Boric Acid Adulteration Detection in Wheat Flour using Solvent Extraction Followed by ATR-FTIR Spectra Collection and Applying Feed Forward Neural Network

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ABSTRACT: Food adulteration is very common and thus its detection requires easy, fast and reliable methods. It is self-evident that availability of easy detection methods discourages adulteration. Current study is striving to develop fast and easy method for detection of boric acid adulteration in wheat flour by using by using Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) spectra collection along with Artificial Neural Network (ANN) classification of spectra. Thus objective of study was to help in solving challenge of adulteration detection using easy to use methods. Extraction of adulterated samples was done both with water and alternatively with methanol. In case of methanol used as solvent for extraction, later on methanol was evaporated and samples was re-suspended in water. Finally spectra is collected by applying extract to FTIR crystal. It was assumed that instead of applying samples directly, extraction will increase detection rate. It was discovered that combination solvent extraction with ATR-FTIR spectra and ANN approach can detect equal or more than 2% adulteration of boric acid in wheat flour successfully. Objective of the current study to easily detect adulteration was solved to an extent. It was concluded that improved sample preparation along with more sensitive ATR-FTIR technology can improve detection levels further.

Keywords: Adulteration, Wheat Flour, Boric Acid, ATR FTIR, Neural Network.

INTRODUCTION

Food adulteration is addition or removal of anything which is not explicitly communicated and such adulteration is happens commonly (Medina *et al*., 2019). One such adulteration is wheat flour which is added with boric acid. Wheat flour is added with boric acid because boric acid is insecticide and thus prolongs shelf life of wheat flour. Boric acid acts as antimicrobial agent and insecticide (National Pesticide Information Center, 2013). In high doses boric acid is toxic and it damages vital organs like kidneys (Weir & Fisher, 1972). Boric acid is not allowed as preservative in wheat flour in many jurisdictions including in India (EFSA ANS Panel, 2013; FSSAI, 2011). Thus it was need of time that methods for boric acid detection in wheat flour be developed.

Rapid and non-destructive methods work well for easy adulteration detection. FTIR spectroscopic methods are such methods. FTIR and chemo-metric based methods are already reported in literature for adulteration detection in numerous commodities (Medina *et al.,* 2019). Absorption of light by molecular vibrations and interaction of this vibrations along with overtones become visible in IR spectroscopy and this results in very complex infrared spectra. Because of this complexity of IR spectra it can be hard to attribute specific features present in IR spectra to presence of particular molecule in sample or presence of certain kind of bonds. Calibration techniques depending on multiple variables e.g. partial least squares, principal components analysis, artificial neural networks, are frequently used in IR spectroscopy to churn out required information about undelaying chemical signature (Medina *et al.,* 2019; Santos *et al.*, 2013). Artificial neural networks excel at pattern recognition task and in common at processing complex data which is difficult to process by other methods (Goyal *et al.,* 2021; Medina *et al*., 2019). Therefore in current study IR spectra obtained using solvent extraction of spiked

samples was used to detect adulteration using feed forward artificial networks. ANN used here can be regarded as chemo metric augmentation tool.

MATERIALS AND METHODS

Raw Materials and Sample Preparation. Wheat grains were purchased from local market and was of "MP Tukadi Sharbati" variety. Pure wheat flour was prepared by milling wheat grains in flour mill. AR grade boric acid H_3BO_3 having 99.5% purity was procured from Molychem, Mumbai, India. Boric acid was in dusty fine powdery form. Spiked samples were prepared by taking pure wheat flour by weight and then adding boric acid by weight. Control samples were prepared either form pure wheat flour or from pure boric acid. Adulterated samples were prepared having 8%, to 0.1% boric acid in wheat flour. Saturated boric acid solution in water, 0.16% boric acid in water and 0.02% boric acid in water samples were prepared to see effect of boric acid concentration change on spectra and also these samples served as positive control. As negative control 100% pure what flour extract was used.

Solvent Extraction of Boric Acid. The control and spiked samples were subjected to solvent extraction. For this purpose water or methanol was used as solvent. For extraction 10 g of prepared solid sample was mixed with 50 ml of water or methanol in 100 ml beaker. The content was then mixed for 5 minute by stirring and then it was filtered out using Whatman filter paper number 41. Uniform speed of stirring was maintained for all samples. In case of methanol, the solvent was then evaporated in hot air oven at 70°C till no visible traces of solvent were observed. The residues were then responded in 37 °C warm water. This water suspension was used for spectra collection. For samples extracted with water, extract after filtration was directly used for spectra collection.

Spectra Collection. Cray FTIR 640 spectrophotometer was used to collect spectra. The FTIR machine worked in Attenuated Total Reflectance (ATR) mode and was from Agilent Technologies. ZnSe (zinc selenium) reflectance crystal was used with single light reflection point. The crystal was of brand "Pike Miracle". KBR (potassium bromide) beam splitter was used in FTIR machine. Detector in FTIR machine was "TE cooled DLaTGS" and was set at sensitivity level 4. Speed of spectra collection was 5 kHz. For each sample presented to FTIR instrument spectra was obtained 4 times and averaged out by machine. In other words "number of scans" variable was set to value 4. Wavelength range for spectra collection was 6000 to 600 cm^{-1}. Sampling interval for interferogram as set at value 2. During sample collection1.28 kHz electronic low pass filter was kept active. Symmetric double sided type interferogram was used. Processing of spectral data was performed by Resolution Pro (Version 5) software. Software applied ATR correction to spectra and then outputted data in form of absorbance vs. wavelength values.

To collect spectra first background scan was performed without putting anything on ATR crystal. After that extract of control or spiked samples was kept on ATR crystal. As sample was liquid pressure arm was not used. Sample compartment hatchet was remained closed during sample spectra collection and also during background scan. Spectra was collected for various levels of spiking and these levels are reported in results and discussion section.

For each sample prepared20 readings were taken by repeatedly applying sample and then removing it before applying same sample again. This resultant data set was divided in 50-50% ratio for training and testing ANN.

Artificial Neural Network Parameters. Feed forward type fully connected neural network with hidden layers was used without any bias inputs. Input layer contained exactly same number of neurons as there are data points in data set. Hidden layer always contained 32 more neurons than input layer. 2 neurons were used in output layer corresponding to positive or negative detection. Number of hidden layers was fixed at 1.Output function used was Sigmoid function. Stochastic gradient decent was used as training method. All inputs values were scaled up by dividing them with 0.02. Back propagation with momentum value set at 0.9 was used as raining algorithm. Learning rate was selected based on data. Number of learning epochs were dependent on target error for example when target error was achieved or learning was stopped. Following error function was used.

$$\text{Error} = \frac{1}{2}\sum_{k=1}^{k=n}\left(T_k - O_{o,k}\right)^2$$

Here $k = 1$ to $n$shows output neuron numbers, T_k indicate pre-set expected output on k^{th} output neuron and $O_{o,k}$ indicate actual output on k^{th} neurons in output layer. Maximum acceptable error for learning was set to 0.01 or 0.001 as per experimental requirement and if neurons shows less than 0.35 error on each individual neutron learning is considered successful. Trained networks was tested for correct identification of adulteration on test data set. The sample classification was considered incorrect when total average epoch error was more than 0.1225which was based on above acceptable error criteria about with more is discussed in "results and discussion" section.

Statistical Analysis. Using R software version 3.6.2 available on www.R-project.orgprinciple component analysis was done.

RESULTS AND DISCUSSION

Water Extract Spectra 1 to 8%. FTIR spectra plot for water extraction of 8% to 1% boric acid spiked (green colors) and control samples (blue color) is given in Fig. 1. All the wheat flour samples were extracted with 50 ml of distilled water and after filtering the extract was subjected to spectra collection. Total 120 spectra are superimposed on each other to get clear comparative picture about results. Along with this 120 wheat flour spectra, 10 spectra of each saturated, 0.16% and 0.02% boric acid solution in distilled water are also provided in same figure (gray color) for comparison.
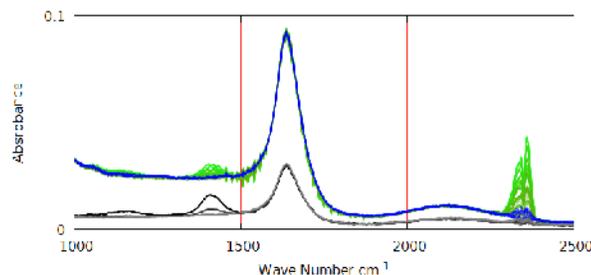
Fig. 1. FTIR spectra, 8% to 1% spiked (green colors) and control samples (blue color) water extraction and pure boric acid solutions (gray colors) in water.

Form the visual inspection of FTIR spectra it is clear that for detection of boric acid one can focus on 1360 to 1470 cm^{-1} region as well as on 2290 to 2390 cm^{-1} region. As 2290 to 2390 cm^{-1} region is giving better spikes that region was selected to train and test ANN. It must be noted that in 1360 to 1470 cm^{-1} region we can see bumps in pure boric acid solutions spectra's in proportion to strength of boric acid in such solutions but such distinct response is absent in 2290 to 2390 cm^{-1} region. The 1420 cm^{-1} peak can be attributed to B-O bonds in boric acid as suggested by literature (*Boric Acid*, n.d.; Patnaik, 2004). Peak at 1640 cm^{-1} is due to water as such peak is present in water IR spectra (*Water*, n.d.) but water IR spectra does not have strong peak at 2290 to 2390 cm^{-1}. As relatively large peak at 2290 to 2390 cm^{-1} is absent both from control sample and pure boric acid solutions we can assume that interaction of sample boric acid and water should be considered responsible for peak amplification.

Methanol Extraction Spectra 1 to 8% and Beyond. FTIR spectra plot for methanol extraction of 8% to 1% boric acid spiked (green colors) and control samples (blue color) is given in Fig. 2. All the wheat flour samples were extracted with 50 ml of methanol by mixing and then filtering was done. The methanol was evaporated. The residues were re-dissolved in 10 ml distilled water for spectra collection. Total 120 spectra are superimposed on each other to get clear comparative picture about results. Along with this 120 wheat flour spectra, 10 spectra of each saturated, 0.16% and 0.02% boric acid solutions in distilled water are also provided in same figure (gray color) for comparison.

It is clear from visual inspection of FTIR spectra that only region which can provide boric acid detection is 1370 to 1470 cm^{-1}. Thus this region was selected for further study. Similar spectra was obtained when data was plotted for 0.1 to 1% adulteration and thus plot is not shown here.

PCA Plots. In PCA plot, figures like 8.0 _17 indicate that the text is representing position of 8.0% adulteration at 17th replication. For control samples Ctrl_5 indicates position of 5th replication of control sample.

Fig. 3 shows PCA of spectra obtained by water extraction in 2290 to 2390 cm^{-1} region. From Fig. 3 one can clearly see that up to 1% adulteration detection is possible. Fig. 4 shows PCA of spectra obtained by alcohol extraction in 1 to 8% adulteration range in 1370

to 1470 cm^{-1} region. From Fig. 4 one can clearly see that up to 1% adulteration detection is possible.
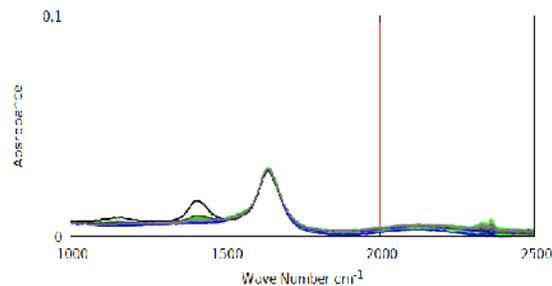
Fig. 2. FTIR spectra, 8% to 1% spiked (green colors) and control samples (blue color) methanol extraction and pure boric acid solutions (gray colors) in water.
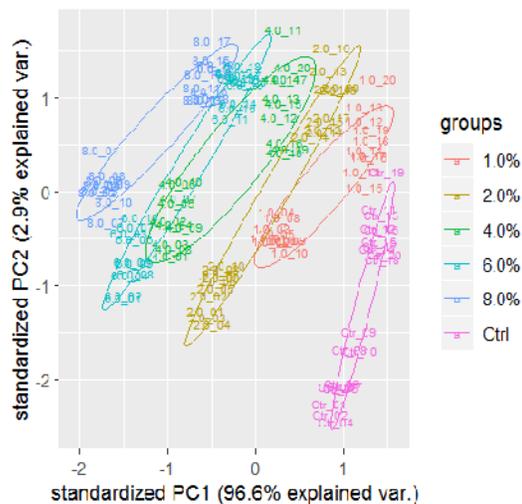
Fig. 3. PCA plot for water extract 1-8% adulteration.

Fig. 5 shows PCA of spectra obtained by alcohol extraction in 0.1 to 1% adulteration range in 1370 to 1470 cm^{-1} region. From Fig. 4 one can clearly see that adulteration detection below 1% level is difficult as indicated by mixing of groups.

From PCA analysis, it is evident that separation of adulterated samples from control samples is difficult at lower levels of adulteration. The same result was confirmed when data was analyzed using artificial neural networks. Use of PCA for data analysis along with chemometrics is well reported in literature (Amsaraj *et al.*, 2021).
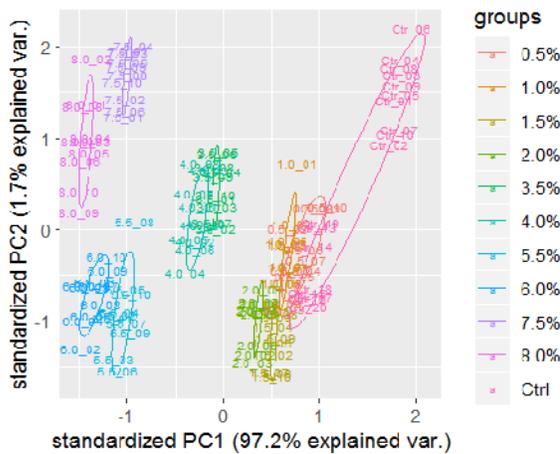
Fig. 4. PCA plot for alcohol extract 1-8% adulteration.
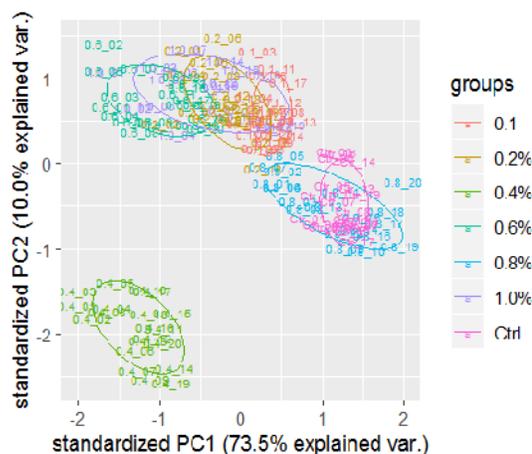
Fig. 5. PCA plot for alcohol extract 0.1-1% adulteration.

ANN Study. Selection of hyper parameters of ANN is somewhat arbitrary choice as there exist no clear cut rules though some guidelines are suggested in literature (Smith, 2018). Some ANN hyper parameters were chosen considering need of study. Deciding other ANN hyper parameters can be considered empirical choices. For example number of input and output neurons were decided by input and output requirements. Scaling of inputs was found necessary and helpful as otherwise all inputs were clustered around zero which gave similar output when used as inputs of sigmoidal function making learning difficult. It was observed by authors that inputs varying between -4 to 4 in equally distributed way are ideal for sigmoid function output variation. Scaling in present study was step in this direction. Scaling of inputs was done by multiplying inputs by 50 and taking results as inputs.

Identification of pure wheat flour vs. adulterated samples was tried. For this neutral network was assigned to give output as given in Table 1.

Table 1: Neutral network output table.

Output Neuron	Output Pure Sample	Output Adulterated Samples
First	1	0
Second	0	1

Choice of 0.1225 as maximum allowed output error originates from criteria that maximum allowed error on each output neuron should not exceed 0.35. Considered two output neurons value of error function must be less than $\frac{1}{2}(0.35^2 + 0.35^2) = 0.35^2 = 0.1225$. Samples giving more than 0.1225 errors are counted as not classified correctly. Maximum allowed error for training was set to 0.04 on each neuron and this translated to 0.0016 in terms of value of error function and this allowed training till error is fairly small. If error was less than 0.0016 that sample did not contribute to ANN training.

Learning rate for ANN was chosen based on try and error method. Too high learning rate was found preventing learning at all i.e. all output became 0.5 and remained so. Too low learning rate caused need of too many epochs to bring error under control. Learning rate

of 0.064 was found satisfactory as it give almost smooth continuous decrease in error with epochs. It must be noted that low learning rate give ability to train ANN to very low errors at the expense of epoch count but too low error targeting on train set and too many epoch cause over fitting of ANN to train data. To avoid over fitting this study limits target error at 0.0016.

By running ANN at various adulteration levels and using one to one comparison with control sample it was possible to detect adulteration in 100% cases at 2% or greater levels. At lower levels some replications were not giving detection and up to 20% of test set samples were miss classified. Earlier reported results for similar study has shown that at 2% spiking level 15% samples miss classify. But in that earlier study no extraction was performed i.e. samples were directly used for spectra collection in solid form. Thus solvent extraction improved detection but only marginally.

Apart from result mentioned above, examination of literature reveals that use of ANN to detect adulteration with FTIR in wheat flour is not reported anywhere though many similar studies for various adulterants using various chemometric tools are reported (Akin *et al.*, 2019; Amsaraj *et al.*, 2021; Andrade *et al.*, 2019; Limm *et al.*, 2018). Thus finding of this study that "ANN can be employed to detect boric adulteration in wheat flour" is in harmony with reported literature.

CONCLUSION

Feed forward artificial neural networks can be successfully used to detect boric acid adulteration in wheat flour at more than or equal to2% levels using FTIR spectra. It can be concluded that detection level is on higher side and improvement scope remains on side of sample preparation and spectra collection techniques rather than on tweaking ANN.

Conflict of Interest. None.

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