DETERMINATION OF URIC ACID IN WHEAT FLOUR INFESTED BY TRIBOLIUM CASTANEUM DUV., USING PAPER CHROMATOGRAPHY[1]

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ABSTRACT

The uric acid present in infested wheat flour was separated by paper chromatography and quantitatively estimated using Benedict's uric acid reagent. The results agreed with those obtained by a direct colorimetric method of determining uric acid in protein-free aqueous extracts of infested flour. The uric acid content in the infested flour increased steadily with the progress of infestation. No uric acid could be detected in the extract of control uninfested flour. The protein-free aqueous extract of control uninfested wheat flour, however, contained small amounts of substances (other than uric acid) which reacted with the Benedict's uric acid reagent and yielded an "apparent" uric acid value. This value was low, as compared with those found in the infested flour.

Earlier investigations from this laboratory (5,7,8) have shown that uric acid, which is an important constituent in the excreta of insects, can serve as a good index of the degree of infestation and unhygienic conditions in infested food grains. In the above studies, uric acid was determined in the protein-free aqueous extracts of foodstuffs by a colorimetric method using Benedict's uric acid reagent (3). Protein-free aqueous extracts of uninfested food samples also contained relatively small amounts of substances which reacted with the uric acid reagent and gave some "apparent" uric acid values. In view of the fact that Benedict's reagent is not specific for uric acid (3), it was felt desirable to determine the uric acid content of infested foods using a more specific method. In the present investigation, the uric acid content of wheat flour infested by *Tribolium castaneum* Duv. has been determined by paper chromatography and the results have been compared with those obtained by direct colorimetric method.

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Materials and Methods

The samples of wheat flour infested by *Tribolium castaneum* Duv. and the control uninfested wheat flour used in the present study were the same as those described in the preceding paper (9).

Determination of Uric Acid in Wheat Flour by Direct Colorimetry. The method used in the present study is a slight modification of that described earlier (5) and is briefly described below: Wheat flour (5–20 g.), containing about 1–5 mg. of uric acid, was suspended in 200 ml. of water. The mixture was allowed to stand for 2 hours with occasional stirring, and then mixed in a Waring Blendor for 10 minutes. It was centrifuged at 2000 r.p.m. for 10 minutes. To 100 ml. of the clear centrifugate, 10 ml. of 10% sodium tungstate were added. After mixing, 10 ml. of 0.667*N* sulfuric acid were added to precipitate the proteins present in the extract. The mixture was allowed to stand for 5 minutes and then filtered. Aliquots of the filtrate containing about 50–100 γ of uric acid were used for the colorimetric determination according to Hawk *et al.* (3). The color intensity was determined in a Klett-Summerson photoelectric colorimeter with 520 mμ filter.

Determination of Uric Acid by Paper Chromatography. Tilden (6) used the paper chromatographic technique for separation and estimation of uric acid in infested fruit products. Johnson (4) and Dikstein *et al.* (2) have described paper-chromatographic techniques for the assay of uric acid in urine. After a preliminary study of the different solvent systems suggested by the above authors, a simple solvent system consisting of 1-butanol-acetic acid-water (4:1:5) was found to be quite satisfactory for the separation of uric acid from extracts of infested wheat flour. The paper-chromatographic procedure finally adopted was as follows:

Extraction of Uric Acid. Wheat flour (10–20 g.) containing about 1–10 mg. of uric acid (as determined by direct colorimetric assay) was suspended in 100 ml. of water at room temperature (24°–29°C.). The mixture was allowed to stand for 2 hours with frequent stirring and was then mixed thoroughly for 10 minutes in a Waring Blendor. It was then centrifuged at 2000 r.p.m. for 15 minutes. The centrifugate was filtered and 20 ml. of the clear filtrate were evaporated on a water bath, so that 1 ml. of concentrated extract contained about 0.1–0.2 mg. of uric acid.

Paper-Chromatographic Technique. The separation of uric acid from the extracts of wheat flour was effected by the descending paper chromatographic technique on Whatman No. 1 paper using 1-butanol-acetic acid-water (4:1:5). The chromatographic chamber was similar

to that described by Block and Bolling (1). Known volumes of the concentrated extract of wheat flour containing about 10–15 γ of uric acid were spotted at intervals of about 4 cm. on Whatman No. 1 filter paper (45–50 cm. long and 18 cm. wide) on a line drawn about 10 cm. from one end of the paper. A total of 50–100 μl. of the extract could be applied to one spot, by repeatedly spotting in 10-μl. quantities and drying the spot in a current of warm air. Known quantities of standard uric acid (15 γ) were also spotted next to each unknown spot. The filter paper sheet was suspended from a glass trough containing the solvent mixture and fitted near the top of a rectangular glass chamber (1). The whole assembly was kept at room temperature (24°–29°C.), and the chromatogram was allowed to develop for 48 hours. At the end of this period, the chromatograms were removed and dried at 50°C. and sprayed with a saturated solution of sodium carbonate followed by arsenophosphotungstic acid reagent, according to Johnson (4). Blue spots were obtained at points where uric acid was located. The R_f value of uric acid was found to be 0.41 in the solvent mixture used. Using the developed chromatogram as a guide, the positions of uric acid spots were located in the untreated portion of the chromatogram. The areas corresponding to that of the uric acid spots were marked with pencil allowing ample space all around the spot, and the spots were cut out. Each cut strip was placed in a test tube and the uric acid present was extracted twice with 5 ml. of phosphate buffer (pH 6.8) at 60°C., and filtered. The uric acid in the filtrate was determined colorimetrically, as in the direct method, according to Hawk *et al.* (3) after 4 ml. of sodium cyanide solution and 1 ml. of Benedict's uric acid reagent were added.

Results and Discussion

Table I shows that the results obtained by the two methods are in close agreement. The results of chromatographic studies also showed that only one spot corresponding to the standard uric acid, with R_f value of 0.41, could be observed in the chromatogram obtained from the extract of infested wheat flour. No uric acid could be detected in the chromatogram developed with the extract of control wheat flour. The recovery of uric acid added to control and infested flours was of the order of 90–100%. In the present study an "apparent" uric acid value of about 5 mg. per 100 g. was found in the extract of control wheat flour by the direct colorimetric method. Since no uric acid could be found in the extract by paper chromatography, this "apparent" uric acid value is due to the presence of other substances which react with Benedict's uric acid reagent. In view of the fact that the

TABLE I

URIC ACID CONTENT OF WHEAT FLOUR INFESTED BY *Tribolium castaneum Duv.*, DETERMINED BY TWO METHODS[a]

PERIOD OF STORAGE	UNINFESTED WHEAT FLOUR		INFESTED WHEAT FLOUR		
	Direct Colorimetric Procedure	Paper Chromatography	Direct Colorimetric Procedure		Paper Chromatography
			Total Uric Acid	Corrected Uric Acid[b]	
months					
0	5.8	nil	5.7	nil	nil
1	5.4	nil	16.0	10.3	12.8
2	4.6	nil	43.9	38.2	37.5
3	5.2	nil	76.0	70.3	73.2
4	4.8	nil	112.7	107.0	105.4
5	4.5	nil	161.4	155.7	159.4

[a] All uric acid values have been expressed as mg. per 100 g. of flour on 14% moisture basis.
[b] Corrected uric acid values were obtained by subtracting "apparent" uric acid value of the uninfested flour at the beginning of the experiment from the total uric acid values.

"apparent" uric acid values obtained for unifested grains are low (5,7,8) as compared with the high uric acid values generally found in infested grains, the direct colorimetric determination of uric acid described in this paper can be used for the routine assay of the uric acid content of infested cereals and cereal products.

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