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Determination of uric acid in foodstuffs by thin-layer chromatography

When the enzymatic method for the determination of uric acid in foodstuffs¹ could not be used for some reason, the Benedict method has been used, in an attempt to correlate uric acid content with insect infestation². Although the latter method is known not to be strictly specific for uric acid³, its use in foodstuffs, giving no colour in the extract, has been tolerable. But in certain foodstuffs, such as spices, condiments and pulses, which usually yield some colour in the extract, the method is obviously not suitable because a blank value of fixed magnitude for a particular food cannot be obtained. In this paper a thin-layer chromatographic (TLC) method is reported for the detection and determination of uric acid in foodstuffs.

A paper chromatographic technique for the separation and determination of uric acid was used by TILDEN⁴ in infested fruit products, by JOHNSON⁵ and DIKSTEIN *et al.*⁶ in urine and by VENKATRAO *et al.*⁷ in wheat flour. There are some references⁸⁻¹⁰ to the use of TLC for separating uric acid, but their aims were different from that of this work. The solvents used by all these previous workers and by a few others for the development of the chromatogram have been tried in the present determination, but one, butanol-5 *N* acetic acid (2:1), has been found to give the best separation and spot formation, the saturation time being 1 h. Cellulose powder (No. 123, Schleicher & Schüll) was found to be a better support than Silica Gel G.

Procedure

Glass plates (20 × 10 cm) with a 250 μm thin layer were air-dried for about 12 h as it is known that the resolving properties of cellulose layers improve on long exposure to air¹¹.

Standard solutions of uric acid (prepared as described by HAWK *et al.*¹², containing up to 10 μg of uric acid, were spotted onto the plate, which was placed in the solvent chamber and allowed to develop to a height of 10 cm. The run took about 30 min. The plate was then dried in air to remove the solvent completely and sprayed with 5% sodium cyanide solution³ followed by arsenophosphotungstic acid reagent³. Blue spots appeared against a white background with an R_F value of 0.51 and a sensitivity (limit of detection) of 5 μg . The spots of graded concentration then gave the standard curve by colorimetric determination after elution with 5 ml of water or directly by densitometer.

A 2-g amount of a representative sample of food was pulverised and suspended in 20 ml of water at room temperature. The mixture was allowed to stand for 2 h with occasional stirring and then mixed in a blender for 5 min. The whole mixture was then centrifuged and the supernatant was treated with about 5 g of alumina, which adsorbed the natural colours to some extent. Alternatively, the centrifugate can be passed through a small column containing alumina. Thus cleaned-up, the solution was treated as described in the previous paragraph.

A number of samples, both infested and uninfested, of turmeric, coriander, pulses and amchur (a seasoned powder of green mango usually used as a condiment in India) have been investigated. In all instances, the uninfested samples showed some colorimetric reading by the Benedict method but none by the TLC method. As

TABLE I

DETERMINATION OF URIC ACID (mg-%) IN UNINFESTED AND INFESTED FOODSTUFFS

Sample	Uninfested foodstuff		Infested foodstuff		
	Colorimetric method	TLC method	Colorimetric method	Corrected ^a colorimetric value	TLC method
Turmeric	4.2	Nil	10.5	6.3	8.1
	4.3	Nil	21.3	17.0	19.6
	4.4	Nil	12.4	8.0	10.2
Coriander	4.2	Nil	11.2	7.0	9.4
	5.6	Nil	37.9	32.3	35.2
	5.4	Nil	70.6	65.2	66.8
	5.4	Nil	69.4	64.0	66.8
Pulses	5.5	Nil	71.3	65.8	67.3
	4.7	Nil	45.8	41.1	44.5
	4.7	Nil	161.4	156.7	159.0
	4.8	Nil	65.2	60.4	64.6
Amchur	4.7	Nil	80.4	75.7	78.6
	10.8	Nil	112.6	—	105.0
	12.5	Nil	103.8	—	94.5
	11.0	Nil	110.9	—	104.2
	10.8	Nil	113.0	—	106.0

^a Infested minus uninfested.

expected, the infested samples showed higher contents of uric acid by the colorimetric method than by the TLC method. The recovery of uric acid added to uninfested food has also been found to be satisfactory by the TLC method. The results for a few typical samples are given in Table I. In all the samples except amchur, the uninfested specimen was infested and uric acid was determined after a lapse of a few months. In amchur, stray (unrelated) samples, infested and uninfested, were analysed.

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