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Research Article

Development of paper-based strip to determine uric acid in wheat flour

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Abstract

The quality of the food products is often deteriorated due to contamination with uric acid and insect fragments which leads to unhygienic condition and makes it unfit for consumption. The uric acid can be detected by various techniques such as HPLC, GC-MS, sensors, and electrophoresis. But the major disadvantage with these techniques is that it is costly and takes a long time to analyse the samples. The major objective of this work is to overcome the problems by developing a paper-based strip to detect uric acid levels in food samples. The principle of the paper-based strip is based on the colour intensity developed by the formation of Prussian blue colour. Based on this principle, the colour intensity was calculated using the MATLAB coding. The colour intensity increases based on an increase in the concentration of uric acid. The linear regression model shows that the R^2 is about 0.993, which depicts that this model is accurate and also other parameters such as RMSE, MAPE, AIC, SBC and APC values are also in lower range. The concentration of uric acid in wheat flour was calculated using the MATLAB code. The results of the analysis and validation showed that the paper-based strip method is accurate and can be used as a cost-effective method for uric acid determination in the food industry.

Keywords: Insect Infestation, Wheat flour, Uric acid, Paper-based strip, MATLAB

1. Introduction

Wheat belongs to the poaceae family and is group as grasses. There are five diverse species of wheat namely diploid einkorn, tetraploid emmer, hexaploid common wheat, durum wheat and spelt. Wheat contains five distinct layers namely pericarp, which is the fruit coat; testa, which is the seed coat; aleurone layer; starchy endosperm, which makes upto 80 to 85 per cent of the grain; Testa, which is the innermost layer (Wieser, Koehler et al. 2020). Globally, the predominant cultivars of wheat include central and west Asia, North Africa, the USA, Russian federation and Australia. Among them India hold the second place accounting to 99.70 million tonnes (Ramadas, Kumar et al. 2019). It is estimated that wheat flour contains moisture, carbohydrate, crude protein, crude fat, crude fibre and total ash of 6.71, 75.47, 10.35, 5.05, 1.33 and 1.09 per cent respectively (Owheruo, Akpoghelie et al. 2023).

Postharvest losses in cereals and grains mostly occur due insect infestation. According to USDA, insect infestation leads to monetary losses of over 470 million dollars per year. The losses include grain consumption, contamination in the form of insect fragments, cast skin and excrements. The wheat infested with insects exhibit deprived protein quality, gluten, sedimentation value and non-reducing sugars. The infested wheat flour also witnesses an increase in the acidity and a decrease in the pH. This is attributed to the presence of uric acid (Sánchez Mariñez, Cortez

Rocha et al. 1997). Uric acid is normally produced as a result of insect excretion and thereby indicates the level of infestation (Ghaedian and Wehling 1996). The major pest that causes an increase in uric acid level and thereby economic loss in wheat flour is *Tribolium castaneum* and is commonly called red flour beetle (Gao, Qi et al. 2022). Research suggested that one number of the insect with a storage period of 21 days, could increase the uric acid level and the microbial count of the flour up to 1.8±0.16 g per kg and 7.34± 0.5 colony forming unit per gram respectively (Negi, Pare et al. 2022).

Generally, uric acid is the end product of purine metabolism (Mazzara, Patella et al. 2021). Detection of uric acid is indispensable as it is an indicator of insect contamination. Chromatographic techniques such as liquid chromatography has emerged as one of the accurate and sensitive methods to detect the uric acid in wheat kernels (Wehling, Wetzel et al. 1984). There are a wide range for techniques for the detection of uric acid in wheat grains. But the detection of uric acid in wheat flour is limited to fewer techniques such as floation method, staining, near infra-red spectroscopy and DNA fingerprinting (Fu, Zhu et al. 2021). Electrochemistry, spectroscopy and chromatography are the three major techniques for the detection of uric acid (Pachla, Reynolds et al. 1987). The most common electrochemical methods include electrochemiluminescence, voltammetry and surface plasmon resonance. Gas and liquid phase chromatography are the most used chromatographic techniques. The most employed spectral methods for uric acid determination are fluorescence and ultraviolet absorption (Wang, Wen et al. 2020).

Though the aforementioned techniques are accurate and precise, a major drawback lies in the fact that it is extortionate. This research aims at developing a cost-effective method for detecting uric acid in wheat flour. Based on the drawbacks and knowledge on the detection of insect infestation, a simple paper-based technique to detect uric acid in wheat flour was developed. The novelty of research aims at utilizing MATLAB for the development of paper based uric acid detector.

2. Materials and Methods

The different concentrations (0-100 ppm) of standard uric acid solution (UA) and wheat flour with different uric acid concentration (WFUA) (8, 16, 24, 32, 40, 48, 56, 64, 72, 80, 88 and 96 ppm/100g) were prepared. The procedure for uric acid extraction was followed for standardising the developed paper strip technique.

2.1 Extraction method

The different concentration of UA (0-100 ppm) and WFUA (8, 16, 24, 32, 40, 48, 56, 64, 72, 80, 88 and 96 ppm/100g) were taken in centrifuge tubes separately and 7mL of sodium borate (0.02M) was added. It was mixed well with vortex and the pH was adjusted to 8.7 using 0.1N HCl. The standard uric acid and wheat flour with different concentrations of uric acid were centrifuged (REMI: model: C-30BL) at 5000 rpm for 30 minutes. The supernatant was collected and filtered using Whatman filter paper No.1. The filtrate was stored in vials for analysis (Dey and Bhattacharya 2017).

2.2 Development of the paper-based strip to detect uric acid

The paper strip was prepared using Whatman filter paper No.40 as base material with 20 mm of width and 60 mm of height as in Fig 1. Then, ferric chloride (30µl, 0.02M) was added to the surface of the paper strip and dried in shade.

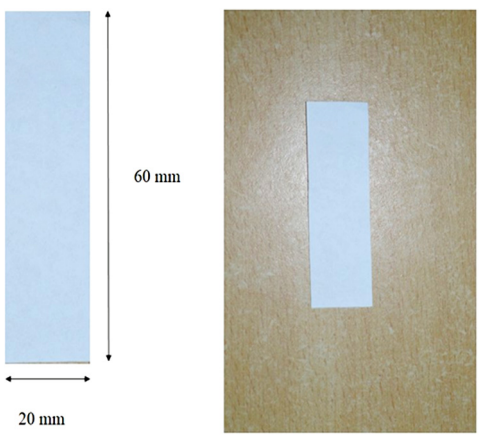

Fig 1: Paper strip to detect uric acid

2.3 Evaluation of paper strip method

The developed paper-strip was evaluated with standard uric acid alone and wheat flour with uric acid. The different concentrations of standard uric acid were added to wheat flour and mixed thoroughly. The filtrate of different concentration of UA (0-100 ppm) and WFUA were treated with potassium ferricyanide (30µl, 0.02M) separately and were applied on a paper-strip surface. The filtrate reacts with potassium ferricyanide to produce potassium ferrocyanide that reacts with ferric chloride

and forms Prussian blue colour. The colour formation was captured by a GigE vision area scan camera (C1600, Genie colour series, DALSA) fitted with a magnifying lens (Zoom 7000, 9mm, Navitar). The captured image was analysed using MATLAB software. The MATLAB code was generated to determine the uric acid present in wheat flour.

2.4 Linear regression analysis using XLSTAT

A linear regression is defined as a statistical analysis applied to a set of data to describe and quantify the relationship between the dependent and independent variables. Regression analysis allows to predict the values of dependent variables based on one independent variable value. In correlation analysis, "r" is a dimensionless number where the value falls from -1 to +1. If the r value falls in -1 indicates that negative relationship whereas it falls in +1 range indicates positive relationship. The linear regression analysis uses an equation ($y = mx+c$) that defines the best fit of line for the relationship between dependent and independent variables. The mean squared error (MSE) measures the average of square deviation between predicted and actual datas. The square root of variance of residuals is defined as root mean squared error (RMSE) (Ul-Saufie, Yahya et al. 2011).

The R^2 indicates the degree of variability of dependent variables due to independent variables. The adjusted R^2 gives the percentage of variations explained by the independent variable that affect the dependent variable. The Akaike's Information Criterion (AIC) indicates by adding more parameters in model that expands the goodness of fit but also increases the penalty imposed by adding more parameters. The measure of quality of predictions made by regression model is known as MAPE (Mean Absolute Percentage Error). The DW (Durbin-Watson statistics) that denotes the autocorrelation coefficients of residual series.

The measure of influence of individual cases in a set of data on linear regression fit is equally impressive is defined as Mallow's C_p criterion (Cp). The Schwarz's Bayesian criterion (SBC) is used to test and locate the changes of variances in normal distribution. The Amemiya's prediction criterion (APC) was proposed by Amemiya in 1980 is used like adjusted R^2 to take account of parsimony of the model (Kumari and Yadav 2018).

3. Result And Discussion

3.1 Colour profiling

A paper strip can be used to detect uric acid contamination in food products and to find the concentration of uric acid in food products using MATLAB coding. The captured paper strip images were analysed. It was observed that, as the UC concentration increases, the captured images showed an increase in colour signal as shown in Fig 2.

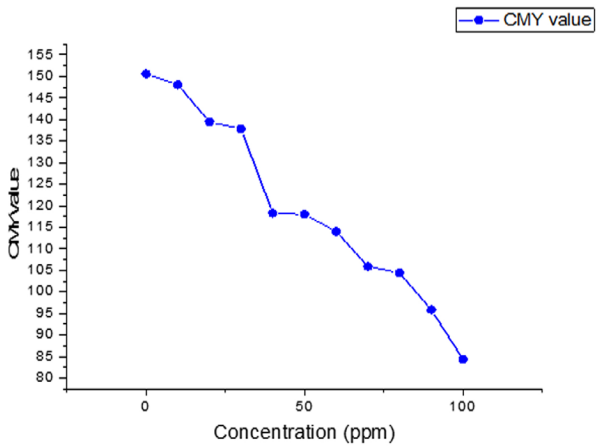

Fig 2: CMY gray value of standard uric acid

Earlier, the activity of two enzymatic papers were quantified by colorimetric analysis by measuring the colour intensity (RGB to Gray scale) of the product (Khan, Thouas et al. 2010). In the same way, Waseem *et al.*, (2011) reported that the uric acid content was determined based on reduction of Fe (III)/ ferricyanide in the presence of uric acid by a flow injection spectrophotometer method. The unreduced Fe (III)/ ferricyanide reacts with in situ reduced ions to produce Prussian blue. This method was useful to determine the uric acid in human urine with a recovery range of 96-105% (Waseem, Yaqoob et al. 2011).

Similarly, a paper strip was developed to detect uric acid on-site in distant areas. A bis-pyrene-based amphiphilic probe was used to detect the uric acid in nanomolar level at pH (7.4) in water. The mechanism was based on the combination of both hydrogen bonding and electrostatic interaction between quaternary nitrogen ends and amide functional groups of probe molecules (Dey and Bhattacharya 2017).

3.2 Determination of uric acid using MATLAB coding

The uric acid was determined using the MATLAB R2019a software. Initially, the RGB values (three-dimensional

matrix) were converted into gray (Black & white) values and denoted as (b). The total pixel value (c) and colour intensity (d) were calculated as mentioned in MATLAB coding. The mean value (m) was calculated from the resulted matrix. The colour signal developed for different concentrations of standard uric acid was shown as a colour chart in Fig 3. The colour intensity of different uric acid concentrations was calculated by MATLAB code showed the correlation coefficient (R^2) of 0.993 against the uric acid concentration and got saturated at a level of 30mg/mL concentration of uric acid (Fig. 4). As the concentration of uric acid increases, colour intensity produced on paper diagnostic device became stronger and intense. The colour signal produced by uric acid in urine solution was captured using a camera and analysed in ImageJ software (Islam, Ahmed et al. 2018). The concentration of standard uric acid and the changes occurs due to colour intensity are shown in Fig.3 and Fig.4.

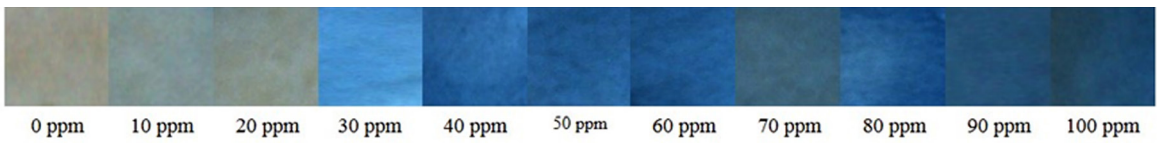

Fig 3: Colour chart of different concentration of standard uric acid

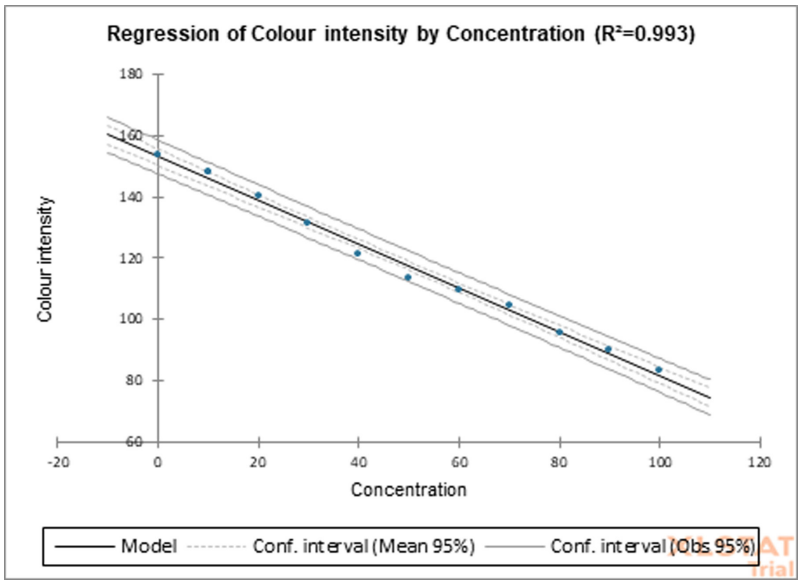

Fig 4: Regression plot of colour intensity by concentration (ppm)

3.4 Validation of linear regression of colour intensity using XLSTAT

The multiple linear regression of colour intensity can be validated in terms of R^2, MSE, RSME, MAPE, DW, Cp, AIC, SBC and PC values that refers to goodness of fit of colour intensity. The data obtained in the present method were analysed and presented R^2, MSE, RSME, MAPE, DW, Cp, AIC, SBC and PC values in Table 1.

In general, the R^2 reveals that 60 per cent of variance is acceptable to consider that the model is fitted. In this method, the R^2 showed 99.3 per cent of variances in colour intensity is accounted by linear regression with the concentration (ppm) and showed that the model of fit test. The adjusted R square (adj. R^2) is used instead of

Table 1: Validation of model parameters that shows Goodness of fit for colour intensity against concentration (ppm)

Comments	Colour intensity
Equation of model	y=153.045-0.713*x
R Squared	0.993
Adjusted R Squared	0.992
MSE	4.420
RMSE	2.102
MAPE	1.358
DW	0.934
Cp	2.000
AIC	18.140
SBC	18.935
APC	0.010

R^2 by adding independent variables to model to raise the R^2. In this analysis, adj. R^2 of 99.2% variation in colour intensity can be attributed to the concentration (ppm) that showed it is an excellent model. In linear regression, the RMSE shows the accuracy of the model which is fit or unfit. The RMSE value less than 7 is acceptable value for the scientifical community. In our cases, the RMSE is about 2.102 for colour intensity showed that the model is accurate and fit. MAPE is an average of absolute percentage error. MAPE value less than 20% is acceptable for the model. MAPE value of colour intensity was 1.358 per cent that showed the model is accurate. The DW is a test for autocorrelation in the residuals from a regression analysis. The DW value less than 2.0 indicates positive autocorrelation. The DW value of 0.934 of colour intensity in the present study showed positive autocorrelation that achieves the consistency of β increases with the intensity with autocorrelation. Similarly AIC and SBC showed that the results were accurate (Kumari and Yadav 2018). Hence the paper-based method of uric acid estimation found to be the accurate method.

Evaluation of paper strip method

The uric acid mixed with different concentration was analysed using the developed paper strip method. The results showed accuracy of the method for estimating the uric acid in food products (Table 2). The results revealed that the paper strip method can be used efficiently for estimating the uric acid contaminations in the food products.

Table 2: Evaluation of paper strip method with different concentration of uric acid in wheat flour

Standard uric acid added in wheat flour (ppm/100g)	Uric acid concentration obtained in paper strip method (ppm/100g)
8	7.69
16	15.68
24	23.59
32	31.98
40	39.14
48	47.58
56	55.60
64	63.13
72	71.11
80	79.17
88	87.71
96	94.98

Conclusion

The paper strip was developed to determine the uric acid in wheat flour. The colour changes were captured using a GigE vision area scan camera and analysed using MATLAB software. The R^2 (0.993) showed the accuracy of colour intensity developed against the standard uric acid. The results revealed that the developed paper strip method can be used for estimating uric acid in the food products during processing and storage. This simple paper strip method will be very useful for food processing industries to determine the uric acid in food products instantly without any additional costly advanced analytical equipment. The food processing industries can assess uric acid level in the raw materials and can reject the infested raw material to develop quality food products for consumers. Based on the outcome of the study, the sensor based uric acid estimation can be a great cost-efficient scope for the detection of uric acid in the near future.

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Author contributions

The conceptualization of research (C.I., M.L. and R.N.); Designing of the experiments (C.I., M.L. and R.N.); Execution of field experiments and data collection (C.I., M.L. and R.N.); Analysis of data and interpretation (C.I., M.L. and R.N.); writing–original draft preparation, C.I., M.L. and R.N.; writing–review and editing, C.I., M.L. and R.N.; Preparation of the manuscript (C.I., M.L. and R.N.).

Conflict of interest

No

Declaration

The authors declare no conflict of interest.

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