

Estimation of Degree of Methylation of Pectin by Pyrolysis-Gas Chromatography

The physicochemical properties of pectin, as related to its function as food fiber, cell wall component in plants, and thickening agents in foods, are determined to a great extent by the degree of methylation of carboxylic acid groups (1). A pectin molecule is represented schematically in Figure 1. Basically pectin is comprised of α -1-4 linked D-galacturonic acid with varying degrees of methylated (DM) carboxyl groups. At points along the chain, neutral sugars, predominantly rhamnose, are attached to form T-junctions (2). The number average degree of polymerization of unaggregated citrus pectins is 50-85 galacturonic units (3). Differences in DM are caused by natural variations in the methyl ester content of pectins as they occur in nature and by the extent of demethylation induced during extraction and purification (4).

Traditionally, DM has been determined by titration of pectin carboxyl groups before and after basic hydrolysis (5), though GC has been proposed more recently to measure methanol release by pectin hydrolysis (6, 7). The ratio of carbomethoxy to carboxyl also may be determined (1) from the ratio of ^{13}C NMR resonances at 171.3 and 172.8 ppm. However, the need exists for a rapid means to determine structural information of pectin. Analytical pyrolysis offers such potential.

Because of their importance in foodstuffs, the thermal decomposition of polysaccharides has been studied extensively. Primarily, these studies have involved slow heating in the presence of air and off-line analysis (8). Reports of polysaccharide decomposition under the anaerobic and almost instantaneous conditions of analytical pyrolysis have suggested that the principal reactions are (1) depolymerization, (2) β -elimination of water, (3) decarbonylation, and (4) retro-aldolization (9). In a preliminary study, the products of pectin pyrolysis were surveyed to ascertain whether structural features could be determined. Some correlations with DM were found but the need for more thorough investigation was recognized (10). The purpose of this research is to further study pectin pyrolysis to develop useful relationships between pyrograms and structure.

INSTRUMENTATION

Pyrolysis-gas chromatography (PY-GC) was carried out in the CDS Model 122 Pyroprobe connected directly to the

capillary injection port of a Varian Model 3700 gas chromatograph. Detection was by flame ionization and resulting data were processed by a Varian CDS 111 data system. The pyrolyzer was equipped with both a ribbon probe and a coiled probe. The former is used with liquid, soluble, or meltable samples and the latter for samples that are insoluble and will not melt. In these cases, samples were contained in a small quartz tube placed within the hollow core of the coiled heating element.

For pyrolysis-gas chromatography-mass spectrometry the pyrolyzer was interfaced directly to the injection port of a Hewlett-Packard Model 5995 gas chromatography-mass spectrometer. Two chromatographic columns were used: a 50-m fused silica capillary (0.2 mm i.d.) coated with free fatty acid phase (FFAP) obtained from Scientific Glass Engineering, Ltd., and a 60-m glass capillary (0.75 mm i.d.) coated with SP-1000 obtained from Supelco, Inc. The former was used for all determinations reported here except for chromatograms shown in Figure 3.

MATERIALS

Commercial citrus pectins with degrees of methyl esterification (DM) of 35, 58-60, and 70 were gifts from Bulmers, Ltd., Hereford, England. Two other pectin samples with DM 37 and 73 were manufactured by Bulmer but were gifts from E. R. Morris and M. J. Gidley at Unilever Co., England. The DM 57 pectin was a gift from Sunkist Growers, Corona, CA. The DM 75 pectin was obtained from the General Foods (GF) Corp. Another DM 73 pectin was obtained by extraction of fresh grapefruit albedo, according to standard procedures (11). Polygalacturonic acid (>98% pure) was obtained from Sigma Chemical Co., St. Louis, MO. Percentage of methyl esterification was determined by the colorimetric method of Wood and Siddiqui (12) and ^{13}C NMR (1). Samples to be neutralized with NaOH were dissolved in 0.01 M phosphate buffer (pH 6.1) containing 0.1 M EDTA, titrated to pH 7 with 0.1 M NaOH, dialyzed against four changes of water over 48 h, centrifuged for 1 h at 30000g to remove insoluble matter, and lyophilized. Dialysis bags were Spectrapor with a molecular weight cutoff of 12000.

To increase the data base, four mixtures were made using sodium polygalacturonate (DM 0) and GF pectin Na^+ salt

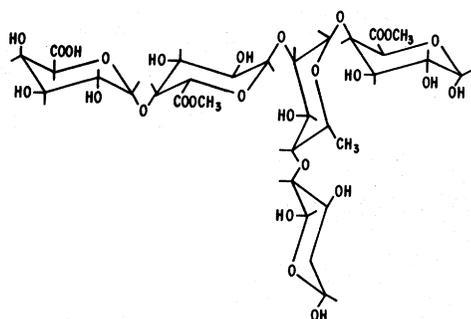


Figure 1. Schematic of pectin molecule indicating methylated and unmethylated carboxyl groups and neutral sugars.

Table I. Conditions

pyrolysis	chromatography
ribbon probe	fused silica capillary, 50 m; FFAP
interface temp, 125 °C	
program	split mode
ramp, 2 °C/ms	program
interval, 2 s	70 °C for 5 min
final temp, 800 °C	5 °C/min to 150 °C
	hold for 5 min

(DM 75), ranging from 20% to 80% sodium polygalacturonate.

PY-GC Procedure. Ten-microliter volumes of 1% aqueous solutions of the various pectins in the Na⁺ form were applied to the ribbon probe with a 10- μ L syringe. The water was removed by using the 20-s heating interval, the longest available, for six consecutive intervals. The probe was then inserted into the interface and purged with the helium carrier gas for 6 min to further reduce the level of moisture present. Then sample pyrolysis, column temperature programming, and data acquisition were initiated. Table I gives the conditions of pyrolysis and chromatography used in collecting data for the statistical analyses. Estimates of percent CO₂ produced during pyrolysis were obtained by PY-GC-MS of pectin samples and calcium carbonate. PY-GC-MS of the pectin samples was as described in Table I with mass spectrometry in the single ion monitoring mode. Pyrolysis of calcium carbonate was as follows: final temperature, 1000 °C, interval 5 s, and ramp off; the column program was as described in Table I and mass spectrometry was in the single ion monitoring mode.

Statistical Methods. The data from the PY-GC procedure were analyzed by stepwise discriminant analysis (13) and stepwise multiple regression analysis (14). Data from repeated runs on two samples (DM 57 and DM 75) representing the areas of each peak larger than 1% were analyzed by stepwise discriminant analysis to determine those peaks that would separate the samples according to DM. These peaks were then used in stepwise regression analysis using backward elimination to determine a predictive relationship between peak area and pectin DM.

RESULTS AND DISCUSSION

When citrus pectins are pyrolyzed at instrument conditions shown in Table I, more than 45 peaks (Figure 2) are observed to elute from a fused silica capillary coated with FFAP and programmed from 70 °C to 150 °C as described in the Experimental Section. Examination of the pyrolyzer probe filament after it was removed from the reactor revealed only scant traces of carbonaceous residue. Repeat pyrolysis of the residue resulted in negligible detector response, indicating pyrolysis to volatile products was essentially complete. Calculation based upon area of CO₂ mass spectral peak referred to CO₂ area produced by CaCO₃ demonstrated that more than 40% of 75% DM pectin appeared in the pyrolysis

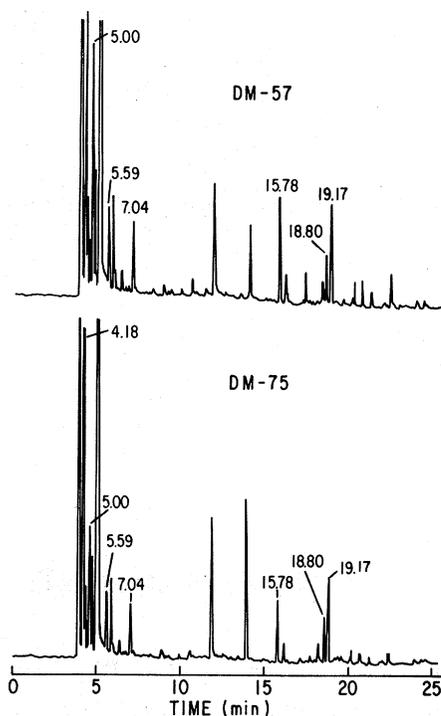


Figure 2. Pyrograms from pectins with 57 and 75% of carboxyl groups methylated (DM). Times indicate peaks correlated with DM. Conditions are given in Table I.

Table II. Variability of PY-GC Peaks from Pectins

retention time, min	range of mean area %	range of std error of mean
4.18	7.9-14.8	0.17-0.39
5.00	0.5-62.4	0.05-1.3
5.59	1.3-3.4	0.03-0.15
7.04	1.1-2.1	0.04-0.09
15.78	1.8-4.6	0.02-0.12
18.80	1.5-2.3	0.12-0.12
19.17	0.02-1.1	0.02-0.18

product as CO₂. Therefore, under the pyrolysis conditions used to assure complete reaction, few multicarbon fragments remain from which to deduce pectin structural information. Nevertheless, stepwise discriminant analysis (13) of all peaks >1% showed that there were differences among pyrograms with different DM. Seven peaks were found to separate samples. The variability of these peaks is shown in Table II. Pyrolysis of the eight citrus pectins was replicated 8-19 times. The standard error was less than 4% of the mean in most cases, demonstrating clearly that pyrolysis with the conditions shown is extremely reproducible.

Stepwise linear regression analysis (14) of normalized areas yielded a model in which a linear combination of three of the seven peaks indicated in Figure 2 was sufficient to predict the degree of methylation with a coefficient of determination (100R²) of 95.5% (78 DF). This approach eliminated from the model peaks that correlated with each other and were therefore redundant. The coefficients of the model are dependent upon experimental conditions and would need to be generated in each laboratory whenever conditions were changed. Subsequently, though, several peaks were identified by a combination of cochromatography of reference compounds and/or mass spectrometry. Of these, methanol (*t*_R ~ 5 min) correlated best (95.2%, 93 DF) with DM. Natural pectins covering the range 0 (polygalacturonate) to 80 DM were not available. Consequently, mixtures of polygalacturonate and 75 DM pectin were prepared and subjected to

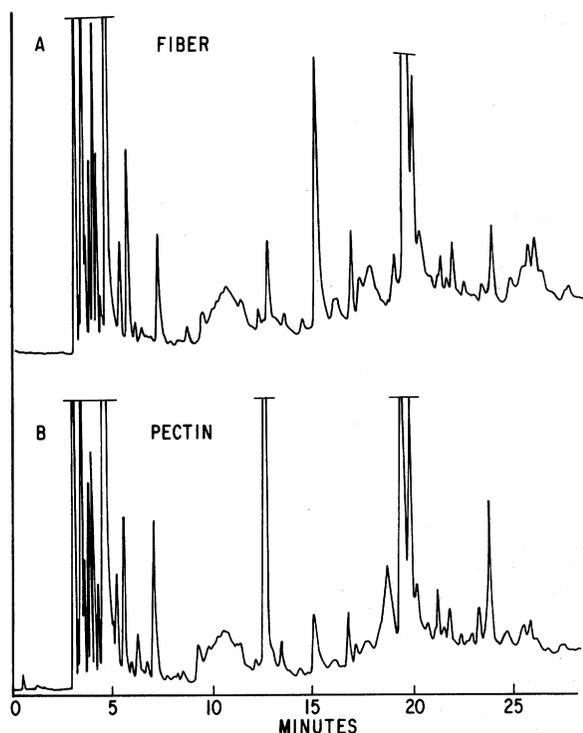


Figure 3. Pyrograms of carrot fiber and pectin derived therefrom.

PY-GC. Statistically similar linear relationships between area of the methanol peak and DM were observed whether or not the mixtures were included in the data analysis. Apparently, even though the chemical environment around methoxy groups for a given DM is different within a polymer molecule than in a mixture of polymers prepared to simulate that DM, the chemistry of methoxy fragmentation is influenced minimally. For conditions described in the Experimental Section, a linear model $\% \text{ DM} = 2.34 \text{ Area (MeOH)} + 1.22$ was derived with coefficient of determination of 95% and coefficient of variation (CV) of 11.71. This CV is comparable to CV reported in the literature for other methods used to determine DM (7). The amount of methanol measured in these pyrolysis experiments was calculated to represent 40–50% of known methylated galacturonate units. This diminished value could result from incomplete pyrolysis or from secondary reaction of methanol. The latter is more probable.

Another peak eluting at 4.2 min, identified tentatively as formaldehyde, correlated negatively (correlation coefficient

$= -0.92$) with DM but to a lesser extent than methanol. This was the last peak discarded during the regression analysis. The major source of formaldehyde likely is the unesterified carboxyl groups.

Figure 3 depicts a pyrogram of carrot pectin together with one of carrot fiber. The similarities of the two profiles and to the profile of citrus pectin are evident. Nevertheless, only 35% DM was calculated from the area percent of methanol and linear model given earlier, whereas the value was known to be greater than 75%. When the three-parameter model was used, 50% DM was found. The discrepancy is likely related to matrix effects and demonstrates that models derived for pectins from one fruit source cannot be generalized to pectins from other plant sources. This observation does not detract from the method presented here but supports the concept that calibrations must be derived from similar substrates for acceptable quantitative information to be generated.

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Registry No. Pectin, 9000-69-5; low-methoxyl pectin, 9049-34-7.

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