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Increasing the purity of polyuronic sorbents

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ABSTRACT

The method of elution ion-exchange chromatography was applied to increase the purity of polyuronic sorbents (apple, carrot and sunflower pectins). The columns is packed with the adsorbent to be purified. Organic solvents *e.g.*, methanol, ethanol and acetone containing HCl are used for the elution at 20, 40 and 50°C. Elimination of mineral substances and nitrogen-containing compounds and an increase in the polyuronic acid content were observed. It was found that efficient purification of pectic substances is possible under the adopted conditions. Partial de-esterification was observed with increase in the pectin purity and the reaction speed was increased by increasing the temperature. Protein–peptide admixtures removed from these pectins, the greatest decrease in the content of nitrogenous substances being observed with carrot pectin.

INTRODUCTION

Pectic substances, pectic acid and alginic acid are polyuronides with well expressed adsorption and ion-exchange properties. Their ability to interact with amines[1], amino acids[2], alkaloids[3], phenols[4], steroids[5], metal ions[6,7], etc., is well known and modified polyuronides have found application in affinity chromatography[8].

Our studies have indicated that polyuronides can be successfully applied as optically active sorbents for the chromatographic resolution of racemic bases[9]. Their good ion-exchange properties make them suitable detoxicants in cases of heavy metal poisoning[5,10] and also as stabilizers of alcoholic drinks against cation turbidity[11].

The good adsorption and ion-exchange properties of polyuronides are also the reason why they retain considerable amounts of admixtures of mineral and organic origin in the process for their production. Therefore, polyuronides are produced with a relatively low purity. However, the requirements for the purity of polyuronides intended for medical and chromatographic purposes have increased recently.

Different methods have been suggested for increasing the purity of pectic substances, *e.g.*, passing dilute pectic solutions through columns with cation exchangers to remove pigments and metal ions[12,13] or static treatment with mineral acids

in an aqueous–alcoholic medium[14–17]. The multiple dissolution of pectin in water and reprecipitation with ethanol acided with HCl has also been applied for purification on the laboratory scale. This method, however, is ineffective and tedious. The low efficiency of static methods is caused by the stability of the salt-like compounds formed from pectins (*e.g.*, pectic acids) and by impurities having a positive electrical charge (polyvalent cations, peptides, amino acids, amines, etc.). The dissociation of such complex salts is possible by column chromatography[9], and we have carried out experiments to increase the purity of pectic preparations by elution of a column with organic solvents containing dilute hydrochloric acid.

EXPERIMENTAL

Materials and reagents

Commercial apple, sunflower and carrot pectins were used. The organic solvents (methanol, ethanol, acetone) were of analytical-reagent grade.

Experimental set-up

The experiments for purification of pectin were carried out as follows. The pectic preparation was hydrated by swelling for 1 h in a mixture of water and the organic solvent (1.5:1) and a chromatographic column (500 mm × 14 mm I.D.) was packed with the material obtained. The pectic preparation acted as an adsorbent. A water-diluted organic solvent containing hydrochloric acid (2.2% and 3.1% HCl) was used as the eluent. In this way the hydrogen ions of the eluent displaced the metal or organic cations bonded with the carboxyl groups of the pectic macromolecules. Moreover, the acidic medium increased the solubility of other organic impurities in organic solvents. After elution, the pectic preparation was removed from the column, mixed with a five times greater volume of 70% ethanol and washed until the washings were neutral.

The purification of pectin by dissolution and precipitating was performed as follows (Table I, experiment 2). A 10-g amount of initial pectin was dissolved in 1000 ml of distilled water and an equal volume of 95% ethanol containing 2.2% HCl was added. The precipitated pectin was filtered, washed until the washings were neutral and dried. The pectin obtained was precipitated twice more in the same way and the pectin dried after the third precipitation was subjected to analysis.

Methods of analysis

The degree of esterification and the purity of pectic preparations were determined by the neutralization method with phenol red as the indicator[17].

The amino acid content of the pectic preparations was determined as follows. A 0.5-g amount of a pectic preparation were mixed with 10 ml of 3 *M* sulphuric acid and heated at 108°C (sulphuric acid boiling point) for 40 h. The acidic hydrolysate was neutralized with calcium hydroxide, the precipitate was filtered and the clear filtrate was subjected to amino acid analysis on an HD-1200 E automatic amino acid analyser (Zavod SNP n.p. Žiar nad HRONOM, Czechoslovakia).

The mineral content of the purified pectins was determined by atomic absorption spectrometry. The ash from each sample was dissolved in 4 ml of concentrated HCl and diluted to 25 ml with demineralized water. These solutions were analysed on a Perkin-Elmer Model 4000 atomic absorption spectrometer.

RESULTS AND DISCUSSION

Initial experiments on the purification of apple pectin by elution in a column with ethanol–hydrochloric acid (2.2% HCl in 40% ethanol) were carried out for 48 h at room temperature. Parallel experiments were performed by dissolving the apple pectin three times in water and reprecipitating it in ethanol–hydrochloric acid. The results obtained are given in Table I.

The results show that both methods of purification give a substantial increase in purity, from 58 to 72–74%, the eluent method being slightly more efficient. Moreover, it should be noted that the removal of the mineral components and nitrogen substances with the elution method is considerably more complete.

It should also be noted that under these conditions a process of de-esterification takes place, which undoubtedly depends on the duration of the acid treatment and is, therefore, more clearly expressed with the eluent method for purification (experiment 1).

No substantial differences in the efficiency of purification of the three pectins (apple, carrot and sunflower) were observed between the three solvents, except with regard to the degree of esterification. The smallest changes were observed with methanol, obviously because of the equilibrium of the de-esterification process in the presence of methanol–hydrochloric acid[15,16].

In another series of experiments, the effect of temperature during elution with ethanol–hydrochloric acid was tested. It was found that an increase in temperature from 20 to 50°C speeded up of the pectin purification, de-esterification also being more strongly expressed.

The data in Table I show that the greatest differences between experiments 1 and 2 are in the nitrogen content of the purified pectins, which is more than halved with the eluent method but remains almost unchanged with the dissolution and reprecipitation method. It was therefore of interest to analyse the tested pectic preparations with respect to this parameter. It has been established that the major part of the nitrogenous compounds in apple and citrus fruit pectins are polypeptides and proteins[18–20], whose concentrations vary from 0.5 to 3.5%. For this reason the samples were subjected to

TABLE I

COMPARATIVE DATA FOR APPLE PECTIN PURIFIED AT 20°C BY ELUTION IN A COLUMN AND REPRECIPITATION

Parameter	*Control pectin sample*	*In a column, 48 h (experiment 1)*	*Pectin precipitated three times from ethanol–HCl (experiment 2)*
Degree of esterification (%)	74.3	65.6	69.3
Purity (%)	58.1	74.4	72.6
Ash content (%)	3.16	0.16	0.75
Nitrogen content (Kjeldahl) (%)	0.39	0.16	0.37
Ethanol consumption (ml per 1 g of pectin)	–	250	300

TABLE II

AMINO ACID COMPOSITION (%) OF PECTIN HYDROLYSATES

Hydrolysis for 40 h in 3 *M* sulphuric acid at 108°C.

Amino acid	*Apple pectin*		*Carrot pectin*		*Sunflower pectin*	
	Initial	*Purified after 48 h elution at 20°C*	*Initial*	*Purified after 80 h elution at 20°C*	*Initial*	*Purified after 50 h elution at 20°C*
Lysine	0.036	0.020	0.064	0.014	0.034	0.026
Histidine	–	–	0.016	–	0.014	Trace
Arginine	–	–	0.032	–	–	–
Aspartic acid	–	–	0.024	0.016	Trace	Trace
Threonine + serine	0.084	0.054	0.108	0.048	0.104	0.036
Glutamic acid	0.032	–	0.094	0.012	0.082	0.076
Proline	–	–	Trace	Trace	Trace	Trace
Glycine	0.036	0.026	0.054	0.016	0.056	0.028
Alanine	0.028	0.016	0.062	0.020	0.068	0.036
Cystine	Trace	–	–	–	–	–
Valine	0.048	0.032	0.048	0.026	0.054	0.044
Methionine	Trace	–	–	–	–	–
Isoleucine	0.034	0.016	0.032	0.014	0.040	0.028
Leucine	0.046	0.028	0.108	0.030	0.074	0.054
Tyrosine	–	–	0.018	0.012	0.024	0.012
Phenylanine	0.022	0.014	0.028	0.016	0.036	0.028
Total	0.366	0.206	0.688	0.224	0.586	0.368

acid hydrolysis and their amino acid composition was determined. Table II gives data for apple, carrot and sunflower pectins. The data for the initial pectin samples show that quantitatively and qualitatively carrot pectin is the richest of the three in amino acids.

Table III gives some data for the cation composition of apple pectin subjected to purification by the eluent method. The analysis was performed with four different

TABLE III

MINERAL CONTENT OF APPLE PECTIN PREPARATIONS OBTAINED AFTER PURIFICATION BY THE ELUTION METHOD

Solvent, 2.2% HCl in 40% ethanol; temperature, 40°C.

Sample	*Duration of purification (h)*	*Amount (mg/g)*							
		Na	*K*	*Mg*	*Ca*	*Cu*	*Zn*	*Mn*	*Fe*
Control	0	0.30	2.10	3.19	5.90	0.14	0.03	0.02	0.75
1	4	0.09	0.08	0.02	0.03	0.02	0.01	0.00	0.18
2	8	0.07	0.02	0.02	0.06	0.02	0.01	0.00	0.16
3	12	0.02	0.02	0.01	0.04	0.02	0.01	0.00	0.13
4	48	0.04	0.02	0.01	0.02	0.02	0.01	0.00	0.10

durations of purification. The results show that the removal of a major part of the mineral admixtures takes place during the first 4 h. Iron cations, however, are removed very slowly and even after 48 h of elution their content is about 0.1 mg/g.

CONCLUSIONS

It has been shown that it is possible to purify efficiently pectins from fruit, vegetables and other plants using column elution with ethanol, methanol or acetone containing hydrochloric acid. An increase in temperature speeds up the removal of admixtures.

Partial de-esterification of pectin was observed with the increase in purity. This process is also speeded with an increase in temperature. De-esterification is slowest using elution with methanol.

A difference was established in removing the protein peptide admixtures of apple, carrot and sunflower pectins. The greatest decrease in amino acid content using the elution method was observed with carrot pectin.

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