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Comparative spectroscopic characterization of different pectins and their sources

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Abstract

The results of atomic absorption spectrometry (AAS) analyses and Fourier transform infrared (FTIR) spectroscopic studies of several pectins obtained from pumpkin and sugar beet, as well as of their vegetable sources, are compared and discussed. Special emphasis is put on the state of carboxylic groups of the polymer backbone and the mineral composition of both the sources and the resulting pectins, including the content of alkaline (Na, K) and alkaline-earth metals (Mg, Ca), as well as traces of heavy metals (V, Fe, Cu, Pb). The pectins were obtained from dried pumpkin pulp by extraction with dilute hydrochloric acid or using a biotechnological process involving the multi-enzyme cell-free culture supernatant from the bacterium *Xanthomonas campestris*; commercial sugar beet pectin extracted by the standard method of acid treatment was obtained from a sugar beet processing plant in Krasnodar (Russia). For comparison, a sample of commercial acid-extracted citrus pectin (Copenhagen, Denmark) was also studied. The results obtained show that potassium seems to occur as a relatively free constituent, whereas a more specific interaction between sodium ions and pectic substances may be assumed depending on the origin of the pectin and obviously on its properties. Much higher amounts of Mg and, especially, Ca found in pumpkin biopectin as compared to all of the three pumpkin, sugar beet and citrus acid-extracted products correlate with a relatively well exhibited capability of pectins to bind these two cations, which is noticeably suppressed in acidic media. The increased content of Ca (and, probably, Mg) may in principle contribute to poorer gelling properties of pumpkin pectin and, in general, of biopectins as compared to the corresponding acid extracts. The results on the mineral fraction of the samples are compared considering the FTIR spectroscopic data for the pectins studied as well as for their sources featuring, in particular, the state of carboxylic groups responsible for metal binding. It has also been found that lead and copper essentially accumulate in pectins upon extraction, whereas iron does not, being relatively more weakly bound by pectic substances (which may, however, depend on its oxidation state) than other heavy metals; the accumulation process is slightly (for Fe and Pb) or not at all (for Cu) suppressed during acid extraction. Comparing the content of vanadium in the pectins and their sources, it may be concluded that this element occurring in plant tissue obviously in different chemical forms may be partly transferred to pectin during its extraction in a proportion similar to that in which it is bound to pectic substances in the plant cell wall, thus indicating its strong binding not affected by acid treatment.

1. Introduction

Pectic substances occurring as structural cementing elements of fruit and vegetable cell walls comprise a diversity of natural carboxypolysaccharides of variable composition which have a common structural fragment of a polygalacturonic acid chain. Thus, structurally, pectins are α(1–4)-linked polygalacturonans with varying lengths of the chain (and therefore essentially different molecular masses) and periodic insertion of rhamnosyl residues, as well as with a number of other possible structural differences (e.g. the degree of methyl

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esterification of carboxylic groups and acetylation of hydroxyls; the presence, length and branching of neutral sugar side chains, etc.) giving a wide range of types (King, 1993; Pilnik, 1990; Selvendran, 1985).

The industrially employed process of pectin extraction from vegetable sources involves relatively severe acid treatment (King, 1993; May, 1990); an alternative method is enzymic extraction using the supernatant from microbial cultures (Matora et al., 1995; Sakai & Okushima, 1980; Zhemerichkin & Ptitchkina, 1995).

Optimal utilization of industrially extracted pectin preparations (May, 1990) requires their physicochemical behaviour in solutions and gels to be determined. In their turn, physicochemical properties of pectins are known to vary widely depending on the source (fruits or vegetables) from which they have been extracted, as well as on the extraction method and conditions applied, which is evidently connected with certain differences in the structure and composition of the resulting pectin macromolecules (Kravtchenko, Voragen, & Pilnik, 1992; May, 1990; Matora et al., 1995; Ptitchikina, Danilova, Doxastakis, Kasapis, & Morris, 1994; Sakai & Okushima, 1980; Zhemerichkin & Ptitchkina, 1995).

An essential role in obtaining the latter characteristics is played by various spectroscopic techniques, including infrared spectroscopy providing valuable analytical and structural information. The advantages of the application of Fourier transform methods, in particular, to mid-infrared spectroscopy (ca. 4000–400 cm^{-1}), which has brought about an essential improvement in instrument performance and renewed interest in the potential of the mid-infrared spectroscopy for food analysis, have recently been overviewed (Wilson, 1990; Wilson & Goodfellow, 1994). A number of excellent examples of identification, authentication and structural investigation of various food constituents and products, including pectins and other polysaccharides, using Fourier transform infrared (FTIR) and conventional infrared (IR) spectroscopy have also appeared in the last several years (Belton et al., 1995; Filippov, 1992; Lai, Kemsley, & Wilson, 1994; Zhbankov, 1992).

Pectins obtained from non-traditional vegetable sources available in Russia (pumpkin and sugar beet) using both of the above two extraction methods have recently been studied from the viewpoint of their monosaccharide composition, physicochemical properties and structure (Kamnev, Ptitchkina, Perfiliev, Shkodina, & Ignatov, 1995a; Kamnev, Ptitchkina, Ristić, Shkodina, & Ignatov, 1995b; Matora et al., 1995; Ptitchkina et al., 1994; Zhemerichkin & Ptitchkina, 1995). In the present communication, our FTIR spectroscopic data and the results of flame (FAAS) or graphite furnace atomic absorption (GFAAS) analyses of the mineral composition (including the content of several heavy metals) are compared for pumpkin and sugar beet pectins (and also for commercial citrus pectin used for comparison) and their sources (Table 1). The data obtained concerning the content of a series of alkaline, alkaline-earth and heavy metals in the above samples of pectins are discussed considering the effect of the extraction method.

2. Experimental

2.1. Materials

Pectins were obtained from pressed dry pumpkin pulp by extraction with dilute acid (Matora et al., 1995; May, 1990) (0.1 M HCl, 1:10 w/w, 2 h, 65°C) or with the multi-enzyme culture supernatant from the bacterium *Xanthomonas campestris* by the general procedure described in detail elsewhere (Matora et al., 1995; Zhemerichkin & Ptitchkina, 1995). The bacterium was cultivated in a medium containing (in g/l): NH_4Cl (1.0), KH_2PO_4 (2.0), $MgSO_4 \cdot 7H_2O$ (0.2), $CaCO_3$ (0.1), partially depectinated pumpkin pulp (10), with further separation of the cell-free supernatant broth by centrifugation. For enzymic extraction of pectin, the weight ratio of the dry pressed pumpkin pulp to the supernatant was applied to be 1:15. The extract was separated by centrifugation, washed in bidistilled water, centrifuged again, and pectic substances were precipitated with two volumes of 96% ethanol. The precipitated pectin was collected by filtration, washed with acidified aqueous alcohol (10 ml HCl in 1 l of 70% v/v ethanol), washed again with pure ethanol, pressed, dried in a flow of warm air (30–40°C), ground and sieved. Other details of the preparation procedures are presented elsewhere (Matora et al., 1995; Ptitchkina et al., 1994; Zhemerichkin & Ptitchkina, 1995). Commercial sugar beet and citrus pectins (the latter was used for comparison in the present study), both extracted by the standard acid treatment method, were obtained from Krasnodar, Russia, and Copenhagen, Denmark, respectively (see Table 1). Sucrose (analytical grade) used for comparison in FTIR spectroscopic measurements was purchased from SERVA.

2.2. Methods

Metal cations were determined in the above pectins and vegetable sources after digestion of precisely weighed samples (ca. 20 mg of dry mass with 1 ml concentrated nitric acid of special purity and 6 ml deionized water, 1 h, 110°C) in a Parr Acid Digestion Bomb No. 4745 (total volume 23 ml, Parr Instruments Company) with further dilution up to 25 ml by flame (FAAS; acetylene–air flame) or, for lowest metal concentrations, graphite furnace atomic absorption spectrometry (GFAAS) using a Perkin–Elmer spectrometer (Model 3110), a graphite furnace (Perkin–Elmer, model HGA

Table 1
General description of the samples of pectins and their sources studied

Sample	Content	Extraction method	Source	Origin
P1	Pectin	Enzymic extraction	Pumpkin	Saratov, Russia
P2	Pectin	Acid treatment	Pumpkin	Saratov, Russia
P3	Pectin	Acid treatment	Sugar beet	Krasnodar, Russia
P4[a]	Pectin	Acid treatment	Citrus peel	Copenhagen, Denmark
Pumpkin	Dry pulp	–	–	Saratov, Russia
Sugar beet	Dry pulp	–	–	Krasnodar, Russia

[a] Used for comparison.

600) and an autosampler (Perkin–Elmer, Model AS-60). Phosphorus (as phosphate) was determined in pectin samples after their oxidative digestion (ca. 20 mg each) with 2.5 g of potassium persulphate and 0.75 ml of 3.75 M NaOH in the above Parr-type bomb (4 h, 110°C) with further dilution up to 50 ml using the ascorbic acid method (American Public Health Organization, 1989).

FTIR spectra were recorded in the transmission mode at room temperature (mid-infrared region, 4000–400 cm^{-1}) using a Perkin–Elmer spectrometer, Model 2000 (Beaconsfield, UK), coupled with a personal computer loaded with an IR Data Manager (IRDM) program supplied by the FTIR manufacturer. A total of 40 scans were measured with a resolution of 4 cm^{-1}. Samples were pressed in pellets with spectroscopically pure potassium bromide (E. Merck, Darmstadt, Germany) or prepared in the form of mulls with nujol obtained from Merck.

3. Results and discussion

Among the variety of applications of pectins comprising both food and non-food industry (King, 1993; May, 1990) one of the most important fields is medicine and prophylaxis based mainly on the capability of pectins to bind and remove toxic cations (heavy metals) from the gastrointestinal tract. The availability of non-esterified carboxyl groups of the rhamnogalacturonan backbone, which may be partly or fully neutralized with alkaline (Na, K) or alkaline-earth (Ca, Mg) cations, are usually considered to be responsible for metal binding and determine the cation-exchange properties of pectic substances (Aimukhamedova & Alieva, 1984; Davarski, Manolov, Petrova, & Mavrov, 1994; Kohn, 1975). It should, however, be noted that an uncontrolled use of pectin preparations as food additives might result in the decrease of availability of vitally necessary microelements (Nair, Asp, Nyman, & Persson, 1987) which is regulated by the relative stability of their complexes with pectins, in particular, at different acidity (Thompson & Weber, 1979).

For the above pectin samples (as well as for dry pumpkin and sugar beet pulp) the content of alkaline (Na, K) and alkaline-earth (Mg, Ca) metals was determined, as well as of iron [an example of an important microelement (Borch-Iohnsen, 1995; Miller, Schricker, Rasmussen, & Van Campen, 1981)], lead, copper [elements usually of anthropogenic origin, which exhibit the well-known toxic effect on the organism (Kohn, Malovikova, Bock, & Dongowski, 1981; Nair et al., 1987; Nriagu, 1978; Thompson & Weber, 1979; Vallee & Ulmer, 1972)] and vanadium [a somewhat 'less anthropogenic' element which, being toxic in excessive quantities, is nevertheless of great biological importance in trace amounts (Butler & Carrano, 1991; Rehder, 1991)]. The results of FAAS or GFAAS (for V, Fe and Pb) analyses for pumpkin and sugar beet pectins P1–P3 and their sources are presented in Table 2.

The content of alkaline metals in pectins P1–P3 (about 0.1 to 0.2% on the average) is comparable with the data reported for fruit (lemon and apple) pectins by Kravtchenko et al. (1992) (note that apple pectin contains essentially more potassium; see Kravtchenko et al., 1992). The fact that pumpkin biopectin P1 obtained under mild extraction conditions contains less K (despite the use of a potassium salt in the medium used for enzymic extraction, see the Experimental section) than acid-extracted pumpkin pectin P2, indirectly indicates that this cation is not specifically bound by pectic substances. This is confirmed by the presence of essentially higher amounts of K in the corresponding vegetable sources than in the resulting pectins P1–P3 (see Table 2).

The behaviour of the Na cation seems to be dependent on the origin of pectin. Thus extraction of the latter from pumpkin both enzymically (P1) and acidically (P2) results in ca. 2–2.5-fold accumulation of sodium, whereas its amount in P3 is depleted 3-fold compared with that in the source (sugar beet). In view of that, a more specific interaction between sodium and pectic substances may be assumed to occur, obviously depending on their origin, which determines their overall physicochemical properties.

The content of Mg and, especially, Ca is noticeably higher in both pumpkin pectins (P1 and P2) than in sugar beet pectin (P3) which, for Ca, evidently correlates with its content in pumpkin (see Table 2).

Table 2
Content of alkaline, alkaline-earth and heavy metals in pectins obtained from pumpkin and sugar beet, as well as in their sources[a]

Metal	Content ± S.D. of metals[b] in samples (see Table 1)				
	P1	P2	P3	Pumpkin	Sugar beet
(mg/g)					
Na	1.22 ± 0.02	1.60 ± 0.01	0.21 ± 0.01	0.64 ± 0.01	0.63 ± 0.01
K	0.65 ± 0.01	2.05 ± 0.02	1.01 ± 0.04	3.17 ± 0.01	5.17 ± 0.23
Mg	2.64 ± 0.03	0.75 ± 0.02	0.39 ± 0.01	0.69 ± 0.01	2.34 ± 0.06
Ca	9.85 ± 0.01	1.09 ± 0.01	0.10 ± 0.01	7.85 ± 0.01	2.11 ± 0.06
(μg/g)					
Pb	36.2 ± 0.9	18.1 ± 0.1	1.96 ± 0.04	4.1 ± 0.4	1
Cu	60 ± 7	96 ± 4	34 ± 5	9.6 ± 0.1	6.2 ± 0.1
Fe	218 ± 1	185 ± 3	66 ± 2	102 ± 4	280 ± 14
V	15.1 ± 0.9	17.4 ± 1.4	14.1 ± 0.4	89.69 ± 0.07	45.83 ± 0.02

[a] For air-dry samples of pectins and vegetable pulp.
[b] Determined using flame (FAAS; for Na, K, Mg, Ca and Cu) and graphite furnace atomic absorption spectrometry (GFAAS; for Pb, Fe and V).

Comparing the data on Mg and Ca for pumpkin biopectin and the acid extract (P1 and P2, respectively), it is clear that acid treatment greatly reduces their content in the resulting pectin as compared to enzymic treatment. In general, it may be concluded that the acid extraction process practically prevents accumulation of Mg in pectin from the source (for P2) or even reduces its content (for P3), while for Ca this reduction is much more pronounced in both cases (for P2 and P3 compared to the corresponding vegetable source).

The mineral composition of pectins P1–P4 (see Table 1), which is determined mainly by alkaline-earth and alkaline cations, is presented in molar units in Table 3 together with the content of phosphate. Considering the usual conditions under which pectins are obtained and their more-or-less acidic character, phosphate should be expected to be present in pectins in the form of its acidic salts (most probably, $H_2PO_4^-$ or at least HPO_4^{2-}). Comparison of the total mineral composition of pectins and the content of phosphate (see Table 3) shows that the latter may bind only a minor part of the cations [with the only exception of P2 where the phosphate content (in equivalent units) is comparable to the total mineral content]. The data on the phosphate content given in Table 3 are comparable with those reported by Kravtchenko et al. (1992) (ca. 0.003 and 0.01 mmol phosphate/g for lemon and apple pectins, respectively)

Thus it may be concluded that alkaline-earth metals (both Mg and Ca) are contained in pumpkin biopectin P1 in significantly higher amounts than in all the other pectins studied, determining its leadership in the total mineral content (see Table 3). While Mg is obviously available from the supernatant (see the Experimental section), the much higher content of calcium in P1 (see Table 1) should be attributed to its higher content in pumpkin (see Table 2), which is significantly decreased during acid treatment and is evidently much less affected by enzymic extraction. In general, much higher amounts of Mg and Ca in biopectin P1 as compared to both acid extracted pectins P2 and P3 correlate with a relatively well exhibited capability of pectins to bind these metals, which is suppressed in acidic media (Camire & Clydesdale, 1981; Kohn, 1975). It is also noteworthy that the essentially higher content of Ca (and, probably, Mg) in biopectin as compared to the acid extract, and in pumpkin pectin as compared to that from, for example, sugar beet, may in principle contribute to poorer gelling properties of pumpkin pectin (Ptitchkina et al., 1984) and, in general, of biopectins as

Table 3
Individual and total molar content of alkaline and alkaline-earth metals and phosphate in pectins P1–P4 (see Table 1)

Pectin	Content of metals in pectins[a] (mmol/g)					Content of phosphate[b] (mmol PO_4^{3-}/g)
	Na	K	Mg	Ca	Total (mg-eq/g)	
P1	0.053	0.017	0.109	0.246	0.78	0.0468 ± 0.0013
P2	0.070	0.052	0.031	0.027	0.24	0.1001 ± 0.0003
P3	0.009	0.026	0.016	0.003	0.07	0.0087 ± 0.0003
P4	0.230	0.021	0.019	0.019	0.33	0.0042 ± 0.0002

[a] Calculated using the data of Table 2.
[b] Determined after oxidative digestion of pectin samples using the ascorbic acid method (American Public Health Organization, 1989) (see Section 2).

compared to the corresponding acid extracts (Matora et al., 1995; Zhemerichkin & Ptitchkina, 1995). The noticeably higher content of phosphate in both pumpkin pectins P1 and P2 correlating with their generally higher content of alkaline-earth cations (see Table 3) may, for example, lead to the formation of heteroligand pectate–phosphate complexes like, e.g. $(R\text{-}COO)^-\ldots Ca^{2+}\ldots H_2PO_4^-$ in dry pectins with their (partial) ionization (dissociation of phosphate) in solution, although this assumption requires further experimental evidence.

Concerning heavy metals (see Table 2), note that the content of vanadium is quite similar in all the pectin samples. This might indicate relatively strong binding of the $V^{IV}O^{2+}$ or $V^{V}O_2^+$ cations [in the form of which this element most probably occurs under physiological conditions (Butler & Carrano, 1991; Rehder, 1991)] by pectins, being relatively less affected by acid treatment. Considering the stability of the vanadium content in pectins, which is nevertheless 3–5-fold lower than in their sources (see Table 2), it may be assumed that this element is obviously transferred from the source to the resulting extract in a proportion similar to that in which it is originally bound to pectic substances in the plant cell wall, whereas the other part of V occurring in the vegetable mass in some other chemical forms remains either not extracted or (e.g. for acid extraction) in the solution.

It is also worth noting that the content of vanadium both in pectins P1–P3 and in the dried vegetables (see Table 2) is noticeably higher than its usual acceptable level in food being of the order of 1 μg/g (Rehder, 1991), which should be taken into account for possible applications.

The content of both Fe and Pb decreases in the following sequence: P1 > P2 >> P3 (see Table 2). Nevertheless, this similarity is only apparent. It should be emphasized that the yield of pectin obtained from a vegetable source is usually approximately 10–20% depending on the source, extraction method and conditions, etc. (King, 1993; Matora et al., 1995). In view of that, when comparing the mineral composition of a vegetable source and the pectin obtained thereof, if the content of a metal in the latter exceeds that in the source by a factor of at least 5 or more, we may conclude that total or the major amount of the cation is transferred from the source to the pectin during extraction (i.e. the case of a strong or even complete accumulation).

Taking into consideration the content of Fe in dry pulp of pumpkin and sugar beet, which is comparable to and even higher (for sugar beet) than that in the resulting pectins, it is evident that iron does not at all (for P3) or essentially (for P1 and P2) accumulate in pectins upon extraction, and its content is somewhat decreased in the case of acid-extracted samples. This agrees with our recent ^{57}Fe Mössbauer spectroscopic data (Kamnev et al., 1995b) which did not reveal any specific binding of ferric ions by pectins in aqueous media. In other reports where iron was shown to be strongly bound by fractions of dietary fibre, including pectic substances (Camire & Clydesdale, 1981; Reinhold, Salvador Garcia, & Garzon, 1981), or where absorption of non-heme iron in rats was shown to be increased when pectin preparations had been added to the diet (Gordon & Chao, 1984; Kim, Atallah, Amarasiriwardena, & Barnes, 1996), the metal was introduced solely in the form of iron(II) salts differing in their complexing ability from ferric ions strongly hydrolysed at physiological pH values. Moreover, in the case of dietary fibres iron could form somewhat stronger complexes with ligands other than carboxyl which may also be present in pectins as trace impurities, e.g. amino acids (Kravtchenko et al., 1992) [as in the case of chitosan where each ferric ion was shown (Nieto, Peniche-Covas, & Del Bosque, 1992) to be strongly coordinated to two amino groups of the glucosamine residue and four water molecules]. As for the differences in the iron behaviour during extraction of pectin from sugar beet (a significant decrease in the Fe content) and pumpkin (some increase in the Fe content, see Table 2), in view of the aforementioned this may indicate the presence of some molecular structures in pumpkin which reduce iron(III) to iron(II), facilitating its binding and transfer to the extracted pectin.

In contrast, the content of Pb in both pumpkin and sugar beet pulp is significantly lower than in the resulting pectins (see Table 2) showing a significant accumulation of this metal in the latter. A clear correlation in the Pb content is observed between the source (vegetable) and the resulting pectin (see above and Table 2). It can also be seen that acid treatment decreases the level of Pb accumulation. In general, these data agree with a higher extent of Pb binding by pectic substances, as compared to, e.g. alkaline-earth cations which, according to Kohn (1987), are bound to carboxylic groups of oligouronates by intramolecular electrostatic bonds.

The content of Cu in pectins obtained from different sources (see Table 2) correlates with that of the latter. It is quite evident that essentially all copper from the vegetable transfers to pectins and that the acid extraction procedure does not at all suppress this process. This agrees with the data reported in the literature on strong stoichiometric binding of copper ions by pectic substances in aqueous solutions (including acidic media, Davarski et al., 1994; Katseva, Kukhta, Panova, & Chirva, 1988; Manzini, Cesaro, Delben, Paoletti, & Reisenhofer, 1984; Nair et al., 1987; Reisenhofer, Cesaro, Delben, Manzini, & Paoletti, 1984; Thompson & Weber, 1979). It should also be noted that Cu may form strong complexes with trace constituents of pectic substances including neutral sugars (and, to a less extent, proteins and phenolic compounds), not all of which are

covalently bound to the pectin molecules (Kravtchenko et al., 1992). The fact that pumpkin biopectin was found to contain fewer neutral sugars than acid-extracted pumpkin pectin (Zemerichkin & Ptitchkina, 1995) may contribute to the reasons for the higher Cu content in P2 than in P1 (see Table 2). The Cu content in P3 is evidently limited by its amount in the vegetable.

Thus the systematic comparison of the mineral fraction of pectins P1–P3 described in part previously (Kamnev et al., 1997) with that of their vegetable sources, considering also the extraction method applied (see Table 2), has made it possible to elucidate the effect of the latter on the degree of accumulation of certain metal cations in pectins during the extraction process.

The above-discussed data on the mineral composition of the samples studied (see in particular Table 3) may in principle contribute to their FTIR spectroscopic characteristics, primarily by affecting the state of carboxylic groups.

A general view of FTIR spectra of pectins P1–P4 is presented in Fig. 1. The spectral data obtained were analysed by comparing the FTIR spectra in the following characteristic regions: O–H stretching band envelope (3100–3600 cm^{-1}); C–H stretching bands (2800–3000 cm^{-1}); the 'fingerprint' region of the spectra (under ca. 2000 cm^{-1}), including the bands contributing to the resonant absorption energy of the pyranose cycle vibrations (950–1200 cm^{-1}), as well as the region 1200–1800 cm^{-1} featuring the state of carboxylic groups (Filippov, 1992; Kamnev et al., 1995a; Zhbankov, 1992).

Despite the general similarity of the spectra (see Fig. 1), detailed analysis shows both the qualitative (i.e. related to band positions) and quantitative differences (i.e. featured by redistribution of relative resonant absorption intensifies) in the characteristic regions which are indicative of certain differences in the

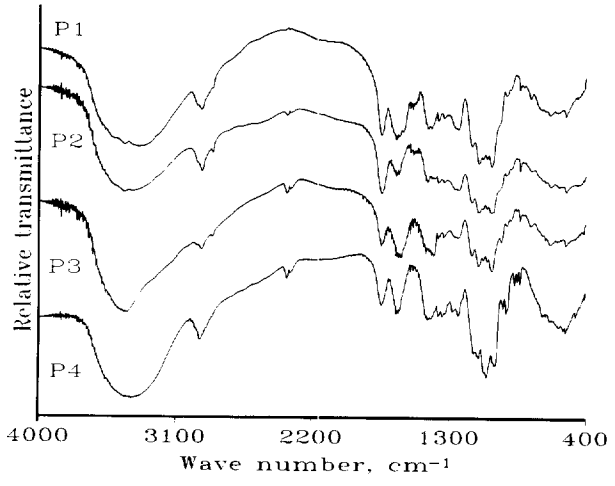

Fig. 1. General view of Fourier transform infrared spectra for pectins P1–P4 (see Table 1) in KBr pellets.

structures and compositions of the pectins studied (Matora et al., 1995; Ptitchkina et al., 1994; Zhemerichkin & Ptitchkina, 1995).

The O–H and C–H stretching regions are less informative owing primarily to the broadness (OH) and relatively small intensity (CH) of bands. Nevertheless, it can be seen even from the general view of Fig. 1 that the contribution of H-bound water (broad absorption at ca. 3200 cm^{-1}) is minimal for P3 and maximal for P1, which correlates with their minimal and maximal mineral content, respectively, differing by over an order of magnitude (see Table 3) and might probably be connected with the presence of hydration water bound to cations. One can also notice somewhat weaker intensity of C–H stretching bands (2800–3000 cm^{-1}) for pectin P3, which may be attributed to an essentially lower degree of methyl esterification of carboxyls in sugar beet acid-extracted pectin (as compared to pumpkin and citrus pectins; Kravtchenko et al., 1992; Zhemerichkin & Ptitchkina, 1995) and its lower acetyl content (Matora et al., 1995).

The fingerprint regions of FTIR spectra for pectins P1–P3 are shown in Fig. 2(a)–(c), respectively. For the pyranose cycle vibrations region, one should note almost identical spectral parts with five bands at 1016–1019, 1052, 1076, 1104 and 1149 cm^{-1} characteristic for pectic substances extracted from different plants (Filippov, 1992). (As is seen from Fig. 1, this region is different for P4 which is connected with the presence of ca. 35% sucrose blended with the commercial citrus pectin; see below.) Pectin P3 [see Fig. 2(c)] exhibits a well resolved band at 954 cm^{-1}, which is evidently absent in the spectrum of P1 [see Fig. 2(a)] and is weakly noticeable only as a shoulder for P2 [see Fig. 2(b)]. In their turn, both pumpkin pectins exhibit a shoulder at 970 cm^{-1} absent in the P3 spectrum. These differences reflect certain variations in the monosaccharide compositions of the above pectins (Matora et al., 1995; Zhemerichkin & Ptitchkina, 1995).

Most interesting is the region featuring the state of carboxylic groups (ca. 1750–1350 cm^{-1}). The band at ca. 1750 cm^{-1} is assigned to the stretching C=O mode of non-ionized (methylated or protonated) carboxyl. Ionization (i.e. the formation of salts) leads to its disappearance, and two new bands appear due to antisymmetric and symmetric stretching modes of COO^- at ca. 1600–1650 and 1400–1450 cm^{-1}, respectively (Filippov, 1992). Thus, in principle, considering the relative intensities of bands in these regions, one may correlate them to the relative amount and degree of esterification of carboxylic groups. Such consideration may, however, be somewhat ambiguous because of overlapping with some other bands (bending H–O–H of trace and bound water, symmetric and antisymmetric bending $-CH_3$ modes), although the latter are usually of smaller intensity and allow one to compare

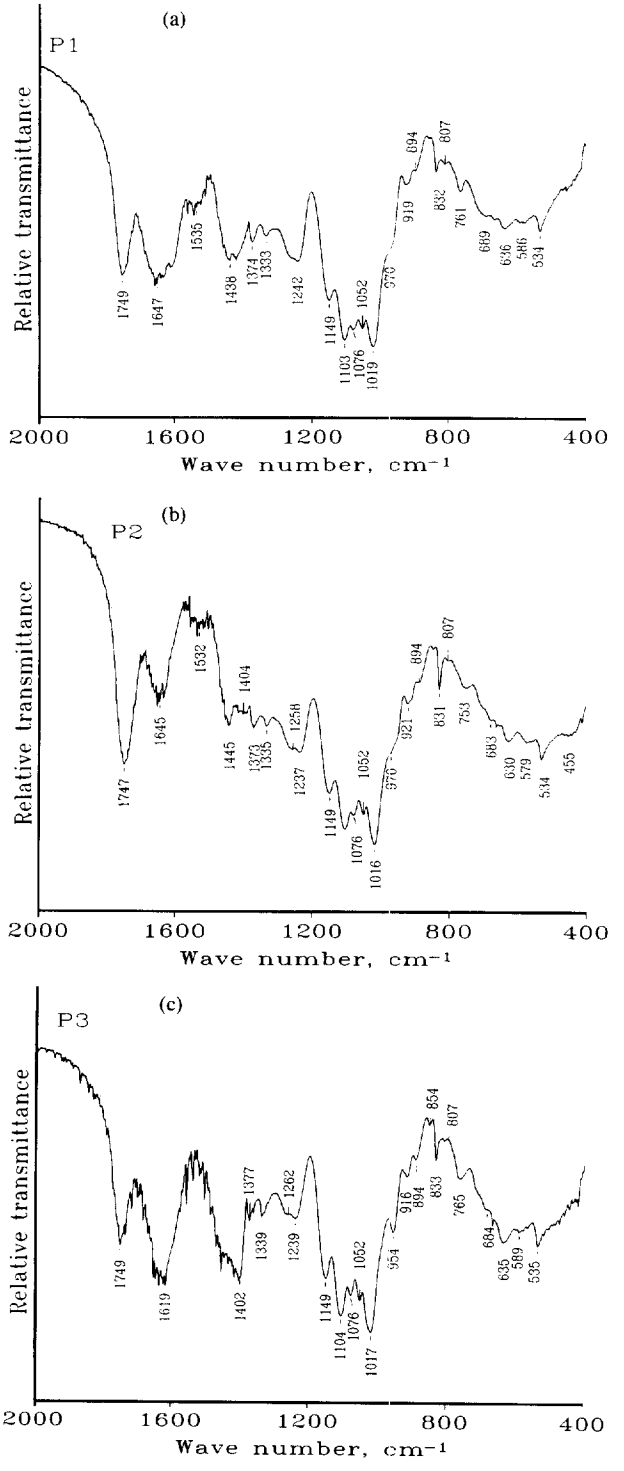

Fig. 2. Fourier transform infrared spectra of (a) pumpkin biopectin P1, (b) pumpkin acid-extracted pectin P2 and (c) sugar beet acid-extracted pectin P3 (see also Table 1) in KBr pellets.

the spectra. Thus, the less intensive C=O band at 1749 cm^{-1} and relatively stronger absorption in the region of ionized carboxyls (1647 cm^{-1}) for P1 (see Fig. 2a) and vice versa for P2 [see Fig. 2(b)] correlate well with the high total metal content in P1 and the lower content of cations in P2 (note also that in the latter an essential part of cations is evidently bound to phosphate; see above and Table 3).

As for P3, its spectral features in the above regions most probably reflect a combination of the following properties of this sample. The relatively low degree of esterification, high proportion of galacturonic acid and relatively low amounts of neutral sugars for sugar beet acid extract P3 (Zhemerichkin & Ptitchkina, 1995) together with its negligible total mineral content (see Table 3) must lead to an increased content of COOH groups. Nevertheless, during preparation of samples for FTIR measurements by grinding and pressing with KBr some cation exchange may occur, which partly leads to the formation of potassium pectate and, in general, may affect the system of hydrogen bonding in the sample studied and result in the formation of new H-bonds (Filippov, 1992; Kamnev et al., 1995a). The most obvious example of this effect is the absence of the sharp intensive band at ca. 3565 cm^{-1}, related to the stretching mode of the non-hydrogen-bonded OH group in sucrose (Olinger & Griffiths, 1993) added to the commercial citrus pectin P4 (see above), whereas this band is well resolved in its FTIR spectrum recorded in nujol, i.e. a hydrophobic diluent [Fig. 3(a) and (b)], in contrast to the other pectins studied containing no sucrose (see Fig. 1). In Fig. 3(b) the C–H stretching region (2800–3000 cm^{-1}) is completely obscured by nujol (paraffin oil) and therefore is not shown.

Nevertheless, the above described effect manifests itself only partly, so that the non-H-bonded OH band in pure sucrose, as well as a number of its other very specific sharp bands are clearly seen even in its spectrum measured in KBr [Fig. 4(a)]. These bands are also noticeable in the spectrum of pectin P4 blended with sucrose (see the region under 2000 cm^{-1} in Fig. 1), as

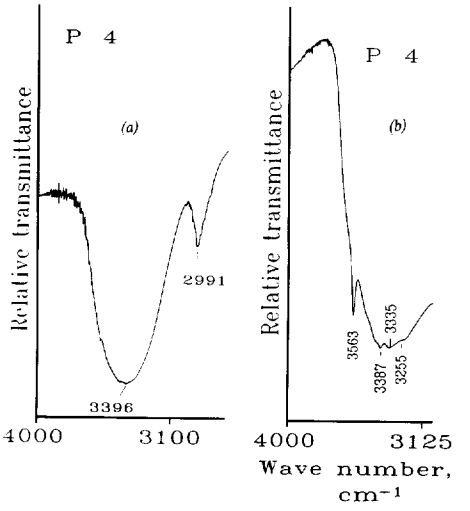

Fig. 3. Fourier transform infrared spectra of commercial citrus acid-extracted pectin P4 (see Table 1) (a) in KBr pellet and (b) in nujol.

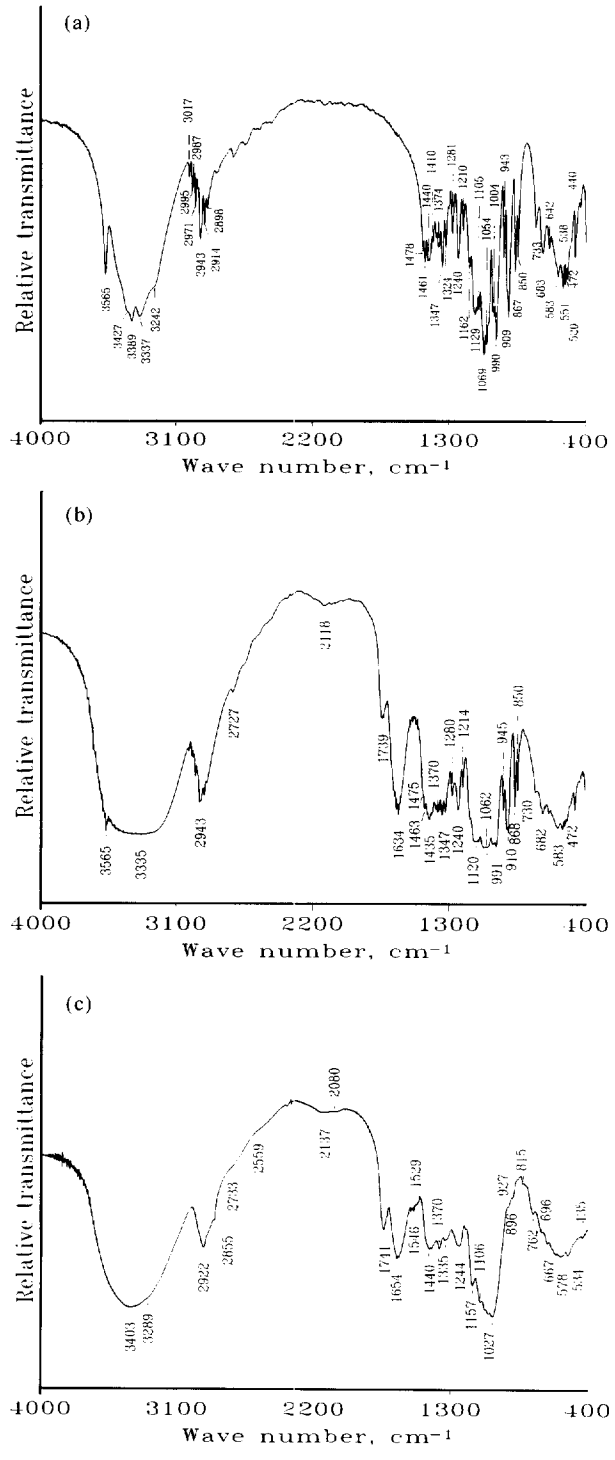

Fig. 4. Fourier transform infrared spectra of (a) sucrose, (b) dry pulp of sugar beet and (c) dry pulp of pumpkin (see also Table 1) in KBr pellets.

well as in the FTIR spectrum of sugar beet dry pulp [Fig. 4(b)], but not in that of pumpkin [Fig. 4(c)]. Note the differences in the characteristic region 950–1200 cm^{-1} for the spectra of pectins P1–P3 [Fig. 2(a)–(c), respectively] and their sources [Fig. 4(b) and (c)].

4. Conclusions

The results of atomic absorption spectrometric analyses for a series of cations have shown that pumpkin biopectin has an unusually high total mineral content due mainly to markedly increased amounts of calcium and magnesium available from the bacterial culture supernatant used for enzymic extraction and/or from the source (i.e. pumpkin found to be especially rich with Ca), which may contribute to the poorer gelling properties observed recently for pumpkin pectins and biopectins in general. Acid extraction significantly decreases the content of the both alkaline-earth metals. As for alkaline cations, potassium seems to be present as a relatively free constituent, whereas a more specific interaction between sodium ions and pectic substances may be assumed depending on the origin of the pectin and obviously on its properties.

Iron does not essentially accumulate in pectins, being relatively more weakly bound by the latter (which may, however, depend on its oxidation state) as compared to other heavy metals. Copper and lead have been shown to essentially accumulate in pectins upon extraction from their sources.

The vanadium content in pectins from pumpkin and sugar beet was found to be quite similar being nevertheless 3–5-fold lower than in their sources. It is assumed that V occurring in plant tissue obviously in different chemical forms may be transferred to pectin during its extraction in a proportion similar to that in which it is bound to pectic substances in the plant cell wall, thus indicating its strong binding not affected by acid treatment.

The content of the other heavy metals studied is either slightly (Fe, Pb) or not at all (Cu) decreased during acid treatment as compared to the enzymic extraction process.

The above results are considered together with Fourier transform infrared spectroscopic data for the samples studied; in particular, with the state of carboxylic groups responsible for metal binding.

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