Monatshefte für Chemie 128, 211–216 (1997)

Monatshefte für Chemie
Chemical Monthly

Atomic Absorption Spectroscopic Investigation of the Mineral Fraction of Pectins Obtained from Pumpkin and Sugar Beet

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Summary. Data on the mineral composition of plant cell wall carboxypolysaccharides (pectins), obtained from non-traditional vegetable sources (pumpkin and sugar beet) using a biotechnological process of enzymatic extraction (biopectin) and the traditional method of acid treatment, are presented. The results have been obtained by flame (FAAS) or graphite furnace atomic absorption spectroscopy (GFAAS) and include trace analyses of the heavy metal content. It is shown that biopectin has an unusually high total mineral content, due mainly to markedly increased contents of Ca and Mg. The content of a series of alkaline, alkaline earth, and heavy metals in the pectins is compared considering the effect of the extraction method.

Keywords. Mineral composition; Heavy metals; Pectin; Pumpkin; Sugar beet; Atomic absorption spectroscopy.

Atomabsorptionsspektroskopische Untersuchung des Mineralanteils von Pektinen aus Kürbissen und Zuckerrüben

Zusammenfassung. Die Zusammensetzung des mineralischen Anteils von Carboxypolysacchariden (Pektinen), die mittels eines biotechnologischen Prozesses (enzymatische Extraktion; "Biopektin") bzw. der üblichen Methode (Behandlung mit Säure) aus den Zellwänden von Pflanzen (Kürbis, Zuckerrübe) gewonnen wurden, wird diskutiert. Die Ergebnisse wurden mittels Flammenatomabsorptionsspektroskopie (FAAS) oder im Graphitofen (GFAAS) bestimmt und beinhalten Spurenanalysen des Schwermetallgehalts. Es wird gezeigt, daß Biopektin einen unüblich hohen Mineralanteil aufweist, der hauptsächlich auf den hohen Gehalt an Ca und Mg zurückzuführen ist. Der Gehalt an einer Reihe von Alkali-, Erdalkali- und Schwermetallen wird verglichen und zur Isolationsmethode der Pektine in Beziehung gesetzt.

Introduction

Pectins, essential components of fruit and vegetable cell walls, comprising a diversity of natural carboxypolysaccharides of variable composition, have found wide application both for medical and industrial purposes [1, 2]. The polymer backbone consists mainly of *D*-galacturonic acid residues joined by $\alpha(1, 4)$-glycosidic linkages, a varying proportion of which occur in the form of their methyl esters, with periodic insertion of rhamnosyl residues. Other neutral sugars are present as side chains. 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 [2, 3].

The industrially employed process of pectin extraction from raw materials involves relatively severe acid treatment [1, 4, 5]; an alternative method is enzymatic extraction using the supernatant from microbial cultures [6–8].

Pectins obtained from non-traditional vegetable sources (pumpkin and sugar beet) using both extraction methods mentioned above have recently been studied from the viewpoint of their monosaccharide composition, physicochemical properties, and structure [8–11]. In the present communication, the results of flame (FAAS) or graphite furnace atomic absorption (GFAAS) analyses of the mineral composition (including the content of heavy metals) are compared for the following samples: pumpkin pectin obtained using a complex of microbial enzymes (biopectin, **P1**), pumpkin pectin obtained using the standard method of acid extraction (**P2**) and commercial sugar beet pectin obtained by the standard acid extraction method (Krasnodar, Russia; **P3**). The data obtained concerning the content of alkaline, alkaline earth, and heavy metals in the above samples are compared considering the effect of the extraction method.

Results and Discussion

For the above three pectin samples, the content of alkaline (Na, K) and alkaline earth (Mg, Ca) metals was determined as well as that of Fe (an example of an important microelement), Pb, Cu (elements usually of anthropogenic origin which exhibit well-known toxic effects on organisms), and V, a somewhat less anthropogenic element which, being toxic in excessive quantities, is nevertheless of great biological importance in trace amounts [12]. The results of FAAS or GFAAS (for Fe, Pb, and V) analyses are presented in Table 1.

The content of alkaline metals in pectins **P1**–**P3** (about 0.1–0.2% on the average) is comparable with the data reported for fruit (lemon and apple) pectins by *Kravtchenko et al.* [13] (note that apple pectin contains appreciably more potassium [13]). The fact that pumpkin biopectin (**P1**) obtained under mild conditions contains less Na and K (despite the use of a potassium salt in the medium used for enzymatic extraction, see above) as compared to acid extracted pumpkin pectin **P2** indirectly reflects that these cations are not specifically bound by pectic substances.

On the contrary, alkaline earth metals (both Mg and Ca) are contained in biopectin **P1** in significantly higher amounts. Whereas Mg is obviously available

Table 1. Contents of metals in different pectins determined by atomic absorption spectroscopy

	Content of metals in pectins[a] (standard deviation)		
	P1	**P2**	**P3**
		(mg/g)	
Na	1.216 (±0.018)	1.598 (±0.008)	0.207 (±0.007)
K	0.648 (±0.005)	2.049 (±0.015)	1.014 (±0.036)
Mg	2.645 (±0.029)	0.751 (±0.024)	0.386 (±0.005)
Ca	9.847 (±0.005)	1.090 (±0.006)	0.101 (±0.007)
		(μg/g)	
Pb	36.22 (±0.94)	18.10 (±0.05)	1.96 (±0.04)
Cu	60 (±7)	96 (±4)	34 (±5)
Fe	217.7 (±1.1)	185.1 (±2.7)	65.9 (±2.4)
V	15.08 (±0.90)	17.40 (±1.36)	14.11 (±0.35)

[a] **P1**: pumpkin pectin obtained *via* enzymatic extraction (biopectin); **P2**: pumpkin pectin obtained by the standard method of acid extraction; **P3**: commercial sugar beet pectin obtained by the standard acid extraction method (Krasnodar, Russia); FAAS data except for Pb, Fe and V (GFAAS)

from the supernatant (see Experimental), the much higher content of Ca in **P1** (see Table 1) should be attributed to its higher content in pumpkin (7.85 ± 0.01 mg Ca per gram of dry pumpkin pulp against 2.11 ± 0.06 mg/g for sugar beet pulp) which is significantly decreased during acid treatment and is evidently much less affected by enzymatic extraction. In general, much higher amounts of Mg and Ca in biopectin **P1** as compared to the acid extracted pectins **P2** and **P3** correlate with the capability of pectins to bind these metals which is suppressed in acidic media [2, 14, 15]. It is also worth mentioning 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 sugar beet, may in principle contribute to the weaker gelling properties of pumpkin pectin [9] and, in general, of biopectins as compared to the corresponding acid extracts [7, 8].

Concerning heavy metals, note that the content of vanadium is quite similar in all samples. This might indicate relatively strong binding of $V^{IV}O^{2+}$ or $V^{V}O_2^{+}$ cations, in the form of which this element most probably occurs under physiological conditions [12], by pectins, being relatively less affected by acid treatment, al-though this assumption requires further experimental evidence. Considering the usual acceptable level of vanadium in food being of the order of 1 μg/g [12], it seems to be accumulated in pectins upon extraction.

The content of Fe and Pb decreases in the following sequence: **P1** > **P2** ≫ **P3** (see Table 1). Nevertheless, this similarity may be misleading. Taking into consideration the yield of pectin from the raw material being about 10 to 20 wt.% at maximum [7], as well as the content of Fe in dry pulp of pumpkin and sugar beet (our analyses showed 102 ± 4 and 280 ± 14 μg Fe/g, respectively, which is com-

parable to and even higher than in the resulting pectins), it is evident that iron does not 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 [11] 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 [15, 16], the metal was introduced solely in the from of iron(II) ions differing in their complexing ability from ferric ions which are strongly hydrolyzed at physiological *pH* values. Moreover, in the case of dietary fibres, iron could form somewhat stronger complexes with ligands other than carboxyl which may be present in pectins as trace impurities, *e.g.* amino acids [13] as is the case with chitosan where each ferric ion was shown to be strongly coordinated to two amino groups of the glucosamine residue and four water molecules [17].

In contrast, the content of Pb in pumpkin and sugar beet pulp (4.1 ± 0.4 and $< 0.1\mu g/g$, respectively) is significantly lower than in the resulting pectins (see Table 1), indicating a noticeable accumulation of this metal in the latter. A clear correlation of the Pb content is observed between the source (raw material) and the resulting pectin (see above and Table 1). It can also be seen that acid treatment decreases to some extent the level of Pb accumulation. In general, these data are in accordance with a higher degree of Pb binding by pectic substances as compared to *e.g.* alkaline earth cations which, according to *Kohn* [18], are bound to carboxyl groups of oligouronates by intramolecular electrostatic bonds.

The content of Cu in pectins obtained from different sources (see Table 1) correlates with that of the latter (9.6 ± 0.1 and $6.2 \pm 0.1\mu g/g$ for pumpkin and sugar beet pulp, respectively). It is evident that copper accumulates in pectins and that the acid extraction procedure does not suppress this process. 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 [13]. The fact that pumpkin biopectin was found to contain fewer neutral sugars than acid extracted pumpkin pectin [8] may contribute to the reasons for a higher Cu content in **P2** than in **P1** (see Table 1).

Conclusions

The results of atomic absorption spectrometric analyses for a series of cations have shown that biopectin has an unusually high total mineral content due mainly to markedly increased amounts of Ca and Mg available from the bacterial culture supernatant used for enzymatic extraction and/or from the source, which may contribute to the weaker gelling properties observed recently for pumpkin pectins and biopectins in general [7–9]. Acid extraction significantly decreases the content of the both alkaline earth metals. Alkaline cations (Na, K) seem to be present as relatively free constituents not specifically bound to pectin.

Iron does not essentially accumulate in pectins from the source, being relatively more weakly bound by pectin as compared to other heavy metals. Those of them studied (V, Cu, Pb) seem to be accumulated in pectin upon extraction from the source. The content of the four heavy metals is either only slightly (Fe, Pb) or

not at all (V, Cu) decreased by acid extraction. It should be taken into account that occurrence and/or accumulation of heavy metals in pectins may be in part related to the presence of trace impurities of substances capable of forming strong complexes with the former as is often the case with pectin preparations.

Experimental

Materials

Pectins were obtained from pressed dry pumpkin pulp by extraction with dilute acid [4, 5, 7] (0.1 *M* HCl, 1:10 w/w, 2 h, 65°C) or with the multi-enzyme culture supernatant from *Xanthomonas campestris* by a procedure described in detail elsewhere [7, 8]. The bacterium was cultivated in a medium containing NH_4Cl (1.0 g/l), KH_2PO_4 (2.0 g/l), $MgSO_4 \cdot 7H_2O$ (0.2 g/l), $CaCO_3$ (0.1 g/l), and partially depectinated pumpkin pulp (10 g/l). Separation of the cell-free supernatant broth was accomplished by centrifugation. For enzymatic extraction of pectin, the weight ratio of the dry pressed pumpkin pulp to the supernatant was chosen to be 1:15. The extract was separated by centrifugation, washed with bidistilled water, centrifuged again, and pectic substances were precipitated with 2 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 to 40°C), ground, and sieved. Other details of the preparation procedures are presented elsewhere [7–9]. Commercial sugar beet pectin extracted by the standard acid treatment method, used for comparison in the present study, was obtained from a sugar beet processing plant in Krasnodar, Russia.

Methods

Metal cations were determined in the above pectins 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 for 1 h at 110°C in a Parr Acid Digestion Bomb No. 4745 (total volume 23 ml, Parr Instruments Company) and further dilution to 25 ml by flame (FAAS; acetylene-air flame) or, for lowest metal concentrations, graphite furnace atomic absorption spectroscopy (GFAAS) using a Perkin-Elmer spectrometer (Model 3110), a graphite furnace (Perkin-Elmer, Model HGA 600), and an autosampler (Perkin-Elmer, Model AS-60).

Acknowledgements

This work was supported in part by the Ministry of Science and Technological Policy of the Russian Federation *A. A. K.* would also like to acknowledge financial support from the Ministry of Science and Technology of France provided in September 1995 *via* the Travel Grant Programme "ACCÉS" which facilitated this cooperation, as well as from the Organizing Committee of the 26th International Symposium on Environmental Analytical Chemistry (April 9–12, 1996, Vienna, Austria) and from the Russian Foundation for Basic Research (travel grant No. 96-03-42520) for participation in the symposium at which this material was presented. The authors thankfully appreciate technical assistance of Drs. *O.M. Tsivileva, O.G. Shkodina* (IBPPM RAS, Saratov, Russia) and *J. Rodriguez* (LQA, FEC, UZ, Maracaibo, Venezuela).

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Received June 5, 1996. Accepted September 30, 1996