

## Note on Fluorometric Method for Determination of Uric Acid in Flour

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A chemical technique used for the regulatory determination of the degree of insect infestation in stored flour is the enzymatic-ultraviolet absorption assay for determining uric acid content (AOAC 1980). Approximately 18% of the total excreta from *Tribolium confusum* is uric acid (Gupta and Sinha 1960). Various investigators (Farn and Smith 1963, Sen 1968, Sen and Smith 1966, Venkatrao et al 1960) have established good correlation between degree of infestation and uric acid content of flour. Although the spectrophotometric assay has good specificity, it is a somewhat lengthy procedure. Turbidity, incomplete enzyme action, and interfering coloration at high pH may further complicate this analytical technique.

Fluorescence spectroscopy, closely related to the widely used analytical technique based on ultraviolet-visible absorption spectroscopy, has assumed an important role in analytical applications. Because its specificity is greater than that of conventional spectroscopy, fluorescence has long been a valuable method of analysis. Fluorometric methods are also simple and accurate. This note discusses the application of a fluorometric technique for the determination of uric acid content in flour and its use as an index for insect infestation.

### MATERIALS AND METHODS

Two species of insects, *Tribolium confusum* (Du Val) and *Tribolium castaneum* (Hbst.) were reared separately in a culture medium prepared according to the method described by Harein and Soderstrom (1966).

Samples (100 g) of hard wheat flour were weighted into 500-ml widemouth glass containers with filter paper inserts in the open metal covers. Two test units, each consisting of three 500-ml containers, were infested with adult *T. confusum* and *T. castaneum*, respectively, at levels of 20, 40, and 60 insects. Two uninfested containers served as controls. All containers were kept in an environmental chamber at 25°C and 55% rh. Three samples from each level of insect infestation, including the controls, were tested after 4, 6, and 8 days. Insects were removed from the flour before analysis by sifting flour through a 50-mesh screen.

A standard curve was prepared for uric acid. One hundred milligrams of accurately weighed uric acid was dissolved in 100 ml of 0.2M sodium acetate buffer adjusted to pH 11.8-12.0 with 2N NaOH. Dilutions of the stock uric acid standard were prepared volumetrically with the acetate buffer to give solutions of 4.0, 12.0, 20.0, 28.0, and 48.0 mg/dl. The samples were read in a fluorometer (Hitachi Model MPF-2A). An excitation wavelength of 330 nm, using a 390-nm filter for the emission monochromator, gave an emission maximum at 420 nm. The plot of emission maxima versus uric acid concentrations was linear.

A blank sample containing 0.5 g of uninfested flour was dispersed in 25 ml of the acetate buffer and stirred for 30 min at 40°C. After stirring, the sample was centrifuged at 3,000 rpm for 10 min and the supernatant filtered through a 45-mm AA millipore filter. This solution was used to zero the instrument. Samples of infested flour were read against this blank.

Samples containing 0.5 g of infested flour were dispersed in 25 ml of acetate buffer for the determination of uric acid content. The extraction procedure was exactly as described for the blank. The uric acid level was determined from the standard curve.

### RESULTS AND DISCUSSION

Fluorescence emission spectra of a solution of uric acid and of extracts from infested and uninfested flour, read with a 390-nm

filter on the emission monochromator, are shown in Fig. 1. Use of a 390-nm filter for the emission monochromator avoids interference from the Raman band in aqueous solvents, which gives rise to large blanks at high sensitivity settings. The 390-nm filter also eliminates scatter peaks from the excitation wavelength, showing only the peak arising from fluorescence (Parker 1968).

The efficiency of uric acid extraction from flour with 0.2M sodium acetate was checked by adding known amounts of uric acid to flour. Table I shows the recovery rate for four flour samples to which amounts of uric acid ranging from 8.0 to 40.0 mg/dl had been added. The average recovery rate of 96.4% ( $\pm 1.4\%$ ) was satisfactory.

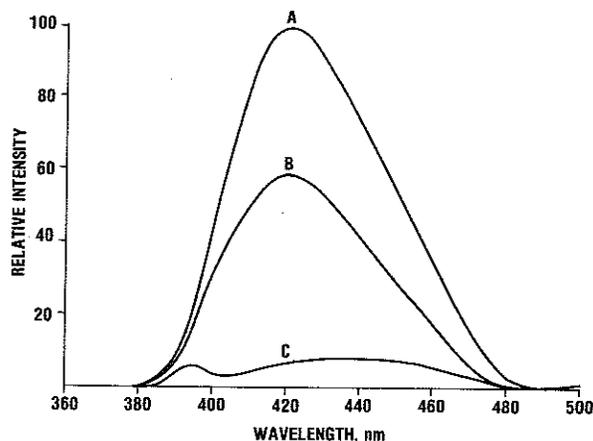


Fig. 1. Fluorescence spectra for solution of A, uric acid (0.5 mg/ml); B, extract of uric acid from infested flour (0.3 mg/ml); and C, extract of uninfested flour (20 mg/ml). Excitation is at 330 nm with an emission maximum (390-nm filter) at 420 nm.

TABLE I  
Analytical Recoveries of Uric Acid Added to Flour

Sample	Uric Acid <sup>a</sup>		Recovery (%)
	Added	Found	
1	8.0	7.7	96.2
2	12.0	11.5	95.8
3	24.0	22.8	95.0
4	48.0	47.3	98.6
Mean recovery <sup>b</sup>			96.4 $\pm$ 1.4

<sup>a</sup> Milligrams per 100 g of flour.

<sup>b</sup> Percent and standard deviation.

TABLE II  
Excretion of Uric Acid<sup>a</sup> by Adult *Tribolium*<sup>b</sup>

Type of Adult	Days	Number of Adult <i>Tribolium</i>		
		20	40	60
<i>T. castaneum</i>	4	3.0 $\pm$ 1.0	4.5 $\pm$ 1.5	6.0 $\pm$ 2.0
<i>T. confusum</i>	4	2.0 $\pm$ 0.5	4.0 $\pm$ 1.5	8.0 $\pm$ 2.5
<i>T. castaneum</i>	6	7.5 $\pm$ 2.0	16.0 $\pm$ 4.0	18.0 $\pm$ 3.0
<i>T. confusum</i>	6	9.0 $\pm$ 1.5	20.0 $\pm$ 3.0	22.0 $\pm$ 2.0
<i>T. castaneum</i>	8	18.5 $\pm$ 3.5	24.0 $\pm$ 4.0	28.5 $\pm$ 3.0
<i>T. confusum</i>	8	17.0 $\pm$ 2.0	27.0 $\pm$ 3.0	29.0 $\pm$ 5.0

<sup>a</sup> Milligrams per 100 g of test flour. Calculated as uric acid content in 0.5-g sample of flour (from standard curve)  $\times$  200.

<sup>b</sup> Average of three replicate measurements.

Table II summarizes the data for *Tribolium*. These data show an increase of uric acid content in flour with time and population. The amount of uric acid excreted by *Tribolium* varies at different stages of its metamorphosis. The larvae produce uric acid at a greater rate than do adult insects; however, considerable amounts of uric acid are excreted by adults, and adults were used in this study to represent natural insect infestation of flour for the determination of measurable quantities of uric acid.

The results of this study suggest that insect infestation of flour can be detected early, while the insect population is relatively low. These data would not necessarily apply to stored flours because extrapolation from laboratory studies to actual storage conditions is difficult. The fluorometric assay offers many advantages over other chemical and physical methods.

In summary, these results demonstrate the use of fluorometry in the determination of uric acid in flour and the correlation of uric acid with insect infestation. The rather simple analytical procedure is rapid and eliminates many of the disadvantages of other chemical methods.

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