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PECTIC SUBSTANCES OF CHERRY FRUITS

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Abstract—Pectic substances from cherry fruits (*Prunus avium*) were studied after extraction successively by water, oxalate, hot dilute HCl and cold dilute NaOH. Each of the fractions contained mainly galacturonic acid and arabinose, but also galactose and rhamnose. The degree of methylation was zero for the alkali-soluble pectic substances and high (> 57.2%) for the other fractions. Heterogeneity in MW distribution as well as in charge was checked by gel filtration on Sepharose CL-2B and DEAE-Sepharose CL-6B, respectively, while their MWs were determined by viscosity measurements.

INTRODUCTION

Pectic substances are polyuronides composed mainly of 1,4-linked α-D-galacturonic acid (or its Me ester) with neutral sugars, typically galactose and arabinose, as side-chains [1]. They are the major components of the primary cell-walls and of the middle lamella of plant tissues [2]. Fruits are particularly rich in pectic substances, where they have been associated with the texture of fresh and processed products [3, 4].

Studies on pectic substances and pectolytic enzymes from cherry fruits (Montmorency cv *Prunus cerasus*) have been carried out for their impact on the firmness during brining [5], bruising [6, 7] or freezing [8]. The pectic substances of the cultivar Bigarreaux Napoléon (*Prunus avium*) have not been studied in spite of the importance of this cultivar and only data concerning their general characteristics are available in the literature [9]. This variety is used in France for the preparation of canned or crystallized fruits but not in jam manufacture.

This preliminary study describes the sequential extraction of pectic substances from Bigarreaux Napoléon cherry fruits and some of their physico-chemical properties.

RESULTS

Sequential extraction

The yield of 80% alcohol-insoluble-residue (AIR) (Table 1) is 1.5% of fresh stoned fruits and the AIR content of pectic substances is 53.8%. These values lead to a pectin content of 0.8% for the fresh stoned fruit.

The use of water, oxalate, hot 0.05 M HCl and cold 0.05 M NaOH, in this order, provides a fractionation of pectic substances (Table 1). These substances are composed mainly of acid-soluble material (HP, 38.1%); water-soluble (WSP), oxalate-soluble (OXP) and alkali-soluble (OHP) materials appeared in similar amounts (*ca* 20%).

Composition

The chemical composition of the pectic substances is shown in Table 2. The anhydrogalacturonic acid content varies from 34.7% in the OHP to 63.5% in the OXP. Total neutral sugars (expressed as anhydroglucose) are *ca* 20% for the OXP, HP and OHP and *ca* 34% for the WSP.

The pectic fractions are composed of the same neutral sugars, as determined by GC: rhamnose, fucose, ribose, arabinose, xylose, mannose, galactose, glucose and the rare sugar, 2-desoxyglucose. These sugars occur in different quantities: arabinose is the most important one (13.9–42.8% of total carbohydrates), the galactose content is much lower (2–8.5% of the total carbohydrates) and the other sugars are present in very low amounts. The content of Me pentoses is in the range 0.7–1.6%.

The degrees of methylation, calculated from the OMe and the anhydrogalacturonic acid contents, are also shown in Table 2. It must be emphasized that the OHP are not methylated whereas the three other pectic fractions proved to be highly methylated (degree of methylation of 65.0%, 57.2%, 66.4% for the WSP, OXP and HP, respectively).

The protein contents vary in the pectic fractions from 8.0% (OXP) to 36.8% (OHP).

Viscometric characteristics

Values of intrinsic viscosities, Huggins coefficients and viscosity-average MWs are given in Table 3. The viscometric characteristics of the OHP were not determined because of their insolubility in the 0.155 M NaCl medium used for the measurements.

Viscosity-average MWs are *ca* 80000 for the OXP and WSP and *ca* 64000 for the HP. The OXP are characterized by a high value (1.20) of the Huggins coefficient.

Ion-exchange chromatography

The pectic substances were chromatographed on DEAE-Sepharose CL-6B (Fig. 1) in order to separate the pectic substances into neutral and acidic molecules. Therefore it was possible to estimate purity and charge

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Table 1. Alcohol-insoluble residue and pectic substances extracted from cherry fruits

Alcohol-insoluble residue*	1.5	
Total pectic substances†	53.8	
WSP		13.0 (24.1)
OXP		10.2 (19.0)
HP		20.5 (38.1)
OHP		10.1 (18.8)

* Dry material, % fr. wt of stoned cherry fruits.

† Total pectic substances are the sum of the dry wt of pectic substances extracted with water (WSP), oxalate (OXP), hydrochloric acid (HP) and sodium hydroxide (OHP), expressed as per cent of dry alcohol-insoluble residue. Values in parenthesis correspond to % total pectic substances.

distribution of the pectic substances. The chromatographic recoveries varied in the range 89–98%.

The materials not bound to the gel in 0.05 M acetate buffer, pH 4.8, contain only neutral sugars. As a consequence, they represent the neutral polysaccharides which are not covalently linked to the pectic backbone. The contaminating neutral polysaccharides represent 8.5%, 1%, 0.8% and 7.5% (as anhydroglucose) of the dry wt of WSP, OXP, HP and OHP, respectively.

The gradient in acetate (0.05–0.8 M) at pH 4.8 leads to the elution of pectic substances. All of them also contain neutral sugars which therefore are covalently linked to the pectic backbone. The OXP, HP and OHP are eluted in a single peak of galacturonic acids and neutral sugars. The HP are characterized by a constant ratio of neutral sugars/galacturonic acids of 0.45 (Fig. 1); this ratio is not constant for the OXP (Fig. 1). The OHP presents a very large peak, chemically heterogeneous which is eluted by an ionic strength of 0.76 (Fig. 1). The WSP are eluted in two peaks (Fig. 1). The first peak eluted by an ionic strength of 0.25 corresponds to pectic polysaccharides very rich in neutral sugars and poor in galacturonic acids (neutral sugars/galacturonic acids = 5–17) which seem to be highly methylated. The second one is composed mainly of galacturonic acids (neutral sugars/galacturonic acids = 0.55–0.70).

Table 3. Viscometric characteristics and viscosity average-molecular weights of pectic substances extracted from cherry fruits with successively water (WSP), oxalate (OXP) and hydrochloric acid (HP)

	Pectic substances		
	WSP	OXP	HP
Intrinsic viscosity (dl/g)	5.33	5.38	4.04
Huggins coefficient	0.64	1.20	0.48
Viscosity average MW ($\bar{M}_v$)	81 500	82 000	63 700

Gel permeation chromatography

Figure 2 shows the elution profile of the pectic substances obtained on Sepharose CL-2B eluted with 0.1 M acetate pH 4. The recoveries vary between 93 and 110%.

No pectic material could be detected at the total volume of the column. The peak at the void volume is always present in varying extents. Except for the OXP (Fig. 2), the pectic substances are chemically heterogeneous, as indicated by the fact that the neutral sugars/galacturonic acids ratio is not constant over the fractionation range. The material at the void volume is generally richer in

Table 2. Composition and degree of methylation of pectic substances extracted from cherry fruits successively with water (WSP), oxalate (OXP), hydrochloric acid (HP) and sodium hydroxide (OHP)

	Pectic substances			
	WSP	OXP	HP	OHP
Galacturonic acid*	39.3 (1.5)†	63.5 (2.2)	53.2 (3.2)	34.7 (1.3)
Rhamnose + fucose	0.9	1.0	1.6	0.7
Ribose	trace	0.1	0.1	0.7
Arabinose	37.6	12.4	14.0	7.0
Xylose	0.7	0.3	0.5	1.0
2-Desoxyglucose	0.6	0.3	0.7	0.2
Mannose	0.4	0.3	0.4	0.6
Galactose	7.5	1.6	1.6	4.3
Glucose	0.9	0.9	0.9	1.2
Total neutral sugars‡	34.2 (3.2)	21.0 (3.2)	22.0 (1.4)	23.4 (3.3)
Proteins ($N \times 6.25$)	12.4	8.0	19.3	36.7
Degree of methylation	65.0 (2.8)	57.2 (1.8)	66.4 (2.9)	0

Results are expressed as % dry wt.

* Sugars expressed as anhydrosugars.

† Values in parenthesis are s.d. (12 determinations).

‡ Determined by the orcinol method as anhydroglucose.

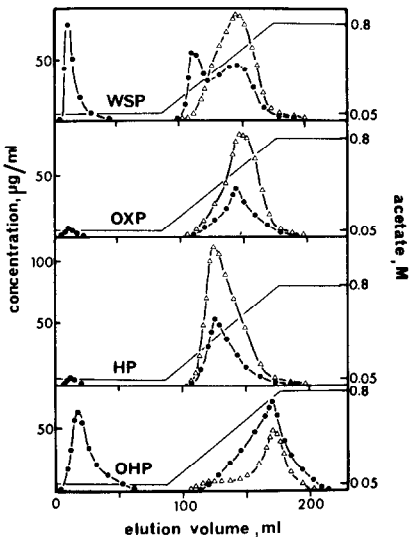

Fig. 1. Ion-exchange chromatography of pectic substances from cherry fruits on DEAE-Sepharose CL-6B (7.5 × 1.6 cm). After sample loading (10 mg), the column was washed with 4 column vol. of 0.05 M acetate buffer, pH 4.8. Bound material was eluted by a linear acetate gradient at pH 4.8 (0.05–0.8 M; 90 ml). Fractions (3 ml) were assayed for neutral sugars (—●—) and galacturonic acids (—△—) by the orcinol method and the *m*-hydroxydiphenyl method, respectively. WSP = water-soluble pectic substances; OXP = oxalate-soluble pectic substances; HP = acid-soluble pectic substances; OHP = alkali-soluble pectic substances.

neutral sugars than in galacturonic acids: the ratio neutral sugars/galacturonic acids is *ca* 2 for the WSP, OXP and OHP and *ca* 1 for the HP. The material eluted in the fractionation range of the column is characterized by a wide galacturonic acids peak with shoulders and by two neutral sugars peaks. The neutral sugars peaks near the total volume of the column correspond to the tails of the galacturonic acids peak. Therefore, the heterogeneity of the pectic substances is greater in the smallest MW range. The elution profile of the OXP is characterized by only two peaks in neutral sugars and in galacturonic acids which are quite well resolved: a peak at the void volume with a constant neutral sugars/galacturonic acids ratio of 2 and a peak eluted at a volume of *ca* 380 ml with a neutral sugars/galacturonic acids ratio of *ca* 0.5.

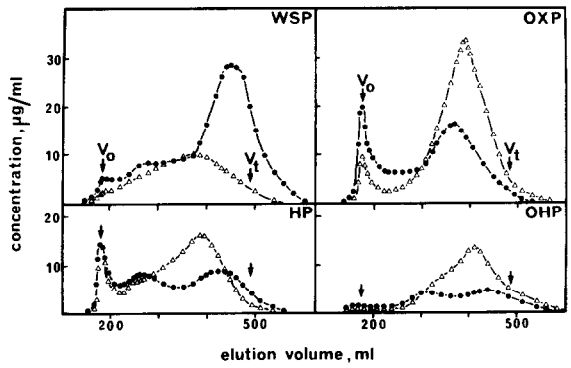

Fig. 2. Gel-permeation chromatography on Sepharose CL-2B (86 × 2.6 cm) of pectic substances from cherry fruits. Samples (10 mg) were injected on the column eluted by 0.1 M acetate buffer pH 4. Fractions (5 ml) were assayed as described in the legend of Fig. 1: —●— neutral sugars, —△— galacturonic acids. V_o and V_t are void volume and total volume of the column, respectively. WSP = water-soluble pectic substances; OXP = oxalate-soluble pectic substances; HP = acid-soluble pectic substances; OHP = alkali-soluble pectic substances.

DISCUSSION

The extraction of pectic substances from cherry fruits was carried out on an AIR. The yield in AIR (1.5 %) is very similar to that (1.7 %) reported by Kawabata [9].

From an AIR, the pectic substances are usually obtained by a sequential extraction with (1) water, (2) oxalate, ethylenediaminetetracetate (EDTA) or hexametaphosphate and (3) hot 0.05 M HCl or cold 0.05 M NaOH, which provides [1] free high-methoxyl pectins, free low-methoxyl pectins or pectates, and protopectin (the insoluble parent of the soluble pectic substances), respectively. Nevertheless, Timmers [10] fractionated the AIR in pectic material and a residue containing hemicellulose and cellulose by a mixture of cold 0.05 M NaOH and 5 mM EDTA.

Van Buren [5] extracted from sweet cherries (*Prunus cerasus*) 20 % of the total pectic substances by water, 29.8 % by hexametaphosphate and 50.2 % by hot 0.05 M HCl. Kawabata [9] obtained from cherries (*Prunus avium*) 16.9 % of water-soluble, 42.6 % of hexametaphosphate-soluble and 40.5 % of hot 0.05 M HCl soluble pectic substances. These figures differ from our results.

The WSP are contaminated by proteins (12.4 %) and by neutral polysaccharides (8.5 %). They are characterized by a high degree of methylation (65 %), a low content of anhydrogalacturonic acids (39.3 %) and a high content of total neutral sugars (34.2 %).

The OXP are highly methylated (degree of methylation 57.2 %). They are characterized by their homogeneous behaviour on gel filtration or ion-exchange chromatography, with a minor contamination by neutral polysaccharides (1 %). These features justify their specific extraction. Different hypotheses can be postulated concerning their extraction by oxalate in spite of their high degree of methylation. One of them could be a blockwise distribution of the methoxyl groups in the pectin which could promote their association with divalent ions such as Ca, even at a degree of methylation up to 60 % [11]. The very high value of the Huggins coefficient (1.20, Table 3) which is usually interpreted as a sign of aggregation [12], would reinforce this hypothesis. However, a generalization should not be made on the basis of this explanation. For instance, pectic substances extracted from apricots with oxalate [13] are quite highly methylated (degree of methylation = 93 %). Another hypothesis can be found in a non-specific role of oxalate towards divalent ions. Salts such as NACl or Na_2SO_4 were reported to be as effective as EDTA or oxalate or polyphosphate for the extraction of pectin from apple marcs [14].

The HP are usually considered as protopectin. Linkages between these substances and other cell-wall constituents are split in a mild acid medium and particularly those of arabinose, fucose and rhamnose [15]. Hot dilute acid would also hydrolyse to a lesser

extent some esters of galacturonic acids and some linkages between galacturonic acid residues [4]. However, the uronic backbone of the pectic substances seems to be stable, as shown be gel filtration (Fig. 2), by viscometry measurements (Table 3) and by their high degree of methylation (66.4%). The acid-stability of the pectic substances is well-known since commercial pectins (protopectins) with high gelling power are obtained by acid treatment from by-products as apple marcs or citrus peels [16, 17]. The HP are characterized by a minor neutral polysaccharide contamination (0.8%) and by a homogeneous distribution in respect to charge and chemical composition (Fig. 1).

The protopectin was not completely extracted by hot dilute HCl since the additional use of cold dilute NaOH extracts another part of the pectic substances (18.8% of total pectic substances). Similar results were found for the tomato pectic substances [18]. The OHP are not methylated, which could explain their insolubility in the 0.155 M NaCl medium necessary for the viscosity measurements. Saponification of this fraction was possible during the extraction since cold dilute alkali can hydrolyse the ester groups of the pectins rather than depolymerize the pectic backbone by β-elimination [4]. Dilute alkali can also split linkages between sugars and amino-acids such as serine [19]. The OHP are characterized by a low galacturonic acid content (34.7%) and a high protein content (36.7%). The contaminating neutral polysaccharides represent 7.5% of the fraction and may be considered as alkali-soluble hemicelluloses [20].

All the pectic substances have some common features. Gel filtration on Sepharose CL-2B (Fig. 2) showed that they have large hydrodynamic volumes and a wide MW distribution. The excluded material, i.e. of the highest MW, is richer in neutral sugars than in galacturonic acids. The same sugars can be identified in the pectic substances: fucose, rhamnose, arabinose, xylose, mannose, glucose, galactose which are frequently found in the pectic substances [1, 21] and the rare sugar 2-desoxyglucose. Galactose and arabinose are known to form side-chains with pectic substances while rhamnose is found as a kink in the polygalacturonic backbone [22, 23]. From the contents of fucose + rhamnose (Table 2), it can be calculated that, in the cherry pectins, there are at the most 3.7 kinks per 100 galacturonic acid residues.

EXPERIMENTAL

Fruits. Ripe cherry fruits (*P. avium* L., cv Bigarreaux Napoléon) were harvested at Station d'Arboriculture Fruitière (INRA Bordeaux, France) in June 1979. Immediately after harvesting, they were stoned, frozen and stored at −20°.

Preparation of alcohol-insoluble residue (AIR). The frozen and stoned cherry fruits (aliquots of *ca* 1.4 kg) were ground for 2–3 min, in 5 vol. of hot 95% EtOH. The slurry was washed with 80% EtOH until the eluate became colorless. The AIR was then dried at 40° and sieved through a 0.5-mm screen by gentle grinding.

Extraction of pectic substances. Pectic substances were extrd from the AIR in four fractions. Each extraction step was repeated ×3.

An aliquot of AIR (*ca* 9 g) was dispersed at room temp. by magnetic stirring in 500 ml of H_2O during 30 min. The slurry was centrifuged at 3000 *g* at room temp. and the supernatant (H_2O-sol. pectic substances, WSP) was collected. 300 ml of 1% K oxalate at pH 4.5 was added to the residue at room temp. The slurry was stirred for 30 min and centrifuged as above. The oxalate-sol. pectic substances (OXP) were collected. 0.05 M HCl (300 ml) was added to the remaining residue. The mixture was heated for 30 min at 100°, centrifuged as above and the acid-sol. pectic substances (HP) were collected. Finally, cold 0.05 M NaOH (300 ml) was added to the residue; the slurry was stirred 30 min at 4°, centrifuged at 4° and 3000 *g* and the extrd material (OHP) was collected, the pH being immediately adjusted to pH 4.5 using 0.1 M HCl.

The four fractions were concd in a rotary evaporator at a temp below 40°. The pectic substances were then pptd by addition of 4 vol. of 95% EtOH, solubilized in H_2O and finally freeze-dried.

Analyses. The anhydrogalacturonic acid and total neutral sugars (expressed as anhydroglucose) were determined automatically by the *m*-hydroxydiphenyl method [24] and the orcinol method [25] after correction for interferences from galacturonic acids, respectively. Degrees of methylation were calculated from the OMe content [26] and the anhydrogalacturonic acid content. N determinations were made by the Kjeldahl method and protein contents were estimated by multiplying the N content by 6.25 [27]. Neutral sugars were determined by GC [8]. Samples (*ca* 50 mg) were hydrolysed with TFA (2 M, 120°, 1.5 hr) [29]. Values are on a dry wt basis.

Viscosity measurements. Intrinsic viscosity ($[\eta]$, dl/g) values were obtained at 25° with an Automatic Fica Viscometer (solvent flow time = 279.58 sec). Pectic substances were dissolved in 0.155 M NaCl and 5 mM EDTA and the viscosity-average MWs ($\bar{M}_v$) were calculated [30].

Gel chromatography. Sepharose CL-2B (Pharmacia) was used for gel permeation chromatography. The column (86 × 2.6 cm) was eluted in an ascending direction with 0.1 M NaOAc buffer pH 4 (ionic strength = 0.1) at 20 ml/hr. Samples of pectic substances in the same buffer (up to 10 mg) were injected and 5 ml fractions were collected.

Ion-exchange chromatography was performed on DEAE-Sepharose CL-6B (Pharmacia) columns (7.5 × 1.6 cm) equilibrated by 0.05 M NaOAc buffer pH 4.8 (ionic strength = 0.05). Pectic substances (up to 10 mg) were loaded onto the column and the gel was washed with four column vols of 0.05 M NaOAc buffer at 40 ml/hr. The bound material was then eluted by a linear NaOAc gradient at pH 4.8 (0.05–0.8 M; 90 ml). Fractions of 3 ml were collected.

Fractions were analysed for their galacturonic acids and neutral sugars contents by the *m*-hydroxydiphenyl and orcinol methods, respectively. Chromatograms were recalculated for the 10 mg of material injected onto the column.

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