Gas chromatographic determination of β-alanylhistidine dipeptides in soup preparations

The spectrophotometric determination of β-alanylhistidine dipeptides in soup preparations[1] is hindered by difficulties in separating β-alanine from other amino acids in the hydrolysate, and in oxidizing it with a view to converting it to 2,4-dinitrophenylhydrazone; these difficulties cause an error of over 10%. A gas chromatographic method has therefore been devised for a better separation and determination of β-alanine.

Experimental

Equipment. A Rotavapor R rotary evaporator was used for the rapid evaporation of the samples under vacuum. The determinations were carried out with a Fractovap D-AI (ex. C. Erba) gas chromatograph fitted with a stainless steel column having a length of 2 m and an internal diameter of 2 mm.

The stationary phase was 1% (w/w) of neopentyl glycol succinate on Chromosorb W support (60–80 mesh) which had previously been washed with acids. The detector sensitivity was set at 1 mV (full scale), and the samples were introduced with a Hamilton microsyringe (1 μl). The conditions of the analysis are given in Fig. 1.

Procedure. The sample, weighing exactly 15 g, was placed in 30 ml of hot distilled water, cooled under the tap until the fat had solidified, and filtered through

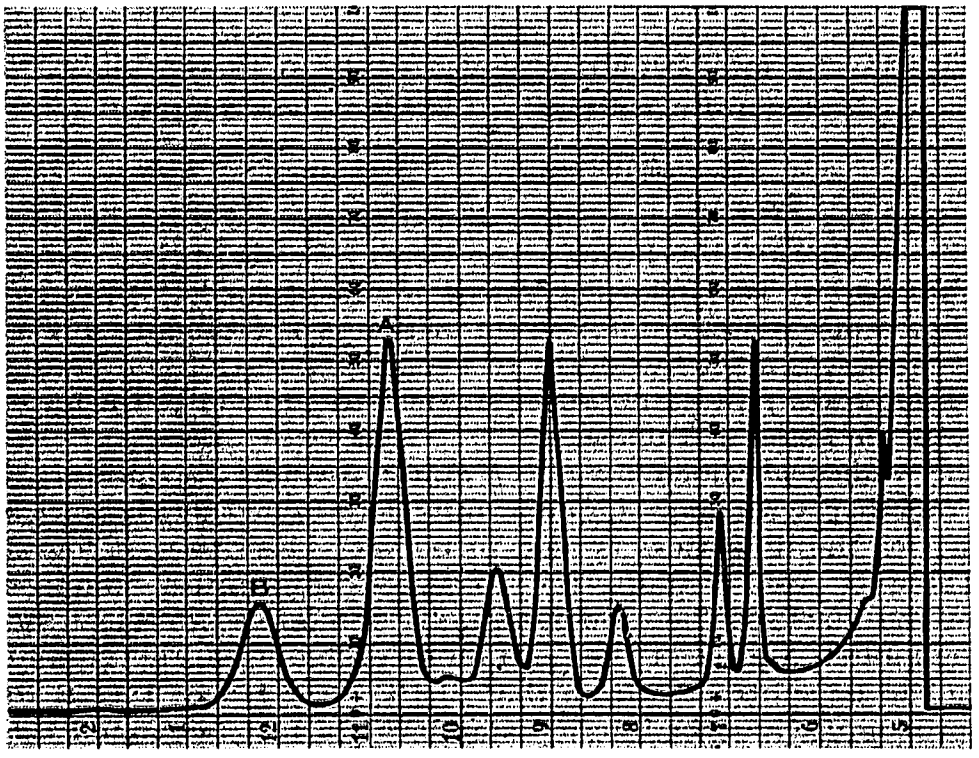

Fig. 1. Typical gas–liquid chromatogram from a soup product without proline standard. A = β-Alanine; B = unknown with the proline peak retention time. Experimental conditions: column temperature 125°; detector temperature 170°; flash heater temperature 200°; nitrogen flow rate, 27 ml/min; split 60 ml/min. Column: stainless steel, 2 m long, I.D. 2 mm. Partition liquid: neopentyl glycol succinate 1% on acid-treated Chromosorb W, 60–80 mesh.

glass wool into a 100 ml volumetric flask. The fat retained on the filter was re-extracted with 10 ml of hot water and the filtrate was combined with that in the volumetric flask, which was then filled up to the mark with 95 % ethanol. In the subsequent operations, carried out as described previously[1], the residue obtained by centrifuging was suspended in 4 ml of 6 *N* HCl and was transferred to a 25 ml volumetric flask. The centrifuge tube and the 100 ml beaker used for the precipitation were washed with four 5 ml portions of 6 *N* HCl and the washings were transferred to the 25 ml flask. The latter was heated for 5 min on a boiling water bath, cooled and filled up to the mark, and its contents were filtered through a Whatman No. 41 paper. Two 10 ml portions of the filtrate were sealed in two 20 ml thick walled vials, and hydrolysis was effected by heating the vials for 4 h at 125° ± 2°. The vials were opened, 5 ml of a standard proline solution prepared by dissolving 0.04 % (w/v) of proline in 0.5 *N* HCl were added to one of them and both hydrolysates were dried in a rotary evaporator at 60°. Methylation, butylation and trifluoroacetylation were then carried out by the method of LAMKIN AND GEHRKE[2].

The N-trifluoroacetylated product was taken up in 1 ml of chloroform, and the sample was injected directly into the gas chromatograph without filtration. We used samples of 1 μl and 0.5 μl according to whether the products contained less than or more than 5 % of meat extract. Both the sample with and that without proline were injected at least twice.

To obtain the calibration curve, samples of 1, 2 and 3 mg of β-alanine were mixed with 5 ml of the standard proline solution, subjected to methylation, butylation, and trifluoroacetylation and then gas chromatographed. The areas of the β-alanine peak and the proline peak were measured and their ratio was plotted against the β-alanine concentration. For calculation of results, the method of the ratio of peak areas using an internal standard corrected for the blank was adopted.

Results and discussion

In the range of 1 to 6 mg of β-alanine, the calibration gave a straight line. The accuracy of the result was checked by eluting seven times 7.60 mg of pure carnosine (Hoffmann La Roche, Basle). The following values were obtained: 7.54, 7.70, 7.52, 7.70, 7.85, 7.55 and 7.63 mg, representing a mean of 100.55 % (standard deviation ± 1.56 %). A further check was made by adding to a soup preparation containing no meat extract various amounts of pure carnosine equivalent to 3 to 8 % of the meat extract content. The results are listed in Table I.

Table II shows the values obtained for the β-alanylhistidine dipeptides in various meat extracts, together with data for creatinine determined by HADORN's method[3]. The mean value for the dipeptides is seen to be very close to that found by other methods[1,4]. Table II also contains the corresponding values obtained for soup preparations on the market. The chromatograms of samples to which no proline had been added exhibited a peak at the position of proline (see Fig. 1), and the area of this peak had to be subtracted from that of the peak B (see Fig. 2) to obtain the true area of proline used as the internal standard. In fact, we found the area of the unknown peak on the chromatogram of the sample without the internal standard, divided it by the area of the β-alanine peak on the same chromatogram, and multiplied the ratio by the area of the β-alanine peak on the chromatogram of the sample containing the internal standard. Within a certain range, the ratio between

TABLE I

PERCENTAGE RECOVERY OF CARNOSINE FROM SOUP PRODUCTS WITHOUT MEAT EXTRACT

Carnosine added (mg)	*Carnosine found (mg)*	*Recovery (%)*
2.42	2.38	98.23
4.00	3.96	98.95
5.63	5.60	99.47
7.50	7.46	99.51
7.60	7.68	101.11
2.58	2.57	99.46
2.58	2.59	100.47
2.58	2.57	99.69
Average recovery:		99.61 % $\sigma \pm 0.88$

TABLE II

DIPEPTIDE AND CREATININE CONTENTS IN MEAT EXTRACTS AND SOUP PRODUCTS

Sample	*Dry matter*	*Dipeptide content % on wet weight*	*Creatinine content % on wet weight*	*Dipeptides/creatinine*
1	77.61	4.43	5.77	0.77
2	80.33	5.16	6.60	0.78
3	78.97	6.19	7.40	0.84
4	82.00	5.69	7.10	0.80
5	82.72	5.24	7.81	0.67
6	77.41	5.13	6.80	0.75
7	81.90	6.05	7.40	0.82
8	83.53	5.49	7.68	0.72
Average	80.56	5.42	7.07	0.77
A		0.60 (11.13) *	0.75 (10.54) *	0.81
B		0.35 (6.40)	0.43 (6.08)	0.81
C		0.40 (7.40)	0.53 (7.50)	0.73
D		0.25 (4.57)	0.31 (4.37)	0.80
E		0.41 (7.58)	0.52 (7.38)	0.79
F		0.23 (4.18)	0.30 (4.19)	0.77
G		0.37 (6.84)	0.52 (7.34)	0.71

* The values in parentheses are meat extract percentages obtained by the dipeptide and creatinine average.

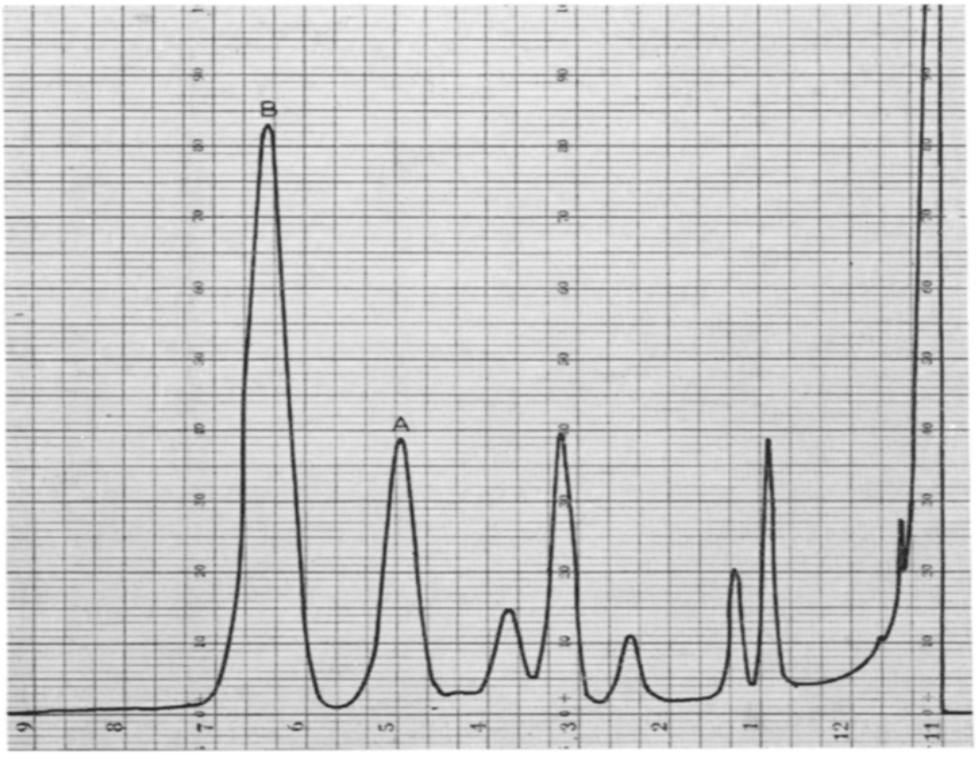

Fig. 2. Typical gas–liquid chromatogram from a soup product with proline standard. A = β-Alanine; B = proline standard + unknown having proline peak retention time. Experimental conditions as in Fig. 1.

the peak area of β-alanine and that of the internal standard varied only to a small extent with the analytical conditions. To ensure accuracy, however, it is advisable to analyze from time to time samples containing 1 mg of β-alanine and the internal standard.

The determinations were carried out at a flow rate of 27 ml/min (measured by a soap bubble flowmeter at the column outlet) and at a column temperature of 125°. Under these conditions the retention time of β-alanine was about 12 min (overall time of analysis 20 min). This enabled us to analyze four consecutive samples in 80–90 min. After this time, we observed the appearance of peaks of compounds less volatile or more polar than proline, which were present in the solution. These peaks would have been superimposed on the peaks of a fifth consecutive sample. This was prevented from happening by rapid heating of the column to 180° and keeping it at this temperature for 15 min. The column temperature was then lowered and kept at 125° for at least 10 min prior to the injection of the first of the next four samples. In the gas chromatographic preparations of amino acid derivatives by LAMKIN's method[2], it was also essential to minimize traces of moisture, since these could change the derivatives and distort the analytical results.

Conclusions

It can be concluded from the data in Table II that the present method is suitable for the determination of the β-alanylhistidine dipeptides in soup preparations. On the basis of these results, one can calculate the meat extract content of these products. This procedure therefore offers an alternative to HADORN's method[3].

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A simple device for making a gradient for chromatography

A number of methods to produce a gradient for use in column chromatography have been described[1–4]. This paper describes the theory and application of a simple inexpensive apparatus* for producing such a gradient. Two similar conical chambers, such as 1000 ml Erlenmeyer flasks, one inverted and one upright, side by side, are joined by glass tubing with a three-way stopcock at their lowest point, from which they feed a chromatography column (see Fig. 1). A gradient may be produced by filling these chambers with solutions of different compositions. When these chambers are full the major contribution to the column will come from the inverted chamber but as they empty this contribution will lessen and the contribution of the upright chamber will increase. The ratio of the contributions of these chambers at any one time is equal to the ratio of the squares of their radii at the level of the top of the solutions at that time. Although the gradient produced by such an arrangement is not quite linear but is slightly sigmoid, its approach to linearity is sufficient for most purposes (see Fig. 2).

The characteristics of each cone of which the two flasks are frustums (segments of cones not including the apices) should be known to properly plan the gradient. The $\Delta r/\Delta h$, where r is the radius of the cone and h is the height of the cone, is needed and is found by R/H, where R is the radius of the base of the cone and H is the total height of the cone. The following method will give R and H.

The lowest portion of the apparatus that is conical should be established by filling the flasks to this level while freely connected to each other and this level well marked and measured from the top or bottom of each flasks as a point of reference. A note of caution should be added at this point. The shape of the flasks, by producing an optical illusion and by the optical effect of the glass, makes the level in the two flasks appear to be different even though the levels are the same. This effect

* Obtainable from Tudor Scientific Glass Co., Belvedere, S.C.