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## ANALYSIS OF WOOL PROTEIN AND CHEMICAL RESIDUES BY PYROLYSIS GAS CHROMATOGRAPHY

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### SYNOPSIS

The coupling of pyrolysis to GC offers a means to analyze nonvolatile and insoluble materials such as wool. Initial studies examined the response of Lissamine Yellow 2G and Mitin FF as model chemical add-ons. These compounds give rise to characteristic peaks easily distinguished from the numerous peaks generated by pyrolysis of wool itself. Peak area responses per unit weight of wool gave somewhat better predictability of add-on (% on weight of fiber) than response per internal standard peak, but the gravimetric approach was tedious. Tyrosine, phenylalanine and tryptophan residues of wool protein could be studied by their respective pyrolysates (Tyr: sum of phenol and p-cresol; Phe: benzyl cyanide; Trp: indole). Trp is difficult to determine by amino acid analysis; its vulnerability during chemical treatment of wool motivated the development of a simple analytical procedure. We found good correlation between the molar ratio of Tyr/Trp and the peak area ratio (phenol + p-cresol) / indole for wool and other proteins. Trp depletion by oxidation (DMSO) or hydrolysis (carbonizing) could be monitored by pyrolysis-GC. Tyr depletion by nitrosation could be followed relative to Phe by monitoring (phenol + p-cresol) / benzyl cyanide, since Phe is stable under nitrosation conditions.

### INTRODUCTION

Pyrolysis gas chromatography (Py-GC) is a useful technique for detecting and quantitating normally nonvolatile and insoluble

substances. Perlstein<sup>1</sup> used the technique for identifying fiber composition based on diagnostic peaks; wool and silk, for example, gave rise to toluene, pyrrole, and p-cresol. Others have used Py-GC to study the emission of volatile and sometimes toxic gases such as HCN, CO<sub>2</sub>, CH<sub>4</sub>, H<sub>2</sub>S, and CS<sub>2</sub><sup>2</sup>. Pyrolysates from protein decomposition have been associated with specific amino acid residues. Phenol and p-cresol, for example, arise from tyrosine (Tyr); indole and skatole are formed from tryptophan (Trp)<sup>3</sup>. Tsuge and Matsubara<sup>4</sup> used Py-GC coupled with mass spectrometry to confirm the pyrolytic products of amino acids and peptides and to quantitate them based on peak area vs. sample weight.

This paper presents our assessment of the utility of Py-GC in wool studies based on two approaches. First, it was desirable to assess the technique as a means of detecting and quantitating chemical add-ons from wool processing. Lissamine Yellow 2G and Mitin FF were selected as model compounds and their characteristic pyrolysates were studied relative to sample weight of wool<sup>5</sup>. Second, we wished to assess the utility of the technique for measuring the content of specific amino acid residues in wool protein and for deriving procedures for tracking changes in those residues during chemical treatment of wool. We concentrated on Trp analysis due to the difficulty of quantitating Trp by conventional amino acid analysis; Trp content was measured against that of Tyr<sup>6</sup>. We also studied Tyr decomposition, wherein Tyr was tracked relative to phenylalanine (Phe)<sup>7</sup>

#### EXPERIMENTAL

Fig. 1 is a schematic diagram of the experimental set-up of hardware for Curie-Point pyrolysis coupled with capillary column GC. Note that initial work for add-on determinations was done using a more polar stationary phase, SP-2330 (cyanopropyl silicone), but later work with amino acid residues utilized the

less polar phase Supelcowax 10 (polyethylene glycol) for better resolution.

Wool fabric pretreated with specific amounts of Mitin FF (Ciba-Geigy) was obtained from the IWS Technical Centre, Ilkley, UK. Wool samples dyed with Lissamine Yellow 2G (ICI) were prepared in our laboratory<sup>5</sup>. Synthetic polypeptides and egg-white lysozyme were obtained from Sigma Chemical Co., St. Louis, USA. Dairy proteins were isolated in-house<sup>6</sup>. Trp in wool was oxidized by dimethyl sulfoxide<sup>8</sup>. Carbonizing was done using 6% v/v sulfuric acid<sup>9</sup>. Nitrosation utilized potassium nitrite in acetic acid<sup>10</sup>. Conventional amino acid analysis was accomplished on a Beckman 119-CL amino acid analyzer<sup>11</sup>. For Trp analysis, samples were hydrolyzed using mercaptoethanesulfonic acid and phenol in sealed, evacuated tubes prior to analysis<sup>12</sup>. Data for the add-on work were analyzed by the method of inverse regression to enable prediction of percent owf and the error for duplicate determinations<sup>13</sup>.

#### RESULTS AND DISCUSSION

Py-GC of wool gives rise to a complex pattern of peaks from pyrolysate products. Very volatile materials, gases such as HCN, CO<sub>2</sub>, CH<sub>4</sub>, H<sub>2</sub>S, and CS<sub>2</sub>, elute rapidly along with such products as benzene and toluene. The major peaks of interest for these studies elute between 10 and 50 minutes. Figure 2 shows a portion of a chromatogram using Supelcowax 10 stationary phase with identification of peaks from Tyr, Trp, and Phe. (BzCN denotes benzyl cyanide.) In add-on studies, in which an SP-2330 stationary phase was used (no figure shown), the Tyr-generated peaks phenol and p-cresol eluted at 21 and 22 min., respectively, a peak from Lissamine Yellow 2G was seen at 18 min. and one from Mitin FF at 32 min.

The areas of the peak from the Lissamine dye were plotted both relative to sample weight (gravimetric approach) and to the sum of the two Tyr-generated peaks (internal standard approach).

Replicate analyses (n=5) for wool with 3 levels of the dye (1.4, 2.7, and 7.6%) were conducted. The following equations describe the linearity of response (peak areas indicate response by flame ionization detection; sample weights are in micrograms; R is the correlation coefficient):

Gravimetric:

(dye-peak area/sample weight) = 1548(% owf) - 901; (R=0.998)

Internal standard:

(dye-peak area/sum of phenol + p-cresol peak areas) = 0.2185(% owf) - 0.0563; (R=0.997)

A similar treatment was afforded the Mitin-FF-treated samples (0.30, 0.60, 1.0 % owf):

Gravimetric:

(Mitin-peak area/sample weight) = 1440(% owf) - 296; (R=0.945)

Internal standard:

(Mitin-peak area/sum of phenol + p-cresol peak areas) = 0.1804(% owf) + 0.0014; (R=0.999)

Despite better linearity by the internal standard approach, treatment of the data by inverse regression analysis projected a better predictability of % owf using the gravimetric approach. Table I demonstrates such predictability as a function of the number of replicate Py-GC analyses (q) carried out:

The gravimetric approach is tedious because of the need to weigh samples in microgram amounts. Subsequent work used the internal standard approach and also used a stationary phase that allowed better peak resolution.

Table I. Predictions of % owf from inverse regression analysis<sup>5</sup>

Agent	Peak Area per Sample Wt <sup>a</sup>	Predicted % owf	Predicted Range <sup>b</sup> of % owf using q replicate determinations, where q =					
			1	2	3	4	5	inf.
Mitin-FF	600	0.62 ±	0.19	0.14	0.12	0.10	0.09	0.05
Lissamine Yellow 2G	6000	4.5 ±	1.6	1.2	1.0	0.9	0.8	0.4

a Hypothetical average of replicate determinations, peak area/microgram wool.

b 95% confidence limits.

Trp analysis of the wool sample by the cumbersome wet chemical method<sup>12</sup> showed 42.2 nmol/mg of wool, with a CV of 12%. Likewise, Tyr content was 287 nmol/mg (CV = 3%). Thus the molar ratio Trp/Tyr was 0.147, or approximately 1 Trp for every 7 Tyr. To assess the Py-GC method for determining the Trp/Tyr ratio, i.e., by determining the peak area ratio of indole/(phenol + p-cresol), 5 Py-GC replicates were run and the ratios determined for each run. The area ratio was 0.179 (CV = 12% for the 5 separately determined ratios). Calibration curves were determined using data from the pyrolysis of known mixtures of pure poly-Tyr and poly-Trp. For Trp/Tyr ratios of 0.50 or under, linear regression analysis gave the following equation:

$$\text{indole}/(\text{phenol} + \text{p-cresol}) = 1.3371(\text{Trp}/\text{Tyr}) + 0.0166; \quad (R=0.999)$$

This enabled a prediction of Trp/Tyr = 0.122, or approximately 1 Trp per every 8 Tyr, instead of the abovementioned 1 per 7 by wet chemical determination. A broader range of standards was used to determine another linear fit, this time for Trp/Tyr up to 2.0. The following equation was determined:

$$\text{indole}/(\text{phenol} + \text{p-cresol}) = 1.1227(\text{Trp}/\text{Tyr}) + 0.0569; \quad (R=0.996)$$

Use of both these equations allowed the prediction of Trp/Tyr ratios for a large number of natural proteins of known ratio. The

results of these predictions, including wool, are shown graphically in Figure 3, where all results are expressed in the inverted ratio of Tyr/Trp. For all proteins with Tyr/Trp of 5 or under, the Py-GC method predicted the proper ratio to the nearest integer. Wool and alpha-s<sub>2</sub>-casein were off by one integer; kappa-casein's results were the least acceptable<sup>6</sup>.

To demonstrate the utility of the technique for following Trp depletion, wool was oxidized over a 24-hr. period with DMSO. The indole/(phenol + p-cresol) ratio of the control (0.179) diminished to 0.060 within 4 hr. (i.e., 66% Trp destruction) and to 0.028 by 24 hr (84% Trp destruction). Another wool sample (peak area ratio = 0.237) was subjected to a standard carbonizing treatment using sulfuric acid. The carbonized wool gave a peak area ratio = 0.204, equivalent to 14% destruction of Trp<sup>6</sup>.

Finally, the Py-GC technique was used to assess the destruction of Tyr under conditions in which Phe remains stable. Samples of wool were subjected to nitrosation using potassium nitrite in acetic acid over a 45-hr. time span. Aliquots of wool were removed and washed at set intervals and analyses were done on them both by Py-GC (n=5) and by conventional amino acid analysis (n=2). A correlation between the Tyr/Phe ratio and the peak area ratio of (phenol + p-cresol) / BzCN (where BzCN = benzyl cyanide) was demonstrated, with the following equation for wool:

$$\begin{aligned} (\text{phenol} + \text{p-cresol})/\text{BzCN} &= 10.05(\text{Tyr}/\text{Phe}) + 0.2788; \\ (R=0.997) \end{aligned}$$

Results are shown graphically in Figure 4. Also shown in Figure 4 are the parallel results from three known mixtures of poly-Tyr/poly-Phe. Whereas in the Trp/Tyr studies the polypeptides gave the same linear regression as the natural protein samples, in the present Tyr/Phe study an entirely different line resulted from the polypeptides vs. the wool samples. This likely was due to the use of the BzCN peak for Phe. Nitriles arise from pyrolysis of N-terminal amino acid residues<sup>4</sup>. Poly-Phe can be expected to contain many more terminal Phe

residues than wool and therefore give rise to a larger BzCN peak relative to (phenol + p-cresol) for the same Phe/Tyr ratio. Thus, in Figure 4, wool with a Tyr/Phe ratio of 1 corresponds to a much greater (phenol + p-cresol)/BzCN ratio than does the polypeptide mixture with the same Phe/Tyr ratio. Nevertheless, the Py-GC technique enables the tracking of Tyr content over the course of nitrosation of a wool sample<sup>7</sup>.

## CONCLUSIONS

Pyrolysis GC can serve as a useful analytical tool for analysis of chemical add-ons to wool as well as changes in the amino acid composition of wool during processing. The use of Py-GC has proven particularly useful for tryptophan determinations of wool and of other natural proteins; the technique obviates use of a very tedious and lengthy wet chemical procedure for Trp determinations. Py-GC was demonstrated to be capable of tracking both the disappearance of Trp and Tyr during chemical processing of wool.

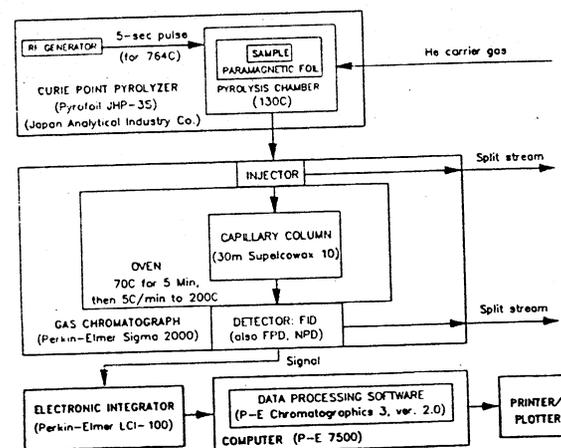


Fig. 1. Hardware configuration for pyrolysis-GC, as used for amino acid studies. For chemical add-on studies, column was 30m SP2330 capillary. Detection for all these studies was by flame ionization.

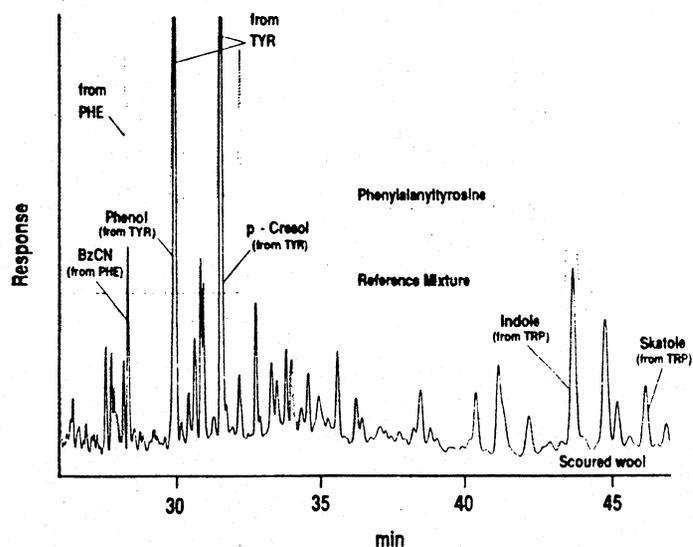


Fig. 2. Pyrolysis-GC chromatogram of wool, 26-47 min. segment, denoting characteristic peaks from phenylalanyl (PHE), tyrosyl (TYR), and tryptophanyl (TRP) residues; column: 30m Supelcowax 10 capillary; BzCN - benzyl cyanide. Overlapping dotted traces show chromatograms of an injected reference mixture of indole and skatole and the pyrogram of the model dipeptide phenylalanyltyrosine.

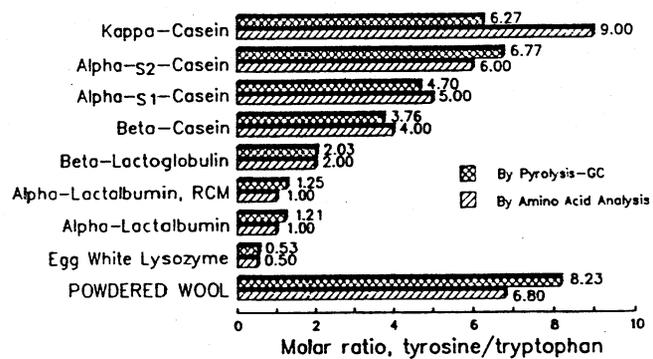


Fig. 3. Tyr/Trp ratios for wool and other natural proteins; comparison of determinations by pyrolysis-GC (upper bars of each pair) and amino acid analysis (lower bars of each pair).

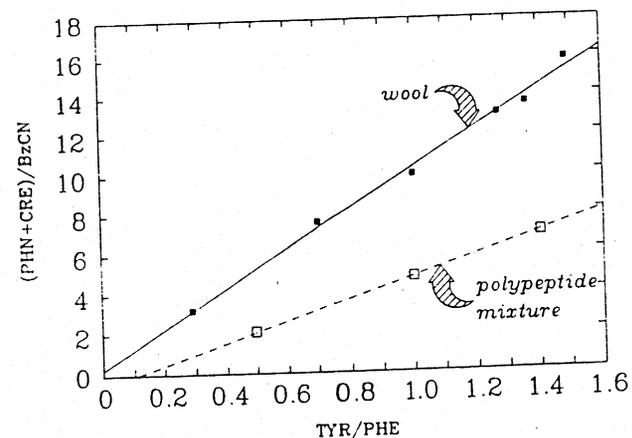


Fig. 4. Destruction of tyrosine in wool by nitrosation (solid line). Correlation of peak area ratios from pyrolysis-GC vs. amino acid analysis. Abbreviations: PHN, phenol; CRE, p-cresol; BzCN, benzyl cyanide. Dashed line indicates parallel analysis of mixtures of poly-Tyr and poly-Phe of three known compositions.

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