International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 18-10-2021, Accepted: 04-11-2021, Published: 19-11-2021
Volume 6, Issue 6, 2021, Page No. 84-86

Influence of host and duration of storage on uric acid contamination in wheat products infested with confused flour beetle, *Tribolium confusum*

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Abstract
The insect infestation in the food commodities affect the quality and reduce the quantity by direct feeding, excretion and contamination with uric acid and fragments. The insect infestation cause contamination such as uric acid and quinone that leads to unpleasant smell from the foods substances. There are different methods to detect internal and external insect infestation that occurs in food samples. A study was undertaken to find out the influence of host and duration storage on uric acid contamination in wheat products infested with confused flour beetle (*Tribolium confusum*) using a spectrophotometric method. The uric acid content in wheat flour was found to be 35.39, 49.63, 67.71 and 71.43 ppb on 7, 14, 21 and 28 days respectively. An increase in uric acid was due to increase in release by the larval stage. But the uric acid level is comparatively less in rava throughout the period when compared to maida and wheat flour which may be due to the variation in preference of food products by *T. confusum* beetle. The results revealed that the infestation in the stored food products leads to uric acid contamination and spoilage of food products in addition to weight loss.

Keywords: insect infestation, uric acid contamination and *Tribolium confusum*

Introduction

The major food source for humans and domesticated animals over worldwide are cereals, oilseeds and legumes. The food commodities have to be stored for a shorter and longer period depends on the requirement and condition of the product. There are possibilities of quality and quantity deterioration of food material during storage by biotic and abiotic factors. The damage of food grains occurs due to the insects, mites, rodents, birds and microorganisms (Vimala Bharathi *et al*. 2017). The primary quality consideration of food products is insect infestation by various insect species. A severe loss may happen to processors by the contamination of food product with the presence of live insects, webbing, protective secretions, feces, cast skins (Indumathi *et al*. 2021) [4]. There are possibilities of reduction in profitable value of food products and returns of product occurs due to the presence of live insects that reduces the consumer confidence because of insect infestation (Johnson, 2013) [5]. The presence of insects has to be identified in the early stage to safeguard the food materials. There are physical and chemical methods to determine the insect infestation in stored food grains. The hidden infestation can be detected by X-ray imaging, specific gravity, insect fragment count, staining techniques, NMR and NIR techniques. The external infestation can be detected by acoustic methods, visual inspection, sampling and sieving, grain probes, insect traps and heat extraction methods (Thanushree *et al*., 2018).

The odor detection techniques are used to detect insect infestation during the storage of food material by way of identifying volatile compounds released by the insects (Olsson *et al*., 2002) [11]. The compounds at lower concentration have to be extracted by distillation and headspace techniques and can be detected by using GC-mass spectroscopy. The E-nose can also be used to detect the insect infestation. A collection of chemical arrays containing in e-nose is used to detect odor compounds from the infested samples (Paolesse *et al*., 2006) [12].

Kumari *et.al*.,(2011) [6] reported that insect produce quinone, uric acid, inoculate bacteria and fungi, also leaves fecal materials and cast off skin that give unpleasant odour. The *T. castaneum* secretes uric acid and it leads to spoil the food materials and it also causes carcinogens (Mehmood *et al*. 2018) [8]. The major insect infesting in food processing facilities, mostly rice and wheat mill is confused flour beetle, *T. confusum*. The infestation leads to contamination in food products due to uric acid, quinone, and fragments of insects. The larva and adult stages of *T. confusum* causes damage to the processed food products. The uric acid can serve as a good index of infestation and unhygienic conditions in infested food grains (Randy *et al*., 1984). The contamination of uric acid depends on the level of infestation, host / food material and also duration of storage of food material. Hence, a study was undertaken to find out the influence of host and duration storage on uric acid contamination in wheat products infested with confused flour beetle (*T. confusum*) using a spectrophotometric method.

Materials and Methods

Insect culture

The confused flour beetle, *T. confusum* was cultured in the glass container with wheat flour as food. The freshly emerged adults of *T. confusum* (50 numbers) were collected from the stock culture, released in the glass container containing 300g of wheat flour and covered with the muslin cloth. It was kept at ambient temperature (34±2°C) for the egg laying. The adults were removed after 24 hrs of egg laying. The eggs grown into larvae, pupae and emerged as uniform aged adults for further studies (Negi *et al*,. 2021) [9].

Infestation of food matrices

The freshly emerged adults of *T. confusum* wereseparated from culture and released 20 adults each in seven glass containers containing the wheat flour, maida, and rava for infestation and covered with the muslin cloth. The infested food materials were kept at ambient temperature (34±2°C) for four weeks. The samples of infested wheat flour, maida, and rava were subjected to extraction of uric acidevery week and the quantity of uric acid was estimated (Negi *et al.*, 2021) [9].

Extraction of Uric acid

One gram of infested product (wheat flour, rava, maida) with 7mL of sodium borate was taken in centrifuge tubes, mixed well in vortex and adjusted the pH to 8.7 using 0.01N HCl. The samples were centrifuged (REMI: model: C-30BL) at 5000rpm for 15 minutes. The supernatant was collected, filtered using Whatman No.1 filter paper and stored the filtrate in vials for further spectrophotometric analysis (Dey & Bhattacharya, 2017) [1].

Estimation of uric acid in Spectrophotometric method

One mL of extracted sample was diluted five times with distilled water in a test tube. Thenone mL of potassium ferricyanide and ferric chloride each were added and kept for 5 minutes. The spectrophotometric readings were taken in a UV-visible spectrophotometer (Shimadzu; model: UV-1800) at the absorbance of 520nm (Teepoo *et al*., 2012) [15] were represented in Fig 1.

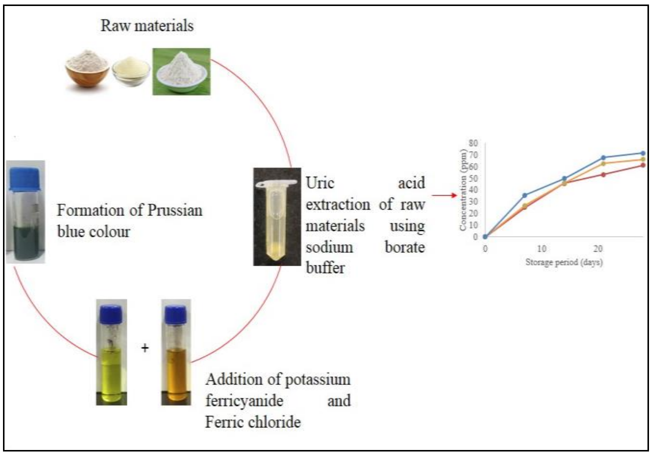

Fig 1: Flowchart for detection of uric acid contamination in food samples

Statistical analysis using Minitab software

The statistical analysis (One-way ANOVA) was carried out using Minitab® 20.4 software. In one-way ANOVA, the major factor is p-value (Tutorial *et al.*, 2015) [17]. The probability that measure the evidence against null hypothesis. The lesser probability affords null hypothesis towards the strong evidence. R^2 is defined as the percentage of variation in response is explained by the model. The higher R^2 shows the better model fit (Bower 2000) [2].

Result and Discussion

The uric acid content in insect infested wheat flour, maida, and rava were extracted and estimated using spectrophotometric method in weekly interval for4weeks.It was observed that the larvae contaminated the food material with uric acid ranged from 25.03 to 35.39 ppb within a week. The results showed a gradual significant increase in uric acid content in all wheat products (Table 1). The uric acid content in wheat flour was found to be 35.39, 49.63, 67.71 and 71.43 ppb on 7, 14, 21 and 28 days respectively. An increase in uric acid was due to increase in release by the larval stage as reported by Holmes, (1980) [3]. But the uric acid level was comparatively less in rava throughout the period when compared to maida and wheat flour which may be due to the variation in preference of food products by *T. confusum* beetle (Ojumoola *et al*. 2020) [10].

Table 1: Influence of storage period on uric acid content in wheat products

Storage period (days)	Uric acid concentration (ppm)*		
	Rava	Maida flour	Wheat flour
0	0.00^e	0.00 ^{e}	0.00^e
7	25.03^d	26.69^d	35.39^d
14	45.54^c	45.78^c	49.63^c
21	53.3^b	62.53^b	67.71^b
28	61.20^a	66.01^a	71.43^a

* Values with different letters in the same column differ significantly (p<0.05)

The uric acid content in maida flour was found to be 26.69, 45.78, 62.53 and 66.01ppb on 7, 14, 21 and 28 days respectively. The uric acid content in rava recorded lesser quantity with 25.03, 45.54, 53.31 and 61.20 ppb on 7, 14, 21 and 28 days respectively when compared to maida and wheat flour. An increase in uric acid in the wheat products may be due to increase in release rate of uric acid by late instar larvae. Similarly, the results showed that there is an increase in uric acid in all wheat products as the storage days' increases. The uric acid concentration of all wheat products still 4th weeks of storage shows significantly difference from each other (p<0.05). Pixton (1965) [13] reported that an increase in uric acid level in infested wheat flour which was related with an increase in larvae production. In their study, 32 number of adult showed an increase in uric acid content during larval stage within 14 days and also showed gradual increase in uric acid level while pupae turns into adult. The gradual increase of uric acid in the present study confirmed that increase in the release of uric acid by the late instar larvae.

The concentration of uric acid in infested rava sample increases from 0 to 61.20 ppb still 4th weeks of storage period, whereas for infested maida and wheat flour shows increase in uric acid concentration from 0 to 66.01 ppb and from 0 to71.43 ppb till storage period of 4th weeks. Similarly Bhattacharya*et.al*. (2017) [1] has reported a significant rise of uric acid in three different types of grains like sorghum, pennisetum and eleusine. The infested sorghum grains showed an increase in uric acid as well as weight loss. Stathers *et al*.(2020) [14] was reported that an increase in protein content also lead to cause contamination in maize samples with the presence of insect body parts and also immature stages of insect in the kernel and excretory wastes like uric acid. Another report showed an increase in density of infestation by rice weevil in wheat flour correspond to the concentration of uric acid (Zakladnoy and Yaitskikh, 2020) [20]. The results of present study and earlier studies supported to conclude that the uric acid concentration increases when the storage period increases which leads to spoilage of food products in addition to weight loss.

Acknowledgements
The authors are thankful to the Indian Institute of Food Processing Technology for the facility provided for conducting the experiments.

Conflict of interest
The authors have no conflict of interest to declare.

References
1. Bhattacharya Nilanjan Dey, Santanu. Nanomolar Level Detection of Uric Acid in Blood Serum and Pest-Infested Grain Samples by an Amphiphilic Probe. Analytical Chemistry,2017:89(19):10376-10383 doi: 10.1021/acs.analchem.7b02344.
2. Bower, Keith M. Analysis of Variance (ANOVA) Using MINITAB. Scientific Computing and Instrumentation,2000:17(3)64-65.
3. Holmes LG. Note on flourometric method for determination of uric acid in flour, Cereal Chem,1980:57(5):371-372.
4. Indumathi C, Manoj D, Loganathan M, Shanmugasundaram S. Impact of Radiofrequency Disinfestation on *Tribolium Castaneum* (Herbst) in Wheat Flour and Its Influence on the Functional Characteristics of Wheat Flour. Journal of Food Processing and Preservation,2021:45(10):1-14. doi: 10.1111/jfpp.15770.
5. Johnson, J. Pest Control in Postharvest Nuts. Woodhead Publishing Limited, 2013, 56-87.
6. Kumari, Prabha, Rekha Sivadasan, Anitha Jose. Microflora Associated with the Red Flour Beetle, *Tribolium Castaneum* (Coleoptera: Tenebrionidae). Journal of Agricultural Technology,2011:7(6):1625-31.
7. Larsen, Torben, and Kasey M. Moyes. Fluorometric Determination of Uric Acid in Bovine Milk. Journal of Dairy Research,2010:77(4):438-444. doi: 10.1017/S0022029910000580.
8. Mehmood, Khalid, Mureed Husain, Muhammad Aslam, Muhammad Shoaib Ahmedani, Azhar Mehmood Aulakh *et al*. Changes in the Nutritional Composition of Maize Flour Due to *Tribolium Castaneum* Infestation and Application of Carbon Dioxide to Manage This Pest. Environmental Science and Pollution Research,2018:25(19):18540-47. doi: 10.1007/s11356-018-2063-6.
9. Negi, Aditi, Akash Pare, Loganathan Manickam, Meenatchi Rajamani. Effects of Defect Action Level of *Tribolium Castaneum* (Herbst) (Coleoptera: Tenebrionidae) Fragments on Quality of Wheat Flour. Journal of the Science of Food and Agriculture, 2021. doi: 10.1002/jsfa.11349.
10. Ojumoola AO, Obikwe E, Oladigbolu AA, Adesiyun AA. Influence of Prior Feeding Experience and Food Deprivation on Flour Selection and Utilization by the Red Flour Beetle, *Tribolium Castaneum* (Herbst). Agro-Science,2020:19(2):41-47. doi: 10.4314/as.v19i2.7.
11. Olsson J, Börjesson T, Lundstedt T, Schnürer J. Detection and Quantification of Ochratoxin A and Deoxynivalenol in Barley Grains by GC-MS and Electronic Nose. International Journal of Food Microbiology,2002:72(3):203-14. doi: 10.1016/S0168-1605(01)00685-7.
12. Paolesse, Roberto, Adriano Alimelli, Eugenio Martinelli, Corrado Di Natale, Arnaldo D’Amico, Maria Grazia D’Egidio, Gabriella Aureli, Alessandra Ricelli, and Corrado Fanelli. Detection of Fungal Contamination of Cereal Grain Samples by an Electronic Nose. Sensors and Actuators, B: Chemical,2006:119(2):425–30. doi: 10.1016/j.snb.2005.12.047.
13. Pixton S.W. Detection of Insect Infestations in Cereals by Measurement of Uric Acid. Cereal Chem,1965:85(42):315-22.
14. Stathers, Tanya E, Sarah EJ Arnold, Corinne J Rumney, Clare Hopson. Measuring the Nutritional Cost of Insect Infestation of Stored Maize and Cowpea. Food Security,2020:12(2):285-308. doi: 10.1007/s12571-019-00997-w.
15. Teepoo S, Chumsaeng P, Jongjinako S, Chantu K, Nolykad W. A New Simple and Rapid Colorimetric Screening Test for Semi-Qualitative Analysis of Vitamin C in Fruit Juices Based on Prussian Blue. Journal of Applied Sciences,2012:12(6):568-74. doi: 10.3923/jas.2012.568.574.
16. Thanushree MP, Vimala BSK, Moses JA, Anandharamakrishnan C. Detection Techniques for Insect Infestation in Stored Grains. Agriculture Engineering Today,2018:42(4):48-56.
17. Tutorial, Minitab, Carpenter Review Source, Example Data Source, One-way Analysis, and Balanced Analysis. Introduction to One-Way ANOVA,2015:1:1-18
18. Vimala Bharathi SK, Vishnu Priya V, Vishnu Eswaran, Moses JA, Alice RP. Sujeetha. Insect Infestation and Losses in Stored Food Grains. Ecology, Environment and Conservation,2017:23(1):286-91.
19. Wehling RL, Wetzel DL, Pedersen JR. Stored Wheat Insect Infestation Related to Uric Acid as Determined by Liquid Chromatography. Journal - Association of Official Analytical Chemists,1984:67(3):644-47. doi: 10.1093/jaoac/67.3.644.
20. Zakladnoya GF, Yaitskikha AV. Dependence of Uric Acid Content in Stored Grain on the Population Density of the Rice Weevil *Sitophilus Oryzae* (L.) (Coleoptera, Dryophthoridae). Entomological Review. 2020:100:170-172. doi: 10.1134/S0013873820020049.