PLSR and ANN. A comparison of the RMSE and R^2 values of the methods used in prediction is given in the Table 2.

Table 2. Comparison of efficiencies of PCR, PLSR and ANN for prediction of Chili powder, Sudan III and Sudan IV

Sample ID	PCR		PLSR		ANN (proposed)	
	RMSE	R^2	RMSE	R^2	RMSE	R^2
1	0.686	0.831	0.685	0.832	0.647	0.847
2	0.724	0.611	0.705	0.631	0.636	0.693
3	0.800	0.512	0.896	0.388	0.698	0.602

(1 = Chili Powder, 2 = Sudan III and 3 = Sudan IV)

From the comparison it can be concluded that ANN fits the best among the all methods used. The R^2 value is high enough. So, the ANN is being proposed with UV spectroscopic data as a method for prediction of Sudan III-IV dyes in chili powder.

Predicted results

In order to show the analytical applicability of the proposed method six commercial samples were then run into the proposed model to predict the amount of chili powder, Sudan III and Sudan IV in the samples. The predicted results are given the Table 3.

Table 3. Predicted percentage of chili powder

Sample name	Predicted chili powder (%)	Sudan dye III (%)	Sudan dye IV (%)
CS1	100.259	0.0002	0.0289
CS2	100.069	0.0012	0.1142
CS3	99.733	0.0058	0.2683
CS4	99.415	0.1856	0.3955
CS5	99.534	0.1089	0.3524
CS6	99.596	0.0788	0.3207

From the predicted values, we can see that all the samples contain almost 100% chili powder and trace amount of Sudan III and Sudan IV. It may be an error of prediction as the R^2 value of model is not equal to 1. So, some prediction error can occur. Apart from the very insignificant error, it can be easily seen that no sample contains any kind of Sudan III or Sudan IV dye.

Conclusion

The report confirms the usefulness of ANN method using UV–visible data for the simultaneous determination of chili powder, Sudan III and Sudan IV in commercial samples. The proposed procedure allows chili powder and Sudan III-IV dyes to be quantified with acceptable errors in concentration level. Results obtained also reveal that the develop method could be used for a routine analysis of chili powder and their quantitative control of adulteration with dyes specifically Sudan III and Sudan IV with minimum time and cost.

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