Determination of the esterification degree of the pectin of different origin and composition by the method of infrared spectroscopy

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The change in infrared spectrum of the pectin films of different origin accompanying the transformation of potassium pectinate into potassium pectate was investigated. It was revealed that the intensity ratio of the bands $v_{as}(COO^-)$ at 1608 cm^{-1} and $v(C=O)_{ester}$ at 1745 cm^{-1} corresponding to fully deesterified pectin and methyl pectate, respectively, is a constant independent of the kind of fruit used for the isolation of pectin as well as of the pectin composition. Thus, it has been proved that the method of the determination of the degree of esterification of the pectin by methanol based on the measurement of absorbances in the maxima of these bands can be used for different kinds of pectins. The degree of esterification of pectin (E %) can be determined with absolute error $\pm 1.3\%$.

Исследовались изменения инфракрасного спектра пленок пектина разного происхождения при изменении пектината калия в пектат калия. Доказано, что отношение интенсивностей полос $v_{as}(COO^-)$ при 1608 см$^{-1}$ и $v(C=O)_{эфир}$ при 1745 см$^{-1}$, соответствующих вполне деэстерифицированному пектину или метилпектату, является константой, независимой от сорта плодов, из которых пектин изолирован, и от его состава. Таким образом доказано, что метод определения степени эстерификации пектина метиловым спиртом, основанный на измерении оптических плотностей в максимумах этих полос, можно применить в случае разных сорт пектинов. Степень эстерификации пектина (E %) определена с абсолютной ошибкой $\pm 1,3\%$.

In the preceding study [1] we proved for the samples of apple pectin that the degree of esterification of the carboxyl groups of pectin by methanol could be determined by infrared spectroscopy (KBr pellets) with an error less than $\pm 3\%$ by measuring the absorbance of the bands $v(C=O)_{ester}$ and $v_{as}(COO^-)$ of potassium

pectinates. The aim of this study was to make clear whether this method might be applied to pectins of different origin and composition. In contrast to preceding investigations, the measurements of spectra were carried out with films.

Experimental

Pectin was isolated from different kinds of fruit by fractional extraction according to [2]. The citrus pectin (Genu Pectin; Københavns Pektinfabrik, Denmark) was used as a standard preparation.

The content of polygalacturonic acid and the degree of esterification of carboxyl groups by methanol were estimated alkalimetrically [3]. The average molecular weight of pectin was determined viscometrically [4].

Procedure

The pectin films prepared as published earlier [5] (10—12 of each kind) were transformed into pectinic acid by dipping into 0.1 M-HCl in 70% ethanol for 30 min. Subsequently, they were submerged into a 0.3 M solution of potassium acetate in 70% ethanol (pH 6.0) for 30 min to give the films of potassium pectinate which were washed with 70 and 96% ethanol for 5 min and dried for 1 h at 50°C. Their alkaline deesterification with 0.05 M-KOH in 70% ethanol at 5°C taking one hour yielded the films of potassium pectate which were washed and dried, as described above.

The spectra of potassium pectinate and pectate were measured in the region of 1500—1950 cm^{-1} always with the same nondamaged film using a spectrometer UR-20 with an external recorder N1 B 102a. The absorbances of the bands $\nu_{as}(COO^-)$ 1608 cm^{-1} and $\nu(C=O)_{ester}$ 1745 cm^{-1} were determined by the base-line method. The base line was drawn through the section of absorption in the region of 1950—1800 cm^{-1} parallel with the abscissa.

Results and discussion

The basis of the method of infrared spectroscopy for determining the degree of esterification of the carboxyl groups of pectin by methanol is the determination of the absorbance ratio (dA) of the bands $\nu(C=O)_{ester}$ and $\nu_{as}(COO^-)$ at 1745 and 1608 cm^{-1}, respectively. The value of dA increases with the increasing degree of esterification of pectin and *vice versa*.

On the basis of a detailed analysis of the spectra of pectin carried out earlier [1], we derived the following equation

$$dA = \frac{A_{1745}}{A_{1608}} = \frac{C e + k(1-e)}{B(1-e)+m} \tag{1}$$

expressing the relationship between the absorbance ratio A_{1745}/A_{1608} and the degree of esterification of pectin (e). The symbols B, C, m, and k stand for the absorbance coefficients of the bands corresponding to $v_{as}(COO^-)$ (1608 cm^{-1}), $v(C=O)_{ester}$ (1745 cm^{-1}), $\delta(H_2O)$ (1608 cm^{-1}) and (1745 cm^{-1}), respectively. The spectra of deuterated samples of pectic acid, potassium pectate, and methyl pectate have shown that the absorption $\delta(H_2O)$ at 1608 cm^{-1} is a constant quantity independent of pectin composition while the absorption $\delta(H_2O)$ at 1745 cm^{-1} appears only with the salts of pectic acid and pectinic acids.

The absolute values of the coefficients B, C, m, and k are not known. But their relative values B/C, m/C, and k/C are easy to be determined.

Hence, eqn (1) may be written in the form

$$dA = \frac{e + \dfrac{k}{C}(1-e)}{\dfrac{B}{C}(1-e) + \dfrac{m}{C}} \tag{2}$$

A series of pectin samples with different degree of esterification (e), different content of polygalacturonic acid (UA), and average molecular weight $\bar{M}_n$ was prepared by extraction from different kinds of fruit (Table 1). These pectin samples were used for the preparation of films of potassium pectinate and pectate and the absorbance of the bands at 1608 and 1745 cm^{-1} was determined. (During the

Table 1

Ratio of the absorbance coefficients B/C of the $v_{as}(COO^-)$ and $v(C=O)_{ester}$ bands for pectins of different origin and composition

Source of pectin	UA %	e	$[\eta]$ dl g^{-1}	$M\eta \cdot 10^3$	B/C	$\Delta(B/C)$
Apricot	42	0.46	3.62	61	1.61	-0.04
	53	0.59	5.84	87	1.70	$+0.05$
	56	0.57	3.80	63	1.61	-0.04
	57	0.60	2.80	50	1.70	$+0.05$
	60	0.66	8.04	111	1.62	-0.03
Sour cherry	59	0.30	3.90	65	1.65	0.00
	69	0.51	2.80	50	1.61	-0.04
Cherry	67	0.52	2.48	46	1.67	$+0.02$
Pear	69	0.75	4.30	69	1.65	0.00
Quince	70	0.65	2.82	51	1.61	-0.04
Apple	71	0.52	1.12	25	1.72	$+0.07$
Citrus	78	0.68	—	—	1.66	$+0.01$
				Average	1.65	

preparation of potassium pectinate no hydrolysis of the methyl esters of carboxyl groups was observed.)

On transforming the film of potassium pectinate into potassium pectate, the absorbance A_{1608} increases proportionally to the number of the —COO^- groups which have been formed during the alkaline hydrolysis of the methyl esters of carboxyl groups. Simultaneously, the absorbance A_{1745} decreases proportionally to the number of the hydrolyzed —$COOCH_3$ groups. The ratio of the absorbance A_{1608} increase to the absorbance A_{1745} decrease during the transformation of pectinate into pectate, therefore, represents the ratio of the absorbance coefficients B/C corresponding to fully deesterified pectin and methyl pectate, respectively. With each pectin sample the value of B/C was determined for the same film. The mean quadratic error of one analysis did not exceed $\sigma = 0.08$ and the coefficient of variation was $w = 4.5\%$. As obvious from Table 1, the deviations of the values of B/C from their average value do not exceed the quantity σ. The maximum of the dispersion of values is within the range 2σ. Therefore, the ratio of the absorbance coefficients B/C of the bands $v_{as}(COO^-)$ and $v(C=O)_{ester}$ may be justifiably regarded as a quantity independent of pectin origin and composition. This fact proves that the method of infrared spectroscopy may be used for different kinds of pectin.

On rounding off and respecting the error of analysis, we have put $B/C = 1.7$. Similarly, we have also determined the coefficients $m/C = 0.06$ and $k/C = 0.013$. By inserting these values into eqn (2), we get

$$dA = \frac{e + 0.013(1-e)}{1.7(1-e) + 0.06} \qquad (3)$$

and after rearranging and rounding off the coefficients, we obtain the simplified form

Table 2

Titrimetric and spectroscopic determination of the degree of esterification (E) of carboxyl groups by methanol for different samples of apple pectin

UA %	E % determined		ΔE
	titrimetrically	spectroscopically	
90.5	16.0	16.8	+0.8
51.7	26.0	25.5	−0.5
90.5	26.5	25.5	−1.0
57.6	32.0	32.8	+0.8
87.0	43.0	42.2	−0.8
54.6	50.0	51.3	+1.3
46.0	76.0	74.5	−1.5

$$e = \frac{1.8\ dA}{1.7\ dA + 1} \qquad (4)$$

For the degree of esterification of pectin E in %, it holds $E = e\ 100$.

In order to verify the validity of the method, we determined the degree of esterification E in a series of samples of apple pectin the degree of esterification of which had been ascertained titrimetrically [3]. The samples were widely different even in the content of polygalacturonic acid found in dry matter (Table 2). The maximum difference between two parallel spectral analyses did not exceed 2.5% (absolute value). The results given in Table 2 show that the differences between titrimetric and spectroscopic determination are only low. They are in the range of experimental errors.

The above results demonstrate that the method of infrared spectroscopy is suited for the determination of the degree of esterification of the pectin carboxyl groups by methanol and may be used for arbitrary kind of pectin.

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