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The Use of NIR Spectrophotometry to Estimate the Pectic Substances in Fruit and Fruit Products

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ABSTRACT

A study was carried out on the possibility of using near infrared (NIR) spectrophotometry for the analysis of the pectic substances in fruit.

The NIR reflectance spectra of the alcohol insoluble solids (AIS) of fruit (peach, apricot, apple) were scanned against teflon powder as a blank and compared with those of some pure polysaccharides (cellulose, hemicellulose, pectins, starch, etc.) usually present in AIS. Since the spectrum obtained from the AIS gave unresolved and broad bands, due to the overlap of the bands from the components, the correlations with chemical analysis were very poor.

Running the spectra against cellulose as a blank gave much better correlations with chemical analysis, for the total pectic substances and their water soluble and insoluble fractions as well as their methoxyl content.

INTRODUCTION

The pectic substances play an important role in regulating the firmness of fruit during ripening and processing. Therefore a knowledge of their composition in fruit is important in the development of fruit processing and preservation techniques. For instance, for the osmotic dehydration of fruit the dependence of the quality of the product on the soluble pectin/protopectin ratio present in the different species of fruit was reported by Forni *et al.* (1986). This implies that it is necessary to know not only the total pectin content, but also the water-soluble, oxalate-soluble and insoluble fractions. Classical analytical methods involve time

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consuming extraction, fractionation and estimation stages. For technological research it is particularly desirable to have a more rapid method. One possibility is near infrared spectrophotometry (NIRS).

The application in food analysis of NIRS has increased rapidly over the last decade, especially for the evaluation of general composition of food, as can be seen from reviews by Polesello and Giangiacomo (1983) and Davies and Grant (1987). In the case of polysaccharides, crude and dietary fiber NIRS analysis has been carried out and applied to a wide number of foods (Bak *et al.*, 1979; Baker & Norris, 1985) and animal feeds (Norris *et al.*, 1976; O'Keefe *et al.*, 1987). Pectin however has not received much attention. Giangiacomo (1983) made a tentative attribution of some NIR bands in commercial pectins, while Horvath *et al.* (1984) estimated pectins as constituents of the dietary fiber in wheat bran by NIRS. The problem arises from the difficulty of selecting specific absorption bands for pectins due to overlap with those associated with accompanying carbohydrates and in some cases proteins (Norris, 1978; Giangiacomo, 1983; Baker & Norris, 1985).

The present paper describes a preliminary investigation to evaluate the possibility of NIRS for the direct estimation of water soluble, oxalate soluble and insoluble pectin fractions in fruit. For this purpose it was first necessary to standardize the chemical methods, in order to characterize the materials used to calibrate the NIRS method.

After having checked some methods quoted in literature, special methods were studied and carried out for the analysis of pectins characterized by their galacturonic acid content (Forni *et al.*, 1987) and by their methanol content as an index of their esterification degree (Forni *et al.*, 1984).

EXPERIMENTAL

The research was performed using the alcohol insoluble solids (AIS) of fresh and osmotically dried peaches and apricots, of fresh and solid-packed apples and commercial pectins of technical grade with different degrees of esterification.

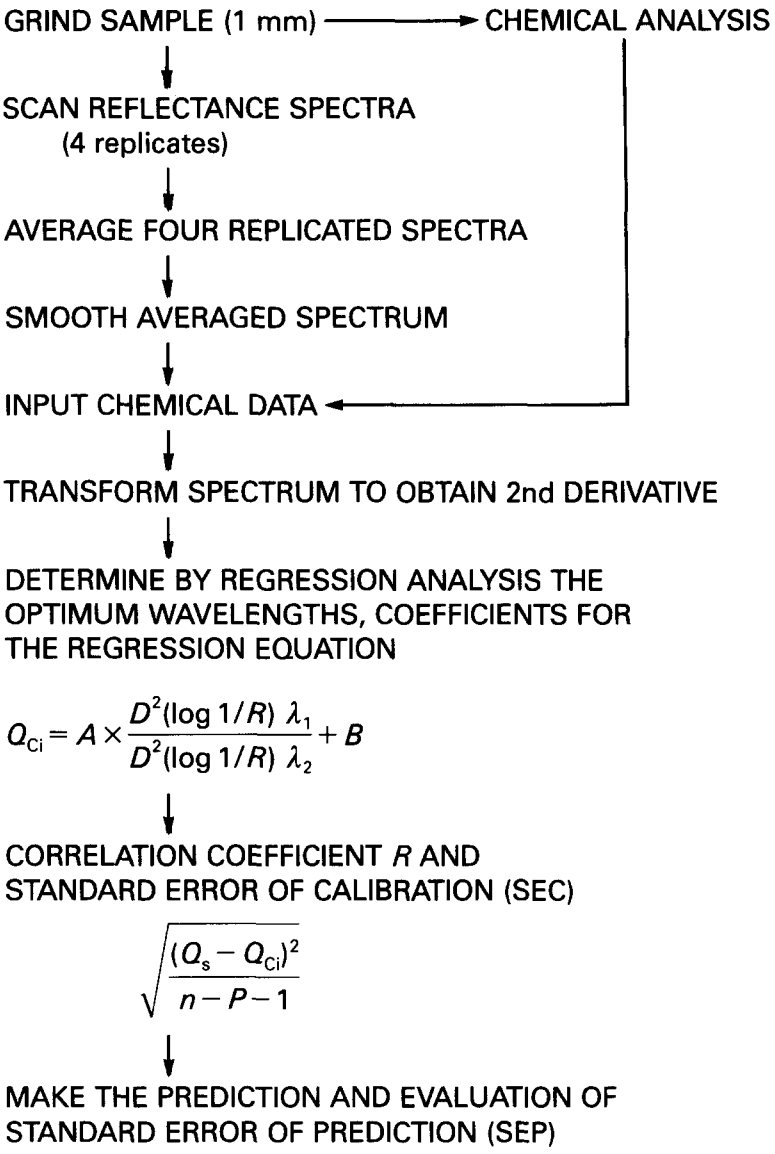
Analytical grade cellulose, starch and polygalacturonic acid were used to obtain reference spectra.

The research involved two operations:

1. The chemical analysis of the samples to be used.
2. The NIRS analysis of the same samples.

The recorded spectra were then processed according to the flow chart shown in Fig. 1.

The experiments performed are summarized in Table 1.



Q_{Ci} = The component concentration calculated in mass percent.
A, B = Regression coefficients.
D^2 = 2nd derivative.
Q_s = The component concentration determined by chemical analysis in mass percent.
n = The number of samples.
P = The number of independent variables.

Fig. 1. Flow-chart for NIR analysis procedure of fruit AIS.

TABLE 1
Experimental Plan

Analysis of fruit AIS using teflon as a reference standard (blank):	
Blank	Teflon powder
No. of samples	32
Wavelength range (nm)	2000–2500
Wavelength increment (nm)	0·5
No. of points	1000
Spectra of pure polysaccharides samples against two different blanks:	
Blank	(a) Teflon powder (b) Cellulose powder MN 300
Wavelength range (nm)	1000–2500
Wavelength increment (nm)	1·5
No. of points	1500
Analysis of commercial pectins and fruit AIS using cellulose as a blank	
Spectra analysis:	
Blank	Cellulose powder
No. of samples	—
Wavelength range (nm)	1000–2500
Wavelength increment (nm)	1·5
No. of points	1500
Calibration:	
Blank	Cellulose powder
No. of samples	30
Wavelength range (nm)	2000–2400
Wavelength increment (nm)	0·5
No. of points	800
Influence of the chemical methods on the calibration	
Blank	Cellulose powder
No. of samples	30
Wavelength range (nm)	2000–2400
Wavelength increment (nm)	0·5
No. of points	800

Chemical methods

Preparation of AIS

This was carried out according to Barbier and Thibault (1982) with some modifications (Forni *et al.*, 1986).

The air-dried material was ground by a hammer mill to pass a 1 mm sieve. Water soluble pectin (WS) and residual protopectin (RP) were extracted from the AIS according to a modification (Forni *et al.*, 1984) of the method of Barbier and Thibault (1982).

The combined enzymatic and HPLC method was used for the evaluation of pectin concentration from their galacturonic acid content (GA) (Forni *et al.*, 1987) and the methoxyl content was determined from measurement of released methanol using gas chromatography as described by Forni *et al.* (1987).

NIRS analysis

A Neotec Spectrocomputer, modified according to the suggestions of USDA Lab. of the Russell Research Institute of Athens, Georgia, USA, was used. It consisted of a modified single beam Cary 14 monochromator connected to a special sample compartment for reflectance with geometry 0°–45° and a PbS detector connected to a photometric unit interfaced with a Micronova MN100 Data General Computer equipped with a twin floppy disk drive.

Spectral data were recorded and stored in the form of $\log 1/R$ (R = relative reflectance) prior to processing according to the procedure shown in Fig. 1.

Thirty fruit AIS samples with a WS–GA ranging from 2·91% to 8·01% RP-GA from 4·23% to 16·29%; WS-Me from 0·49% to 1·13% and RP-Me from 0·91% to 2·00% were used.

Eleven commercial pectins samples ranging in total GA from 58·11% to 71·25% and total Me% from 0·00 to 7·64% were used.

All the analysis and data processing operations were carried out using software kindly given by USDA Russell Research Lab., based on the methods of Norris (1978), Norris and Massie (1981), Kaffka *et al.* (1982) and Norris (1983).

RESULTS AND DISCUSSION

Analysis of fruit AIS using teflon as a blank

The correlation between the spectral data and the chemical data for 30 AIS samples was very poor (Table 2), except in the case of the methanol content of the protopectin.

TABLE 2

Summary of Linear Regression Analyses Relating Data from Chemical Analysis and Value of the 2nd Derivative of log $(1/R)$ Curves at Two Characteristic Wavelengths for 30 AIS Fruit Samples (Teflon as a Blank). A, B: Coefficients of Regression, R: Correlation, SEC: Standard Error of Calibration

	Water soluble pectin		*Insoluble pectin (protopectin)*	
	% GA	*% Me*	*% GA*	*% Me*
λ_1 (nm)	2405	2360	2158	2268
λ_2 (nm)	2224	—	—	—
A	3·22	1·40	0·97	0·52
B	1·82	−53·47	1320·05	151·70
R	0·657	−0·668	0·871	0·955
SEC	0·568	0·085	1·408	0·086

Spectra of pure polysaccharides samples against two different blanks

Figure 2 reports the spectra of pure polygalacturonic acid, starch and cellulose as an example of some AIS components, scanned against teflon powder as a blank.

The characteristic bands of the three polysaccharides overlap complicating the interpretation of spectra from materials containing mixtures of polysaccharides. This problem was previously pointed out by Norris (1978) and by Giangiacomo (1983) as a difficulty in the application of NIR analysis to carbohydrate components.

The spectra obtained by scanning the same polysaccharides using cellulose as a reference blank is shown in Fig. 3. In the polygalacturonic acid spectrum, the large band observed at 2120 nm, with teflon as a blank is now absent, while a band at 2045 nm is apparent.

Analysis of commercial pectins and fruit AIS using cellulose as a blank

Analysis of spectra

The spectra of a group of commercial pectins having different esterification degrees is reported in Fig. 4. A sharp band at 2256 nm is exhibited by the pectins with higher methoxyl contents.

The effect on $\log 1/R$ of dilution of pectins with cellulose is displayed in Fig. 5 where the spectra of different concentrations of pectin mixed with cellulose are reported. The lowest pectin concentration was within the range of concentrations reported for the pectic substances in fruit AIS (Thibault, 1979).

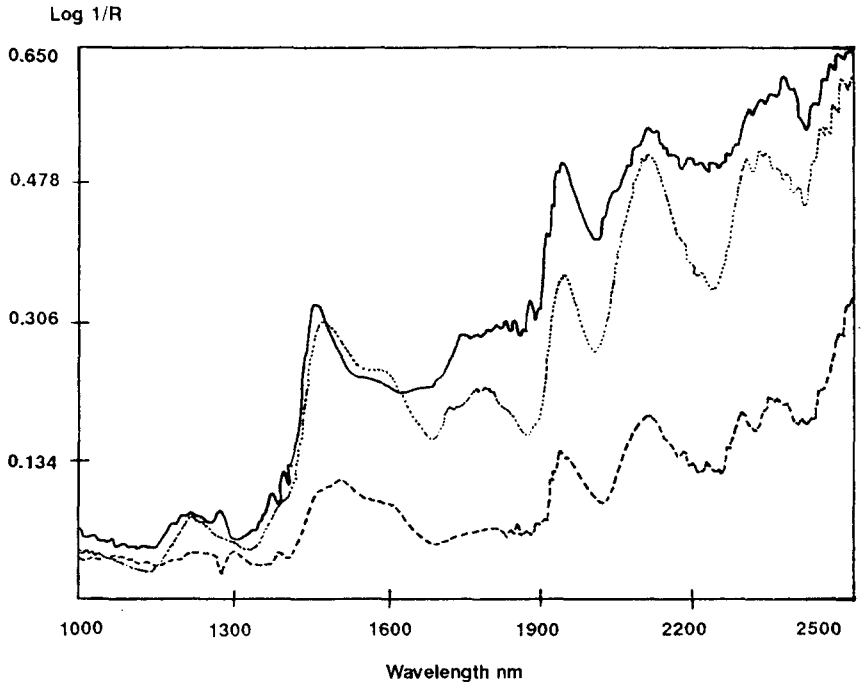


Fig. 2. Spectra of polygalacturonic acid: ———, starch: ····· and cellulose: – – – – scanned against teflon as a blank.

Studies on the calibration curves

Spectra were recorded in the region between 2000 nm and 2400 nm where the most characteristic pectins bands were found by preliminary tests. Moreover in this range no noise was detected. Therefore it was sufficient to record 800 data points, shortening the time required to process the data.

Table 3 reports the coefficients of the regression equation as well as the correlation coefficients R and the Standard Error of Calibration (SEC) according to Norris and Massie (1981).

The best correlation coefficients were achieved for the GA content, with an acceptable correlation coefficient, but a somewhat high SEC. The correlation coefficient for the Me content was low, while the SE was acceptable. The resultant calibration however was much better than that obtained using teflon powder as a blank (Table 2).

The same calibration performed on commercial pectins (where GA and Me content were high) gave rather better results (Table 4), although a small set of 11 samples was used. This stresses the importance of taking into consideration the concentration of the chemical compound to

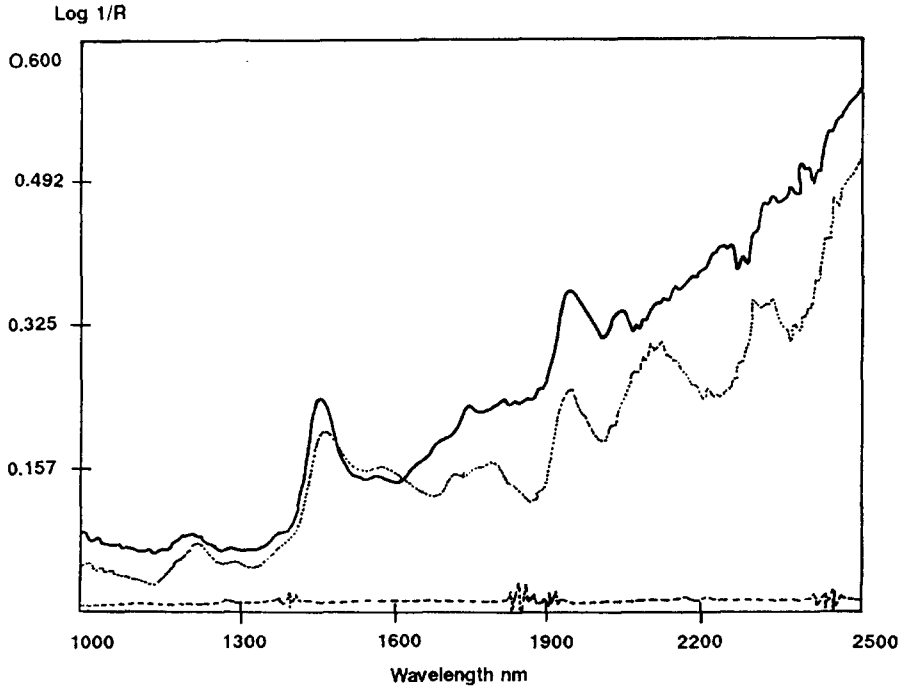


Fig. 3. Spectra of polygalacturonic acid: ———, starch: ····· and cellulose: – – – – – scanned against cellulose as a blank.

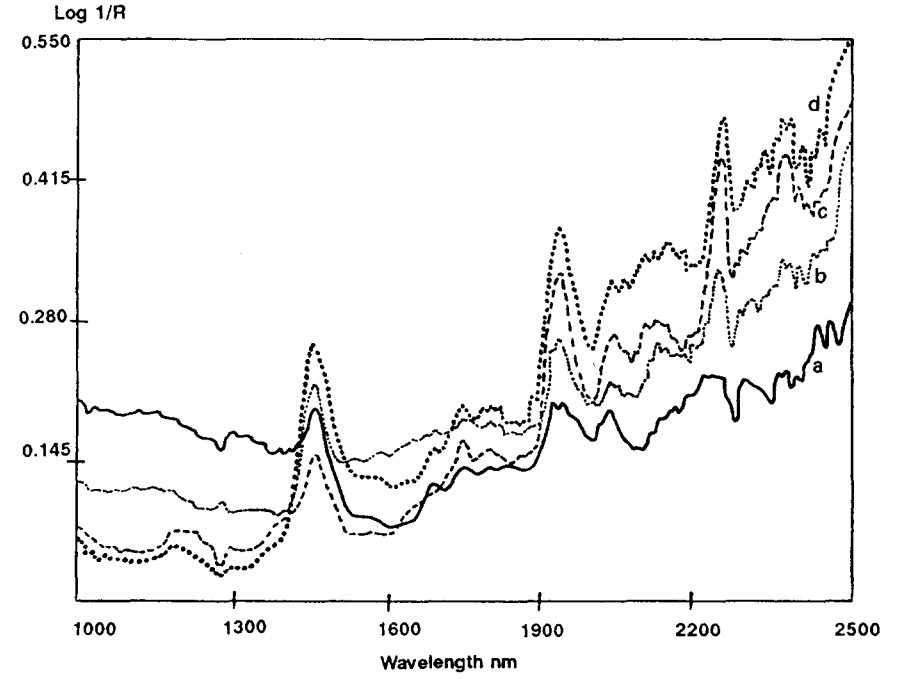


Fig. 4. Spectra of commercial pectins at different degrees of esterification (DE) scanned against cellulose as a blank: a, DE = 10%; b, DE = 38%; c, DE = 65%; d, DE = 75%.

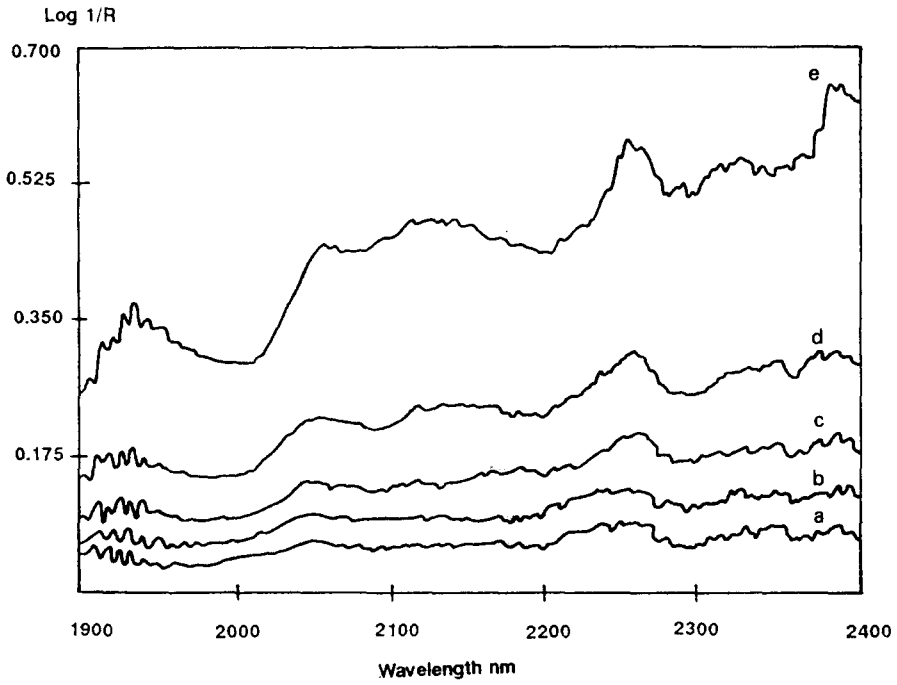


Fig. 5. Spectra of pectins (DE = 65%) and cellulose model mixture scanned against cellulose as a blank: a, 20% pectin–80% cellulose; b, 40% pectin–60% cellulose; c, 60% pectin–40% cellulose; d, 80% pectin–20% cellulose; e, 100% pectin.

TABLE 3

Summary of Linear Regression Analyses Relating Data from Chemical Analysis and Value of the 2nd Derivative of log $(1/R)$ Curves at Two Characteristic Wavelengths for 30 AIS Fruit Samples (Cellulose as a Blank). A, B, R, SEC: see Table 1

	Water soluble pectin		*Insoluble pectin (protopectin)*	
	% GA	*% Me*	*% GA*	*% Me*
λ_1 (nm)	2320	2106	2266	2329
λ_2 (nm)	2254	—	—	2256
A	4·21	−0·11	13·01	1·84
B	3·13	−46·78	−1279·80	0·42
R	0·915	−0·843	−0·915	0·876
SEC	0·466	0·106	1·036	0·111

be estimated when deciding on the number of samples to be included in the calibration.

Influence of the chemical methods on the calibration

Table 5 reports the calibration data obtained using the chemical data obtained by two different methods to evaluate total GA in AIS.

The spectrophotometric data confirmed the greater reliability of the HPLC method compared with the m-hydroxy-diphenyl (MHDP) colori-

TABLE 4
Summary of Linear Regression Analyses Relating Data from Chemical Analysis and Value of the 2nd Derivative of log $(1/R)$ Curves at Two Characteristic Wavelengths for 11 Pectin Samples (Cellulose as a Blank). A, B, R, SEC: see Table 2

	Total pectin	
	% GA	*% Me*
λ_1 (nm)	2190	2257
λ_2 (nm)	2288	—
A	72·25	1·84
B	35·99	93·92
R	0·951	0·975
SEC	2·486	0·633

TABLE 5
Summary of Linear Regression Analyses Relating Data from Chemical Analysis and Values of 2nd Derivative of log $(1/R)$ Curves at Two Characteristic Wavelengths for the GA% in 30 Fruit AIS Samples, Determined with Two Methods (Colorimetric MHDP and HPLC). A, B, R, SEC: see Table 2

	MHDP	*HPLC*
λ_1 (nm)	2080	2171
λ_2 (nm)	2085	2286
A	11·89	19·64
B	33·08	16·09
R	0·672	−0·907
SEC	4·409	0·906

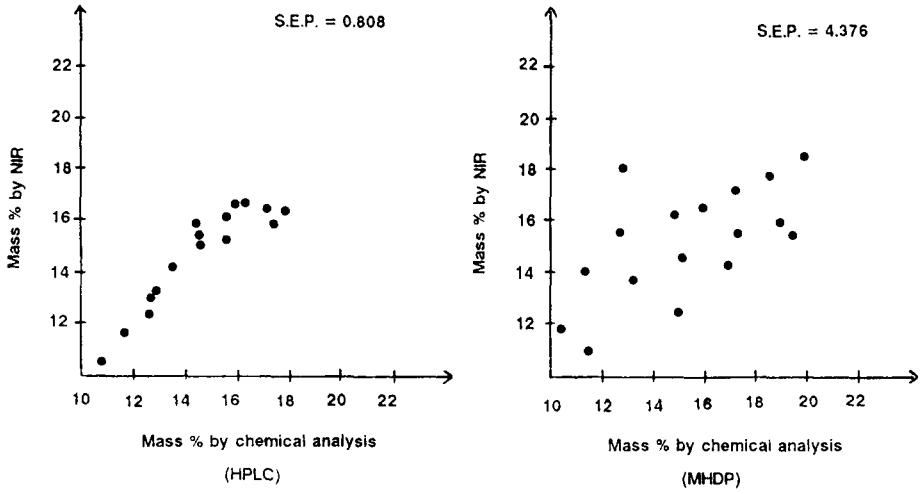


Fig. 6. Relationship between total galacturonic acid content in fruit AIS determined by two methods of chemical analysis and predicted values from the calibration equations.

metric method (Voragen *et al.*, 1983) as earlier reported by Forni *et al.* (1987). This is illustrated in Fig. 6.

CONCLUSION

The application of NIRS to the analysis of the pectic substances in fruit appears to have good prospects when the experimental conditions are optimized.

This preliminary work pointed out some factors influencing the results:

1. The blank used. Using cellulose instead of teflon it was possible to improve the correlation with chemical methods by removing an interfering component.
2. The analytical method chosen to obtain chemical data. The colorimetric MHDP method for galacturonic acid gave a poorer correlation than the HPLC method.
3. The number of samples used in developing the calibration curves depends on the log $1/R$ response of the spectrum, i.e. the concentration of the compounds to be estimated. The calibration obtained with commercial pectins, having a high concentration of galacturonic acid and methanol, were good in spite of the fact that it was made with few samples. In contrast, for fruit AIS, 30 samples gave only a moderately good calibration.
4. The instrument performance. This factor is illustrated by compar-

ing the facilities used by Horvath *et al.* (1984) with ours. Their instrument was able to record 8000 points in the spectrum, while ours just 1700. Therefore Horvath *et al.* (1984) could explore the whole range of the spectrum with precision.

Using 57 samples, Horvath *et al.* (1984) obtained a good correlation for pectins, in spite of the fact that they were estimated by the carbazol method, which we consider is less accurate than the HPLC method used here.

Developing better calibration relationships for the different forms of pectic substances in fruit should be possible with our instrument, using a larger set of samples, with very accurate chemical data.

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