ijlpr 2022; doi 10.22376/ijpbs/lpr.2022.12.1.L200-205

International Journal of Life science and Pharma Research

ISSN 2250-0480

Research Article

Simple and Rapid UV method for Uric acid Determination

Development Of A Simple Rapid Method For Determination Of Uric Acid Using UV-Visible Spectroscopy

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Abstract: Insect infestation is a major problem in the storage of food products and causes quality and quantity loss. The total weight loss of food products is about 10 per cent which occurs due to moisture loss, insect infestation, rodents, microorganism and birds during transportation and storage period. The insect infestation can be identified by detecting the presence of insect fragments, uric acid contamination, and quinone contamination. The uric acid can be determined by various instrumental methods but requires longer duration and high-tech equipment. The major objective of our study is to develop a simple rapid method to determine the uric acid content using UV-visible spectroscopy in the insect infested food materials. The rapid method is required for the food processing industries. A rapid method was developed to determine the uric acid with lesser time and accuracy. In the present study, a preceding method forms an unstable chromophore, whereas in case of a rapid method with UV-visible spectrophotometer, it was based on the formation of Prussian blue colour. The rapid method was validated with the preceding method. The R, R^2, standard error and Durbin-Watson test values of preceding method were 0.996, 0.991, 0.000572 and 1.103 respectively, whereas the values for rapid method were 0.997, 0.994, 0.024806 and 1.713 respectively. The results of the analysis showed that, the rapid spectrophotometric method required less time of 20 minutes for analysis, whereas the preceding method required 40 minutes. The results of the study concluded that, the new rapid method is a simple and easy method to determine the uric acid content in a lesser time. The outcome of the result will help the food processing industries to utilize this simple rapid method for testing the uric acid analysis in the food products in a lesser time.

Keywords: Uric acid, insect infestation, UV-visible spectrophotometer, rapid method, validation.

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Received On 9 November, 2021
Revised On 13 January, 2022
Accepted On 17 January, 2022
Published On 24 January, 2022

Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

Citation Induja C, , Loganathan M, And Shanmugasundaram S , Development Of A Simple Rapid Method For Determination Of Uric Acid Using UV-Visible Spectroscopy.(2022).Int. J. Life Sci. Pharma Res.12(1), L200-205
http://dx.doi.org/10.22376/ijpbs/lpr.2022.12.1.L200-205

1. INTRODUCTION

Insect infestation is the most vital problem in the storage of grains and flours; which results in contamination of uric acid and exuviae in stored food materials. These contaminations reduces the quality of the product and its market value. The periodical monitoring of infestation will help to assess the quality of the grains and flours and to take necessary control measures. The infestation can be monitored by visual inspection, but the contamination in grains has to be detected by insect fragment counts and uric acid estimation. Most of the methods of uric acid detection were based on chemicals, enzymatic, fluorescence and biosensors.[1]The important structural components of insect cuticle are chitin, but it cannot be used as a sign of infestation or contamination. The presence of fungi in stored food products may also indicate insect infestation.[2] But the microbial growth will occur only when the grain or flour stored with high moisture content.[3] It was reported that the major end product of nitrogen metabolism in insect is uric acid and 80 percent of nitrogen from faeces was released by insects.[4] Hence measuring uric acid with accurate and rapid method will help to follow disinfestation method in the food storage system. A colorimetric method was used to measure the uric acid content in infested food samples.[5]A large number of food samples can be analyzed rapidly by this colorimetric method using phosphotungstic acid. The turbidity in food samples (flours and grains) can be avoided by using phosphotungstic acid. It was reported that, the method based on arsenal phosphotungstic acid gave a practically good correlation between uric acid concentration and insect number exists in the grain samples.[4]It had been enhanced by a fusion of uricase enzyme and by use of paper chromatography. An enzyme-based colorimetric method was simpler than an enzymatic-ultraviolet method to determine uric acid in flour. This method is more susceptible and uricase enzyme action can be destroyed by adding hydrochloric acid to the sample. This procedure had not been widely used because of the time required for analysis is too long.[6] The liquid chromatography was used to find out the uric acid content of internally infested wheat kernels by various life stages of granary weevil (*Sitophilus granarius*), rice weevil (*Sitophilus oryzae*) and lesser grain borer (*Rhyzopertha dominica*).[7] It was reported that the correlation exists between insect population and uric acid content of infested grain by a particular stage of an internally infesting stored product insect, with correlation coefficients ranging from 0.970 to 0.998. They also found a detection limit for the analytical procedure of less than 1.0 ppm uric acid for late instar granary weevil larvae. But it is difficult to analyse a large number of samples in these methods in a shorter period. Based on the literature review for determination of uric acid in the food materials, the aim of the present study is to develop a rapid method to determine uric acid contamination due to insect infestation in food products. The major objective of our study is to develop a simple rapid method, to determine the uric acid content using UV-visible spectroscopy in the insect infested food materials during processing, storage and validation of the developed method.

2. MATERIALS AND METHODS

A simple rapid method for uric acid determination was developed with standard uric acid at different concentrations and validated using the colorimetric method (Preceding method). It was also validated statistically.

2.1 Standard Preparation

A stock solution of uric acid was prepared by weighing 100mg of uric acid and 900mL of distilled water and made up to 1litre in volumetric flask. From the stock solution, the working standard of different concentrations (20, 40, 60, 80 and 100 ppm) was prepared in a 50mL volumetric flask and made up to volume with distilled water. The working standard was allowed to stand for 10 days.

2.2 Determination The Uric Acid With Colorimetric Analysis Of Allantoin (Preceding Method)

The different concentration of uric acid (1mL) was taken in a boiling tube and 5mL of distilled water was added and 1mL of NaOH (0.05M) in the boiling tube. It was mixed well using a vortex (DLab; MX-S60HZ) and kept in a water bath at 100°C for 7 minutes. The tubes were cooled with water and adjusted the pH to 2.16 with 0.05M HCl. Then 1 mL of phenylhydrazine hydrochloride (0.023 M) was added and mixed with vortex. The tubes were again kept in a water bath at 100°C for 7 minutes and dumped it immediately into an icy alcohol bath (40% NaOH) for 7 minutes.[8] Then 3 mL of concentrated HCl and 1 mL of potassium ferricyanide (0.05M) were added and kept for 20 minutes. The colour developed was recorded at 522 nm in a UV-visible spectrophotometer (Shimadzu; UV-1800).

2.3 Formation Of Chromophore Based On Reaction Mechanism In Preceding Method

In the preceding method, uric acid was hydrolyzed using weak alkaline (NaOH, 0.05M) at 100°C. The hydrolysed uric acid changed into allantoic acid by the activity of allantoinase enzyme. The allantoic acid degrades into urea and glyoxylic acid in acid solution (HCl, 0.05M) (Fig.1).[8] The phenyl hydrazine hydrochloride reacts with glyoxylic acid to form phenyl hydrazone. The potassium ferricyanide reacts with phenyl hydrazone to form an unstable chromophore.

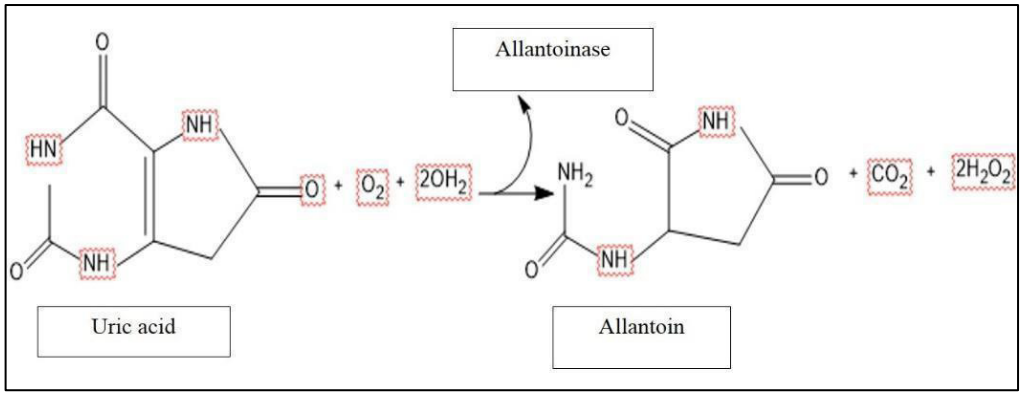

Fig.1.The oxidation reaction of uric acid to allantoin

2.4 Rapid spectrophotometric method to determine uric acid

2.4.1 Developing a simple rapid spectrophotometric method to determine uric acid

A rapid method was developed to reduce the duration of analysis and also to get accuracy. In this method, different concentrations (1mL) of uric acid was diluted five times with distilled water and then 1mL of potassium ferricyanide and ferric chloride (1mL) were added. It was mixed well and kept for 5 minutes.[9] The spectrophotometric readings were taken in a UV-visible spectrophotometer (Shimadzu; model: UV-1800) at the absorbance of 520 nm .

2.4.2 Formation of Prussian blue colour based on reaction mechanism (Rapid method)

The reaction mechanism of uric acid was studied for the simple rapid method to determine the uric acid content using UV-visible spectroscopy. The potassium ferricyanide reacts with the uric acid in infested samples and converts into potassium ferrocyanide (Fig.2). Ferric chloride reacts with potassium ferrocyanide to produce Prussian blue color.[10] The principle of the present rapid method was based on the formation of Prussian blue. The potassium ferricyanide acts with the ferric or ferrous solution to form Prussian blue. The thermal equilibrium occurs between the two solutions and the colour intensity will increase based on the heating process at 25°C.

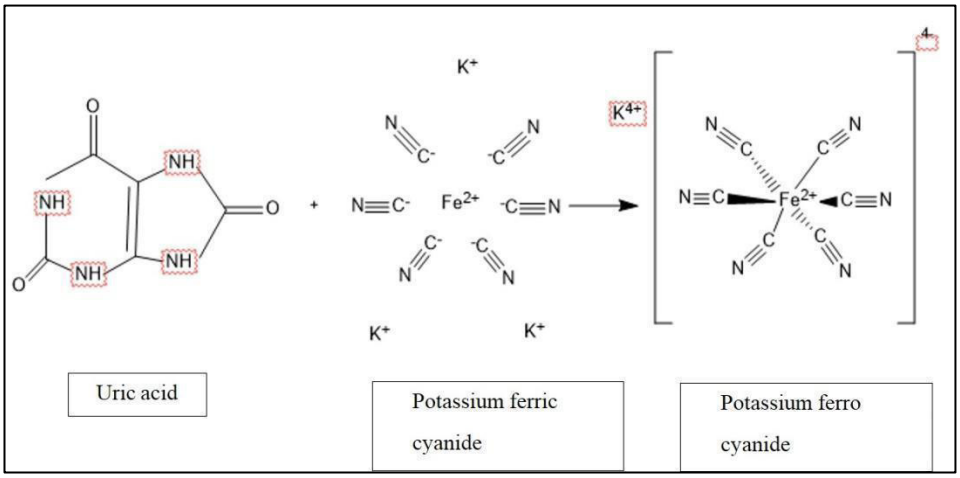

Fig.2.The mechanism of Prussian blue colour formation

2.4.3 Validation Of Rapid Method With The Preceding Method Using Regression Model

A multiple linear regression involves fitting a model for a dependent variable involving more than one independent variable, which is linear in its parameters. The model summary describes R, R^2, Adjusted R^2 and standard error of dependent value (OD) of both the methods. The R value is considered as a measure of quality of prediction of dependent variable (OD). The R^2 ranges from 0 to 1 that represents the proportion of variation in the dependent variable that can be explained by the model explanatory variable. The R^2 represents the coefficient of correlation between dependent and independent variables in the linear regression.[11] The R^2 increases whereas the model is better. The adjusted R^2 is the correction for the number of x variables involved in the predictive model. The adjusted R^2 is close to 1 that indicates the large proportion of variability in the dependent variable that has been explained by the regression model. The adjusted R^2 number which is nearer to 0 indicates that it did not show much variations in the dependent variable. The Durbin-Watson (DW) test is used to test the hypothesis with autocorrelation. If the DW value lies from 0 to 2, it indicates positive autocorrelation. The standard error (SE) represents the average distance that the observed values fall in the regression line. The smaller value of SE indicates that the observation is closer to the fitted line.[12]In case both preceding and rapid methods can be evaluated based on parameters of the regression model.

3. STATISTICAL ANALYSIS

The data obtained were analyzed using XLSTAT software

(Version 2021.2.2.1137). A multiple linear regression model was used to compare the both methods to determine uric acid level based on goodness of fit. The probability value less than 0.05 was assumed in ANOVA.[13]

4. RESULTS AND DISCUSSION

The determination of uric acid in a shorter time is important for analyzing large numbers of food samples. In the present study, a rapid UV-spectrophotometric method was developed to reduce the determination time with more accuracy and validated with the calorimetric method.

4.1 Efficiency of uric acid determination methods

A study using the nanostructured sensor, based on gold nanoparticles and nafion for uric acid determination found that the accuracy and reliability of results have good correlation with the enzymatic spectrophotometric analysis (R^2 = 0.9938).[14,15] Earlier reports on rapid determination of uric acid using electrochemically activated glassy carbon electrode showed a linear range from 0.04 to 2µM with slope of 55.6 A M^{-1}, R^2 of 0.99 and limit of detection of 9 nM.[16–18] In the present study, the correlation coefficient (R^2) of the rapid method using UV-visible spectrophotometer showed 0.994 (Fig.4) and preceding method showed 0.991(Fig.5) which showed the similar accuracy of both the methods. Earlier, the sensors for detecting infestation were used and found that hybrid adapted Neuro-Fuzzy Inference System models (ANFIS) were the best fit to optimize the sensor array detecting infestation and to predict the number of insects (R = 0.999) and uric acid (R = 0.985).[19] A pyrene based amphiphilic receptor was utilized in the nanomolar detection of uric acid content of the aqueous extracts of infested stored grain samples which was used to measure the uric acid at physiological pH in water.[20] A fast method to determine uric acid in milk samples with R^2 value of 0.98 and p value of less than 0.001 was reported.[21] The results of the present study showed that the rapid UV-spectrophotometric method required lesser time of 20 minutes for analysis whereas the preceding method required 40 minutes which explained that the new method required only half of the time to determine uric acid compared to the preceding method. The results revealed that the simple rapid UV-spectrophotometric method is having good accuracy and correlation coefficient with lesser time for determination of uric acid in food samples.

4.2 Validation of rapid method and preceding method using regression model

4.2.1 *Comparison of linear regression for preceding and rapid method*

The linear regression is normally used for the comparison of analytical methods It was reported that aromatic characteristics of 2-cyano-N-(3-cyano-7-tetrahydrobenzo[b]thiophen-2-yl)-acetamide using multiple linear regression models showed an R value of 0.754. Whereas present research work, the model outline of linear regression describes about the R value of 0.996 and 0.997 for preceding and rapid method respectively which showed the R value of both methods indicates good sign of prediction in regression model.[22] The purine derivatives against c-Src tyrosine kinase showed best fit with R^2 of 0.802 was explained by earlier workers.[23] Similarly, the present study showed the R^2 of 0.991 and 0.994 for preceding and rapid method respectively (Fig 3 and 4). These results revealed that the R^2 value of 0.994 is more accurate and the rapid method is more fit in the regression model. The adjusted R^2 of 0.990 and 0.994 were found for preceding and rapid method respectively.

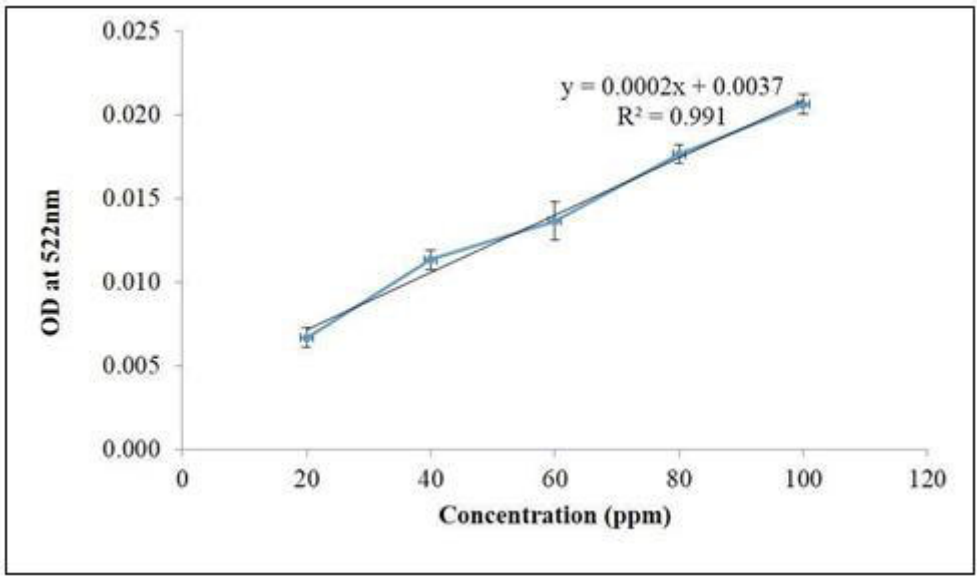

Fig.3. Calibration curve of the preceding method

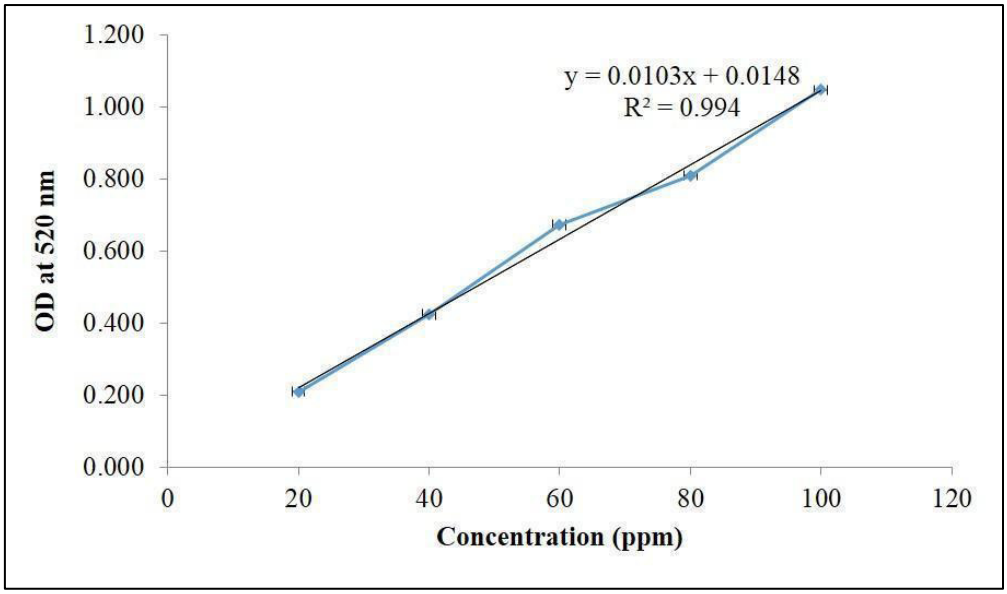

Fig.4. Calibration curve of the rapid method

The SE of model fit is a measure of precision. The SE of preceding and rapid method are 0.000572 and 0.024806 which are less than 2 as reported earlier.[24] The DW test indicated the autocorrelation of preceding and rapid method were 1.103 and 1.713 respectively which are less than 2 (Table 1).

Table 1. Validation of preceding and rapid method

Parameters	Preceding method	Rapid method*
R	0.996	0.997
R^2	0.991	0.997
Adjusted R^2	0.990	0.994
SE of the estimate	0.000572	0.024806
DW test	1.103	1.713
Time required per sample (min)	40	20

*Rapid method showed good fit with lesser time for each sample

The accuracy of the methods were predicted using multiple linear regression model with R, R^2 and SE in the present study (Table 1) which is similar to that the prediction of imidazo[1,2-a]pyrazine derivatives against cancer cell lines Log HepG-2 using multiple linear regression model with the terms of R, R^2, SE value.[25,26] The results of the present study support the earlier report of uric acid detection can be used as an index of insect infestation.[27] It also demonstrated a positive linear correlation between the amount of uric acid and the density of rice weevil, *S. oryzae* in wheat grain.[28,29]

5. CONCLUSION

The preceding method is used to detect the uric acid indirectly by converting the uric acid to allantoin. The uric acid was treated with a weak alkaline solution to convert uric acid to allantoic acid by the activity of allantoinase enzyme. The extraction procedure and colorimetric analysis also required more time (40 minutes) for each sample. But the rapid method is a direct method to determine the uric acid by the formation of Prussian blue colour. This method required less time (20 minutes) compared with the preceding method. The rapid method and preceding method were accurate whereas the $R^2$of rapid method is 0.997 showed good fit compared with the preceding method. The results concluded that, the rapid method is a simple and easy method to determine the uric acid content in a lesser time. The method can be very useful for the food processing industries to monitor the infestation of raw materials and food products.

6. ACKNOWLEDGEMENTS

The authors are thankful to the National Institute of Food Technology, Entrepreneurship and Management-Thanjavur (NIFTEM-T) for the facility provided for conducting the experiments.

7. AUTHORS CONTRIBUTION STATEMENT

Induja, C did this research work under the guidance of Dr. M. Loganathan. Dr. Shanmugasundaram S helped in analysis and drafting of the paper. All the authors read and approved the final version of the manuscript.

8. CONFLICT OF INTEREST

Conflict of interest declared none.

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