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GAS CHROMATOGRAPHIC DETERMINATION OF THIAMINE IN MEATS, VEGETABLES AND CEREALS WITH A NITROGEN–PHOSPHORUS DETECTOR

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SUMMARY

A gas chromatograph with a nitrogen–phosphorus detector has been used to quantitate thiamine in a number of meats, vegetables and cereals. Thiamine concentrations found in the foods investigated are in the acceptable range of concentrations determined by spectrophotometric methods. For most foods, deviation from an experimental mean concentration of ten trials was usually within ± 10% when reported at the 95% confidence level.

INTRODUCTION

The accepted standard procedure for analyzing thiamine is based on the thiochrome method[1], first reported in 1936. The cleanup procedure requires some type of chromatography[2,3] and suffers from the disadvantages of interferences from impurities, quantitative dilutions, curve fitting, and a complex conversion into thiochrome. The many chemical modifications that have been introduced usually depend on the production of thiochrome as the detectable species[3].

The physical method of liquid chromatography has been used in determining thiamine[4,5]. Ang and Moseley[6] adapted the method of high-performance liquid chromatography to the quantitation of thiamine in meats. Their procedure did not use decalso ion-exchange in the cleanup but still made use of thiochrome as the final detectable species, thereby requiring the normal standard solutions and curve-fitting associated with thiochrome.

Hilker and Mee[7] quantitated thiamine by gas chromatography (GC), which involves changing the vitamin into a volatile derivative. Dwivedi and Arnold[8] were the first to report the GC determination of thiamine by cleaving[8] thiamine into 4-methyl-5-(2-hydroxyethyl)thiazole using $NaHSO_3$, but the procedure suffered from the state of the art in column development.

Echols *et al.*[9] modified the procedure of Dwivedi and Arnold by using internal standards and modern equipment to determine thiamine in standard solutions and pharmaceutical vitamin formulations using a flame ionization detector (FID) and a nitro-

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gen–phosphorus detector (NPD). Application of this procedure to the quantitation of thiamine in naturally occurring materials, however, has not been reported, presumably because of the low thiamine levels and large amounts of impurities in these materials. This paper describes a GC procedure which uses the NPD to quantitate thiamine in meats, vegetables, and cereals. The meats were also analyzed by the A.O.A.C. method[10] for comparison purposes.

EXPERIMENTAL

Materials

Pork chops, round steaks, chicken thighs, pork liver, beef liver, cereals, mustard greens, dried (kidney) red beans and carrots were purchased from a local supermarket. The data obtained from these products may or may not be representative of the thiamine content in the general population of such products, since they were obtained from only one source. All solvents were of nanograde quality.

Instrument and settings

A glass-lined Perkin-Elmer Sigma I GC equipped with NPD and FID was used. Columns used in the analyses were 6 ft. × 2 mm I.D. coiled glass packed with 5% OV-17 and 5% OV-101 on Chromosorbs W AW DMCS (80–100 mesh).

The instrument was programmed to run an analysis isothermally at an oven temperature of 180°C, 200°C, or 220°C for 5 min, after which the temperature rose to 245°C for 10 min. The injector and detector temperatures were set 25°C above the initial oven temperature. Nitrogen was used as the carrier gas. The flow-rate of hydrogen and the detector current were set so that the NPD totally discriminated against 1 μl of solvent (methanol) at an attenuation factor of 10.

Meat preparation

All meats were cut into small pieces and pooled. Samples for the GC and A.O.A.C. methods were taken from the pool. Thiamine for both procedures was extracted by the A.O.A.C. method.

The meat (5 or 10 g) was homogenized in 0.1 *M* hydrochloric acid (75 ml). The pH was checked and adjusted, if needed, to 1–2 with concentrated hydrochloric acid. The mixture was then heated at 100°C for 1 h, cooled, and adjusted to a pH of 4.5–5 with sodium acetate (2.5 *M*), after which mylase 100 (250 mg) and papain (500 mg) were added with stirring. The resulting enzyme mixture was heated at least 4 h at 45–50°C. $NaHSO_3$ or Na_2SO_3 (10 g) was added, and the pH was readjusted to 4–5 with concentrated hydrochloric acid.

The mixture was again heated for 2 h at 100°C. Following the addition of 5 ml of trichloroacetic acid (TCA), the mixture was cooled to 5°C and filtered. The flask and precipitate were rinsed with 25 ml of water. Sodium chloride (10 g or 0.1 g/ml) was added, and the pH adjusted to 11–12 using sodium hydroxide (5 *M*).

The solution was then extracted three times with chloroform (50 ml each). The chloroform was evaporated to dryness on a flash evaporator at 45°C in a cone-shaped flask. The residue was taken up with 1 ml of the internal standard; to this solution were added 2 ml of methanol, previously used to rinse the flask. The volume was reduced to 0.5 ml on a thermolyne dri-bath at 45°C. The resulting sample was stored for analysis.

Vegetable and cereal preparation

Cereal (2 g) or vegetable (10–15 g) was blended in 0.1 *M* hydrochloric acid. Steps involving heating in acid and incubation with enzymes were omitted. After the sulfite and TCA steps, 2 g of filter aid (Celite) were added, and the resulting mixture was filtered under vacuum (water aspirator) with a No. 3 buchner funnel. The residue was rinsed with distilled water. The rest of the procedure follows the above meat procedure.

Preparation of internal standards

Internal standard solutions of 10 μg/ml and 20 μg/ml of 2-(2-hydroxyethyl)pyridine (b.p. 114–116°C/9 mm Hg, Aldrich Milwaukee, WI, U.S.A.) in methanol were prepared by the dilution of a 2000 μg/ml solution.

Preparation of calibration standards from the thiazole derivative

Standard solutions of 5, 10, 20 and 30 μg/ml of 4-methyl-5-(2-hydroxyethyl-thiazole (Aldrich) in methanol were prepared by dilution of a 2000 μg/ml stock solution. A 1-ml volume of the 4-methyl-5-(2-hydroxyethyl)thiazole solution was mixed with 1 ml of the 2-(2-hydroxyethyl)pyridine standard, evaporated on a dry-bath to *ca.* 0.5 ml, and stored for use each day before starting the vitamin analyses. The instrument was checked for linearity and adjusted to read the calibration standard correctly by adjusting the detector current or response factor in the calculation.

Preparation of calibration standards from thiamine

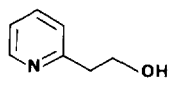
The desired known concentration of thiamine (standard solution) was hydrolyzed by the above procedure for vegetables, and the end product was mixed with the proper concentration of internal standard to give the calibration standard. The resulting solution, which contains the thiazole derivative, can be converted into its equivalent thiamine concentration by the relationship,

$$\frac{\text{mol. wt. of thiamine}}{\text{mol. wt. of thiazole deriv.}} \times (\mu\text{g of thiazole})/\text{unit} = (\mu\text{g of thiamine})/\text{unit}$$

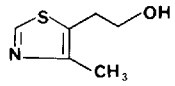
The response factor can be changed to give any unit of concentration desired.

RESULTS AND DISCUSSION

Echols *et al.*[9] used NPD and an external standard to quantitate thiamine in several commercial multivitamin preparations. The low thiamine concentrations found in naturally occurring materials, coupled with the significant error introduced by dilutions, makes the external-standard method unfeasible for routine analyses. After testing several nitrogen-containing compounds, 2-(2-hydroxyethyl)pyridine (I) and 2- and 3-pyr-



I



II

idylcarbinol were chosen for testing because of their similaries to 4-methyl-5-(2-hydroxyethyl)thiazole (II), the detectable derivative of thiamine.

The compound chosen for the internal standard was 2-(2-hydroxyethyl)pyridine (I). Its side-chain and boiling point make it ideal for use as an internal standard for the quantitation of compound II. By proper temperature programming of the instrument, the difference in the retention times of compounds I and II can be made to vary from less than 1 min to more than 5 min (Fig. 1). This variation is important because of other products which show up in thiamine analyses of natural products.

2-(2-Hydroxyethyl)pyridine would be even more desirable as an internal standard if it were less volatile (or at least did not evaporate faster than II) and less soluble in aqueous solutions. In the absence of methanol, a significant amount of I is lost at 40°C over a 24-h period. Although in the presence of methanol there is no detectable loss of I and II when methanol is allowed to evaporate continously over a 3-day period, caution should still be taken not to allow the sample to evaporate to dryness, since I actually does evaporate faster than II.

Because of the solubility of I, attempts to add the standard in an earlier stage of the procedure to minimize errors and ease the burden of quantitative recovery in each step failed. The amount of I recovered from the extraction ranged from 70 to 85% of the amount added.

The pyridines –2- and 3-pyridylcarbinol– could not be retarded on the column unless low temperatures (145–150°C) were maintained. Such low temperatures, however, caused difficulties in quantitation by increasing the retention time of II, thereby increasing the minimum detectable amount of this compound.

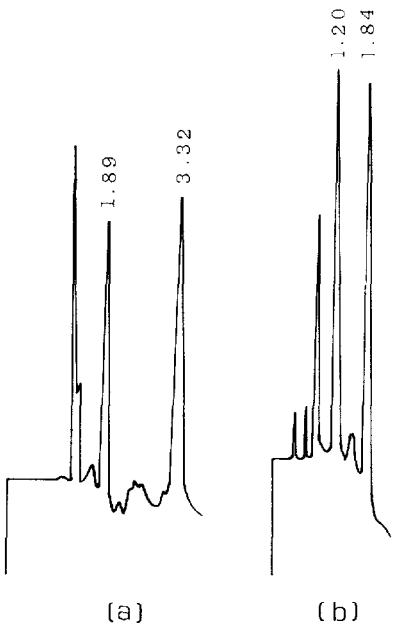


Fig. 1. Representative chromatograms for thiamine analyses: (a) chicken run at 180°C, (b) pork chops run at 200°C. The first and second peaks over which the retention times (in minutes) appear are, respectively, the standard and the thiazole derivative.

The addition of TCA aids in the filtration of meat samples and the extraction of all samples. Without TCA the chloroform–water–tissue dispersion takes overnight or longer to separate. A certain amount of caution must be exercised in the addition of TCA because of bubbling caused by the release of SO_2 from the H_2SO_3 produced. In the cereal and vegetable samples (especially beans), the thick emulsion could not be filtered without the addition of Celite and the use of suction. A No. 3 or larger buchner funnel was needed to give more surface area.

Because of the many compounds in natural foods, the samples were run under different conditions to verify that no foreign compounds were contributing to the area under the peaks for the standard or the 4-methyl-5-(2-hydroxyethyl)thiazole. The meat samples showed minor peaks in the range 2–4 min, whereas the cereal and vegetable samples were nearly as clean as the calibration standard in this range.

To keep residual compounds from building up on the column, the temperature had to be programmed to 240°C for each of the meat-sample runs; but for the cereals and vegetables, as many as 10 runs could be made before temperature programming became necessary. Being able to perform analyses without temperature programming is very important from a timesaving viewpoint. For temperature-programmed runs, *ca.* 30 min are required between sample injections, whereas for isothermal runs, only 5–6 min are required between injections.

Attempts to use the FID were not particularly successful because the effective concentration has to be relatively high (*ca.* 100 μg/ml); moreover, column bleeding interfers with FID quantitation. With cereal (which consists principally of added thiamine), these two problems can be largely overcome by using larger samples; but the use of larger samples of meats and vegetables presents certain mechanical problems (blending, extracting, filtering), along with vast increases in impurities. In a typical meat-sample run using NPD, *ca.* 20 peaks are produced; when FID is used, more than 50 peaks are produced.

Consistently the concentration of a given standard generated by the cleavage of thiamine was found to agree to within 5% with a calibration standard of the same concentration prepared from 4-methyl-5-(2-hydroxyethyl)thiazole. For each food, however, deviation from an experimental mean of ten trials was usually within ±10% when reported at the 95% confidence level (Tables I and II).

Values obtained for the meat analyses by the A.O.A.C. and GC methods are given in Table I. In general the values are higher for the GC method. In analyzing

TABLE I

THIAMINE IN MEATS

Type	*GC method*			*μg/g by A.O.A.C.*
	μg/5 g	*μg/5 g + 20 μg thia.*	*μg/g*	
Calf liver	25.3 ± 4.0	46.1 ± 5.9	5.1 ± 0.8	4.5 ± 0.9
Pork liver	20.2 ± 2.0	42.3 ± 3.8	4.0 ± 0.4	3.4 ± 0.5
Pork chops	54.6 ± 1.6	74.2 ± 2.1	10.9 ± 0.3	8.8 ± 0.8
Chicken	7.2 ± 1.5	28.2 ± 0.2	1.4 ± 0.3	1.8 ± 0.2
Ground beef	10.5 ± 0.9	30.5 ± 2.4	2.1 ± 0.2	1.5 ± 0.3

TABLE II

THIAMINE IN VEGETABLES AND CEREALS

Type	*Concentration*	
	mg/oz.	*mg/lb.*
Corn flakes	0.362 ± 0.025	
Bran flakes	0.387 ± 0.015	
Total*	1.5 ± 0.2	
Red (kidney) beans		1.75 ± 0.2
Carrots		0.33 ± 0.03
Mustard greens		0.77 ± 0.06

* Trade name.

meats with low thiamine concentrations, the GC method is plagued by high concentrations of impurities. These impurities produce large deviations caused by column bleeding, as shown by the liver and chicken analyses. The ground beef does not contain a significant quantity of interfering impurities. By adding thiamine (Table I), the amounts of materials needed for analyses are reduced; the percentage of impurities is decreased; and the precision of the GC method is increased. Lower values for the A.O.A.C. method are attributed to loss occurring in the cleanup procedure[11].

Table II gives results of analyses of vegetables and cereals. The vegetable values are comparable to U.S.[12–14] values (in parentheses). The vegetable values in mg/lb. are red (kidney) beans, 1.75 (2.3); carrots, 0.33 (0.27); and mustard greens, 0.77 (0.80). U.S. Department of Agriculture values for thiamine concentrations in cereals are essentially the same as those found here. The GC method is easier for materials containing large amounts of thiamine but inferior for low concentrations of thiamine.

The enzymes mylase and papain were checked for thiamine content and found to have 2 μg of thiamine per quantity used in the meat preparation. This amount was subtracted out of the calculations.

CONCLUSION

Thiamine concentrations found in foods investigated in this study are in the acceptable range of concentrations determined by spectrophotometric methods. Thiamine contents in meats were generally found to be higher than those reported elsewhere. Added thiamine was totally recovered.

Advantages of the GC method over the standard method are simplicity of technique, ease of standardization, lack of cleanup steps, and the option of storing the samples for a long period in a minimum of space. The GC procedure can be interrupted for an indefinite period of time at any point except the enzyme step (bacteria attack under these conditions). Whereas the results of the A.O.A.C. method is partially dependent on the operator's technique[2], the GC method is more nearly operator-independent. Samples do not show any bacteria attack when left at room temperature for 2 months.

Disadvantages of the GC method are the relatively large amount of sample required and the expense of the instrumentation. The time required to run a GC analysis is substantially longer than that required for the A.O.A.C. method, once the A.O.A.C. conditions are set up.

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