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DETERMINATION OF CREATININE IN SOUPS AND SOUP PREPARATIONS BY ION-EXCHANGE CHROMATOGRAPHY

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SUMMARY

Ion-exchange chromatography, coupled with continuous spectrophotometric monitoring in the UV region, was used to determine the total creatinine in soup preparations, dehydrated soups and tinned soups, containing meat extracts. This is an accurate method because it permits the separation of interfering substances that simulate creatinine in JAFFÉ's test. The recovery of creatinine in the soup preparations, dehydrated soups, and tinned soups amounted to 99.8 ± 0.8 %, 100.3 ± 0.9 %, and 100.2 ± 1.1 %, respectively.

INTRODUCTION

In our previous chromatographic analysis of soup preparations[1], an unknown compound, sometimes present in a large amount, gave a peak so close to that of adenine as to interfere with its determination. From its UV absorption spectrum, JAFFÉ's test[2] and its retention time, this compound was identified as creatinine. The first aim was to separate these two compounds by a suitable choice of the molarity and the pH of the eluent buffer. It was decided to use ion-exchange chromatography to separate the creatinine in soup and soup preparations from interfering substances which sometimes introduce an element of uncertainty into HADORN's method[3], so far the only official method for the determination of the meat-extract content of these products[4]. The insufficient specificity of JAFFÉ's test had previously led others to effect this separation by ion-exchange chromatography, coupled with UV spectrophotometric determination[5-7]. However, this technique was not suitable for our purpose, so with some modifications we used the previously developed method for the determination of ribonucleotides[1]; this new method will be described below.

EXPERIMENTAL

Apparatus and reagents

The chromatographic column (30 × 0.9 cm) was packed to a height of 5 cm with the cation-exchange resin Aminex A_5 (Bio-Rad Labs., Richmond, Calif., U.S.A.), the spherical particles of which had a diameter of 8–12 μ. The column was used in

conjuction with a Mini-Pump (Milton Roy Co., Fla., U.S.A.) and a Beckman DB-G double-beam spectrophotometer fitted with a 10-in. Beckman recorder and a microcell with a quartz window, a light path of 10 mm, and a total volume of 0.3 ml (catalogue Beckman No. 97290).

The reagents were: HCl, 2 *N*; Brockmann's III alumina; Buffer pH 4.30 = 0.3 *M* sodium acetate, adjusted to pH 4.30 ± 0.02 with glacial acetic acid; Buffer pH 3.70 = 0.3 *M* acetic acid, adjusted to pH 3.70 ± 0.02 with 40% NaOH; 1 m*M* cytosine in buffer pH 3.70; 0.5 m*M* creatinine in buffer pH 3.70. 0.1 ml/l of caprilyc acid was added to all buffers as preservative.

Procedure

A sample of a soup preparation, a dehydrated soup, or a tinned soup was weighed out accurately and dissolved in distilled water in such a way as to obtain a solution containing about 0.5 μmole of creatinine per ml. Whenever necessary, the fat and the insoluble components were removed by treating part of the solution with kieselguhr and by filtering it through a Whatman No. 40 paper. About 20 ml of the filtrate were collected, and exactly 10 ml were transferred with a pipette into a flat-bottomed porcelain dish. 5 ml of 2 *N* HCl were added, and the mixture was evaporated to dryness on a boiling water bath. The dry residue was taken up in 5 ml of 2 *N* HCl and again evaporated to dryness. When the residue was very dark (particularly in the case of soups), this operation was repeated for a third time. The residue was finally dissolved in exactly 10 ml of distilled water and filtered through a Whatman No. 40 paper. The filtrate was then passed through an Allihn tube (I.D. 0.9 cm), packed with 2.5 g of Brockmann's alumina. About 6 ml of the eluate were collected, and 5 ml were then transferred with a pipette into a flat-bottomed porcelain dish. 1 ml of 2 *N* HCl was added, and the solution was evaporated to dryness on a boiling water bath. The residue was dissolved in exactly 5 ml of a 1 m*M* solution of cytosine in pH 3.7 buffer, and, whenever necessary, filtered through a Whatman No. 42 paper.

About 1 ml of the filtrate was put on the chromatographic column and eluted with a pH 4.30 buffer at a rate of 120 ml/h and at a temperature of 56°. The eluate was monitored for creatinine in the UV region at 240 mμ and at a slit width of 1.5 mm.

The column needs to be regenerated only when loss of efficiency or resolution is noticed. Regeneration is carried out by passing 10 ml of 2 *N* HCl through the column and then some 1 *N* NaOH until the NaOH front, which is darker, reaches the bottom of the column. The latter is then re-equilibrated with 30–40 ml of a pH 4.30 buffer.

RESULTS AND DISCUSSION

The data in Table I indicate that this method gives accurate and reproducible results. These results were obtained for (a) a laboratory soup preparation, (b) a commercial soup preparation, (c) a dehydrated soup and (d) a tomato soup. An accurately known amount of creatinine was added to each of these, and sample (b) also contained some meat extract, in which the creatinine had been accurately measured. The added creatinine was always recovered with a good accuracy, and the standard deviation never exceeded ± 1.1%.

Sample (d) was the most interesting because it contained some substances that simulate creatinine in JAFFÉ's test (*i.e.* give the same color when reacted with sodium

TABLE I

RECOVERY OF CREATININE ADDED TO A SOUP AND A SOUP PREPARATION

a = Soup preparation without meat extract; b = soup preparation with meat extract; c = dehydrated vegetable soup; d = tinned tomato soup.

Sample	*Creatinine added (mg)*	*Creatinine found (mg)*	*Recovery (%)*
1a	27.00	27.11	100.4
2a	27.00	26.81	99.3
3a	27.00	26.81	99.3
4a	27.00	26.95	99.8
5a	27.00	27.11	100.4
6a	27.00	26.95	99.8
7a	27.00	27.29	101.1
8a	27.00	26.78	99.2
		Average recovery	99.9 ± 0.7
1b	27.00	27.13	100.5
2b	27.00	26.78	99.2
3b	27.00	26.70	98.9
4b	27.00	27.16	100.6
5b	27.00	27.00	100.0
		Average recovery	99.8 ± 0.8
1c	56.50	55.93	99.0
2c	56.50	56.44	99.9
3c	56.50	56.73	100.4
4c	56.50	57.12	101.1
5c	56.50	57.01	100.9
		Average recovery	100.3 ± 0.9
1d	56.50	56.50	100.0
2d	56.50	55.71	98.6
3d	56.50	56.50	100.0
4d	56.50	57.40	101.6
5d	56.50	56.95	100.8
		Average recovery	100.2 ± 1.1

picrate in an alkaline medium), so that this test gives a creatinine content for tomato soup not containing meat extract.

These interfering substances cannot be removed by passing the sample through alumina or by extracting it with diethyl ether. The present method permits the chromatographic separation of creatinine from interfering substances, since these emerge in the first 10 min of the elution, while creatinine appears only after 17 min.

The creatinine zone generally did not contain other substances absorbing in the UV region. This was confirmed analyzing a protein hydrolysate, a yeast autolysate and various fresh and dehydrated vegetables by the proposed method. In fact, it was only in the case of tomato that a peak appeared which was not well resolved from the creatinine peak. Under the present conditions, however, this peak was small and did not interfere with the analysis (*cf.* Table I), also because it was not superimposed on the creatinine peak. Furthermore, this substance has a slightly shorter retention time than creatinine and cannot be mistaken for the latter when present by itself. This can be seen from Fig. 1, showing the graphs obtained in the analysis of tomato soup with and without a meat extract, as well as in the analysis of a standard mixture.

The present method has dispensed with the ether extraction which is time-consuming, rather difficult and gives rise to inaccuracies. Our method is thus rapid, accurate and easy to carry out. The use of an internal standard, cytosine, which closely followed creatinine in the elution, means that the amount of sample placed on the column need not be accurately known.

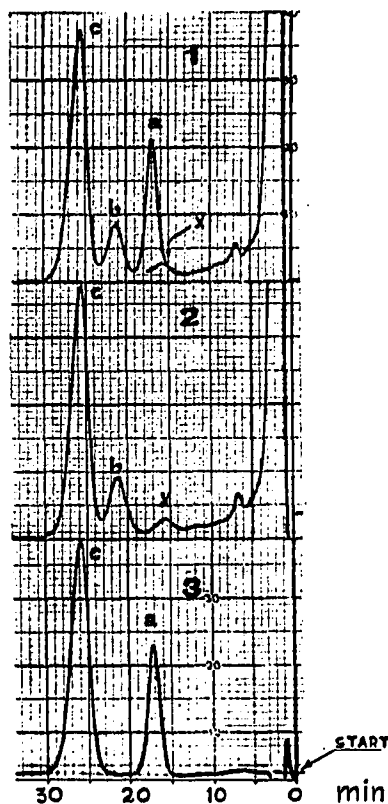

Fig. 1. (1) Chromatogram of a tomato soup with meat extract; (2) chromatogram of a tomato soup without meat extract; (3) chromatogram of a standard mixture of creatinine and cytosine. a = Creatinine; b = adenine; c = cytosine; x = unknown from tomato.

For calculation of results, the method of the ratio of peak areas using an internal standard was adopted. At creatinine concentrations of 0.2–1 μmole/ml, the calibration curve was a straight line. To obtain very accurate results, it is useful therefore to elute occasionally a standard mixture, containing cytosine in the same amount as added to the samples to be analyzed, also containing creatinine in an amount very close to what is expected to be present in the samples to be analyzed. This operation minimizes the inevitable instrumental error.

CONCLUSIONS

The method proposed for the determination of creatinine in soup preparations and soups containing meat extracts is more accurate and less time-consuming than the methods used so far. Furthermore, it enables one to avoid the inconvenience caused by creatinine-like interfering substances which make the analysis inaccurate in the case of tomato soup.

REFERENCES

1 A. Carisano, M. Riva and A. Bonecchi, *J. Chromatog.*, 40 (1969) 386.
2 M. Jaffé, *Z. Physiol. Chem.*, 10 (1886) 399.
3 H. Hadorn, *Mitt. Lebens. u. Hyg.*, 37 (1946) 342.
4 *Analytical Methods for the Soup Industry*, Association of Swiss Soup Manufacturers, Berne, 1961, 1/5.
5 A. C. Tager-Nilsson, *Scand. J. Clin. Lab. Invest.*, 13 (1961) 326.
6 W. S. Adams, F. W. Davis and L. E. Hansen, *Anal. Chem.*, 34 (1962) 854.
7 W. S. Adams, F. W. Davis and L. E. Hansen, *Anal. Chem.*, 36 (1964) 2209.