Characterization and degradation of pectin derived from Budimka apple

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Abstract: The characterization of apple pectin and its oligogalacturonic fractions derived from the autochthones apple variety Budimka, characteristic for central Serbia, is described in this paper. After extraction, the apple pectin was subjected to controlled enzymatic hydrolysis by polygalacturonase (PG) and pectin lyase (PL) from *Aspergillus niger* and then fractionated by ion-exchange column chromatography on Dowex 1X-8 (200–400 mesh). Saturated oligogalacturonic acids, obtained by controlled hydrolysis with PG, were efficiently separated by elution with a gradient of Na acetate buffer (pH 6.0), while unsaturated oligogalacturonic acids, obtained by controlled hydrolysis with PL, were separated on the same resin, using a gradient of Na formate buffer (pH 4.7) as the eluent. The yields of the fractions with the particular degree of polymerization (*DP*) were also determined. The total content of neutral saccharides in the original Budimka apple pectin was detected by HPLC analysis of the 4-nitrobenzoyl derivatives of the sugar, and amounted to 5.31 %. Among the neutral saccharides, contents of galactose, glucose, rhamnose, arabinose, xylose and mannose were detected.

Keywords: degradation; fractionation; oligogalacturonic acids; Budimka apple pectin; polygalacturonase; pectin lyase; neutral saccharides.

INTRODUCTION

Budimka apple is an autochthon apple variety specific for the region of central Serbia. This apple type is very resistant to climate and ecological conditions and has low agricultural demands, making it a good candidate for organic food products. In addition, it is well known for its sharp flavor and deep yellow to red color, which sometimes extends into the fruit, as well as for its good balance of acids and sugars. It is highly sought after by industry to bring a real apple flavor to juices and ciders. For these reasons, the Budimka is treasured in Serbia both

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fresh and for baked dishes. Moreover, the beneficial and even anticancerogenic health effects of the apple pectin and phenols are well documented.[1,2]

Despite a large range of industrial utilization and export of these apples or apple-based products, including commercial pectin, no systematic study on the degradation and characterization of this pectin has been performed. Significant changes related to the characteristics and technological properties of fruit products during maturation, storing and canning are related to the physico–chemical transformations of pectins caused by the action of pectic enzymes. Both naturally present (endogenous) and introduced enzymes (exogenous) catalyze the decomposition of pectins.

Previously,[3] the extraction of apple pectin from Budimka apples was studied and some general properties of the isolated pectin were determined, such as its high degree of polymerization (*DP*) and high degree of esterification (*DE*). These characteristics, together with the particular content of phenolics and neutral saccharides largely determine the technological properties and specific quality of products derived from Budimka apple.

The aim of the present study was to characterize chemically Budimka apple pectin and the fractions obtained by enzymatic degradation. For these purposes, homologous series of saturated and unsaturated oligogalacturonic acids were obtained by enzymatic hydrolysis of pectin compounds from the Budimka apple using polygalacturonase (PG) and pectin lyase (PL) from *Aspergillus niger*.

<div align="center">EXPERIMENTAL</div>

Pectin extraction and purification

Budimka apples from the Arilje locality (central Serbia) were collected for investigation during the year 2004. The extraction and purification of polygalacturonic acids from Budimka apple was described previously.[3] The resulting pectin preparations had a degree of esterification (*DE*) of 75 %, an average degree of polymerization of 134 % and consisted of 93 % galacturonic acids.

Enzymes and chemicals

Enzymes. The pectic acid was degraded by polygalacturonase (PG; EC.3.2.1.15) from *Aspergillus niger* (Sigma, declared activity of 5–20 U/mg) to obtain a mixture of saturated oligogalacturonides and hydrolyzed by pectin lyase (PL; EC.4.2.2.10) also from *A. niger* (Sigma, declared activity of 50–100 U/mg).

Chemicals. D(+)-galacturonic acid monohydrate (Sigma, USA) was used as the standard for chromatographic analysis. Sugars and sugar alcohols were purchased from Merck (Darmstadt, Germany). 4-Nitrobenzoyl chloride (4-NBCl; analytical grade, Fluka), used to obtain 4-nitrobenzoyl sugar derivates, was recrystallized once from petroleum ether (Sigma) b.p. 60–70 °C. The melting point of 4-NBCl after recrystallization was between 71–73 °C. Analytical grade pyridine (Fluka), used for derivatization, was refluxed for 3 h with NaOH (Merck), distilled (b.p. 115–116 °C) and stored over NaOH. 4-Dimethylaminopyridine *purum* was also purchased from Fluka. The reagent solution for derivatization of the neutral saccharides was prepared by dissolving 100 mg of 4-NBCl in 1 ml of pyridine with gentle warming. All other reagents and solvents were of analytical or HPLC grade.

Enzymatic hydrolysis

Preparation of saturated oligogalacturonides. Mixtures of saturated oligogalacturonic acids were obtained by incubating apple pectin in Erlenmeyer flasks in a thermostated water bath with shaking (150 rpm) with an appropriate amount of *A. niger* fungal polygalacturonase (FPG) at 35 °C for different reaction times. The reaction mixture usually contained 1 % PGA (w/v), 0.15 M NaCl in acetate buffer (0.05 M) and 0.15 mg ml^{-1} of FPG (w/v). In order to obtain different types of oligogalacturonides, the following conditions were applied: for pentamer to octamer 30 to 60 min at pH 6.0; for dimer to pentamer 12 h at pH 6.0; for dimer to tetramer 20 h at pH 6.0; for dimer to trimer 30 h at pH 6.0. The dimer was obtained by incubation at pH 3.5 for 48 h. The enzymatic reactions were terminated by addition of 10 g of celite and activated charcoal *per* 1000 ml and by heating for 5 min at 100 °C. This warm suspension was then filtered and the filtrate loaded onto a Dowex-50W (H$^+$) column and eluted with two volumes of bidistilled water (flow rate 48.9 cm h^{-1}). In this way, the uronides were transformed into free acids. The eluate was concentrated under vacuum to about 10 % (w/v) of galacturonic acids. The mixture of the obtained saturated oligogalacturonides was stored in a refrigerator until further separation of the particular oligogalacturonic fractions was performed.

Preparation of unsaturated oligogalacturonides. The samples were prepared in the same way as for the saturated oligogalacturonides except that they were incubated with 0.1 mg ml^{-1} PL from *A. niger*. Also, the same incubation times were applied to collect the unsaturated oligogalacturonides of particular chain length. Upon termination of the enzymatic reaction, the suspension was filtered and the filtrate loaded onto a Dowex-50W (H$^+$), as described above, in order to transform the uronides into free acids. When a hydrolysate contained too much monomer (D-galacturonic acid), this compound was removed from the pectin hydrolysate by precipitation as the sodium strontium salt. The removal of the monomer enabled a better separation of the higher oligogalacturonic acids. The eluate was concentrated under vacuum to about 10 % (w/v) of galacturonic acids. The mixture was refrigerated until further separation of the particular oligogalacturonic fractions was performed.

Analytical methods

The anhydrogalacturonic acid (AGA) content in the pectin preparations was determined by a photometric method with carbazole in 80 % sulfuric acid and borate ions were added. The absorbance of the solutions was compared to that obtained from standard solutions of galacturonic acid subjected to the same procedure.[4]

The unsaturated oligogalacturonides content was monitored by measuring the absorbance increase in the reaction mixture at 232 nm, due to double C_4–C_5 bond formation in the pectin molecule during the enzymatic reaction. The absorbance was measured using a Zeiss PMQ II spectrophotometer. The degree of degradation of the pectin was calculated from the ratio of the measured increase of the absorbance and the theoretical increase that should have occurred if the enzyme were to break all the glycoside bonds. The theoretical absorbance increase was calculated from the concentration of pectin acids, the degree of polymerization and the molar extinction coefficient (ε) for unsaturated bonds according to Macmillan and Vaughn.[5]

The degree of polymerization (*DP*) of the substrate was estimated by determining the ratio of AGA to the content of reducing groups (AGA/CHO), according to Liu and Luh.[6] The reducing group content was determined using the method of Somogyi.[7]

The degree of esterification (*DE*) was evaluated by the cuprizonic method of Keijbets and Pilnik.[8]

The methanol content was determined using chromotropic acid.[9]

The phenolics were determined after saponification of pectin substrate with 1 M NaOH. The phenolics were measured using the Folin–Ciocalteu reagent (FCR) and vanillin–sulfuric

acid reagent (VSR). FCR reacted with monomers and higher phenolic polymers according to the method of Ribereau–Gayon.[10] VSR reacted approximately stoichiometrically with phenollic compounds of medium and lower degrees of polymerization, as described by Goldstein and Swain.[11] Changes in the degree of polymerization of the phenolics in the samples were monitored from the ratio of the values obtained by the two methods.

The molecular mass, M_r, was estimated by determining the end-groups according to Voragen *et al.*[12]

Ion-exchange chromatography of the oligogalacturonic acids

The saturated oligogalacturonic acids were fractionated using a modified procedure of Nagel and Wilson.[13] The material obtained after digestion of 10 g of pectin was separated by column chromatography employing a Dowex 1X-8 column (200–400 mesh). A typical column measured 30×700 mm^2 and had a volume of about 500 ml. The column was loaded with 150 ml of the 10 % oligogalacturonic acid solution. Saturated oligogalacturonic acids were eluted using sodium acetate buffer (pH 6.0) at a linear concentration gradient from 0.2 to 0.8 M. Before elution, the column was washed with 1 l of distilled water. Unsaturated oligogalacturonic acids were separated using the same column but with a different eluent. They were eluted using sodium formate buffer (pH 4.7) at a concentration gradient of 0.1–0.6 M. In this way, the fractions which presented homologous series of saturated and unsaturated oligogalacturonic acids from dimer to octamer, were eluted. The fractions collected were tested for the content of uronide using the carbazole method[4] and for the content of unsaturated uronide by measuring the absorbance increase at 232 nm.[5]

Purification of the oligogalacturonic acids

After analysis for their uronide content, the fractions of the oligogalacturonic acids from the individual peaks were pooled and precipitated with $SrCl_2$. The $SrCl_2$ was added in 100 % excess and the salts of the uronic acids were precipitated in 4 volumes of 92 % ethanol. Then the dimers and trimers were precipitated in 70 % ethanol and finally in 60 % ethanol, while the oligogalacturonic acids with longer chains were precipitated in 50 % ethanol. After filtration, the precipitates were dried under vacuum over $CaCl_2$. To ensure the purity of the individual uronides, the salts from several column runs were combined, converted to the free acids by treatment with Dowex-50 W (H$^+$) and rechromatographed. Only those fractions shown by TLC to contain the desired oligogalacturonides were pooled.

TLC of the oligogalacturonic acids

After column separation, the obtained fractions were identified and determined by thin layer chromatography, TLC. The TLC analyses were performed on cellulose TLC plates (Eastman E-13255). Each spot contained 35–85 μg (depending on the experiment) of the sample. The plates were developed in an ascending direction at 23 °C with ethyl acetate: acetic acid: water (4:2:3. v/v/v). The spots were visualized by spraying the plates with 10 % ammonia solution followed with bromophenol blue according to Liu and Luh.[6] For quantitative assay, an aliquot of 85 μg of hydrolysate was applied for each spot. Quantitative analysis of the oligogalacturonic acid was performed by scraping the acidic spots from the thin-layer plates and analyzing for the content of anhydrogalacturonic acid by the carbazole method described above.

Determination of neutral saccharides

The neutral saccharides were determined by liquid chromatography (HPLC) analysis on a Spectra-Physics Sp 8000 instrument, with a Rheodyne fixed injector (20 μl) using a Bio Rad HPX-87P column (30 cm×7.8 mm, packed with 9 μm spherical sulfonated polystyrene–divinylbenzene beads with 8 % of cross-linked bonds). The neutral saccharides present in the

samples of the oligogalacturonic acid fractions were derivatized prior to the HPLC analysis. Detection of the derivates was performed at 260 nm, which is the extinction maximum for 4-nitrobenzoyl sugar derivatives at ambient temperature. An acetonitrile–water mixture (400:100) was used as the eluent. The flow rate was 1.0 ml min⁻¹. The derivatization of saccharides was performed with 4-NBCl in pyridine according to a modified procedure described by Nachtmann et $al.$[14,15] The reaction was performed in stoppered 10-ml centrifuge tubes. Fifty µl of the samples with 10 % (w/v) of galacturonic acids where mixed with 150 µl of the reagent solution (prepared by dissolving 100 mg of 4-NBCl in 1 ml of pyridine with gentle warming), well shaken, and reacted for 10 min at room temperature. After the reaction, the derivatives were extracted. The pyridine was first removed in a desiccator under a water suction vacuum. The centrifuge tubes were then flushed with an air or nitrogen stream and 2 ml of a 5 % $NaHCO_3$ solution containing 5 mg of 4-dimethylaminopyridine was added. The excess reagent was hydrolyzed after 5 min of treatment in an ultrasonic bath. A blank treated simultaneously should yield a clear solution. The derivatives were then extracted with 2 ml of chloroform and treated with 2 ml of a 5 % $NaHCO_3$ solution and twice with 3 ml of a 0.05 M HCl solution containing 5 % NaCl. This led to the quantitative isolation of the derivatives and complete exclusion of excess reagent and pyridine.

RESULTS AND DISCUSSION

Separation of oligogalacturonides

The yields of saturated and unsaturated oligogalacturonic acids obtained by enzymatic decomposition of pectin preparations from Budimka apple are given in Table I. The saturated and unsaturated di- to octagalacturonides were separated using the same Dowex-1X8 ion-exchange column chromatography but with different eluents. As shown in Table I, the overall yield of saturated oligogalacturonic acids was 60.13 % (w/w), which was higher than the overall yield of unsaturated oligogalacturonic acids, amounting to 53.45 %. In both cases, trimers were obtained in the largest amount (16.76 % for saturated oligogalacturonic acids and 12.25 % for unsaturated oligogalacturonic acids), followed by dimers (12.45 % for saturated oligogalacturonic acids and 9.61 % for unsaturated oligogalacturonic acids) and tetramers (9.21 and 9.02 %, respectively), and the amounts decreased towards oligogalacturonic acids with higher degrees of polymerization, up to octamer. The method employed for the determination of the degree of polymerization (DP) of the fractions of the oligogalacturonic acids, $i.e.$, according to the ratio of AGA to the content of reducing groups (AGA/CHO), was found to be an effective and precise method for both the saturated and unsaturated oligogalacturonic acids (Table I).

The fractions of oligogalacturonic acids present in the incubation mixtures obtained by enzyme action were identified by TLC on the basis of the R_{ga} values, which are presented in Table I (the R_{ga} values represent the relationship between the migration distance of the sample spot and that of the standard). It is obvious that the oligogalacturonic acid fractions identified by the R_{ga} values corresponded very well to the DP calculated on the basis of the AGA and CHO contents. The purity of the identified TLC spots, which was determined by scraping of the

acidic spots from the thin-layer plates and analyzing for the content of anhydro-galacturonic acid by the carbazole method (data not presented), was more than 98 %, thus indicating that a very good separation was achieved.

TABLE I. Yield of saturated and unsaturated oligogalacturonic acids (*DP* 2–8) obtained from 10.0 g of pectin preparation (extracted from Budimka apple) containing 93 % polygalacturonic acid (*DE* 75, *DP* 134)

Oligogalactouronic acid	*DP*	R_{ga}	Saturated			Unsaturated		
			Obtained amount, g	Yield w/w %	AGA/CHO	Obtained amount, g	Yield w/w %	AGA/CHO
Dimer	2	0.67	1.16	12.45	2.01	0.92	9.61	2.02
Trimer	3	0.51	1.56	16.76	3.09	1.19	12.25	3.07
Tetramer	4	0.39	0.86	9.21	3.97	0.87	9.02	4.06
Pentamer	5	0.28	0.69	7.43	4.99	0.68	6.98	4.98
Hexamer	6	0.21	0.54	5.84	6.01	0.63	6.45	5.97
Heptamer	7	0.15	0.46	4.95	7.02	0.52	5.39	7.03
Octamer	8	0.11	0.32	3.49	7.98	0.38	3.95	8.05
Total	–	–	5.59	60.13	–	5.19	53.65	–

The presented results demonstrate that it was possible to employ the same column but with different eluents to separate the unsaturated and saturated uronides. It was noticed that the elution power of the acetate buffer used for the separation of the saturated oligogalacturonides was stronger than the elution power of the formate buffer used for the separation of the unsaturated oligogalacturonides. Thus, a higher concentration of the eluent was applied for the elution of the unsaturated uronides than that used for the elution of the saturated uronides of the same chain length.

The elution profiles for the saturated and unsaturated oligogalacturonic acids are shown in Figs. 1 and 2, respectively. It can be seen that the unsaturated uronides were eluted as broader peaks (Fig. 2). The first small peak at the beginning of the elution profile of the saturated oligogalacturonic acids (Fig. 1) was not part of the homologous series of oligalactouronides. Moreover, the first two small peaks of the profile of the unsaturated oligogalacturonic acids (Fig. 2) were also not part of the homologous series of oligalactouronides. This was confirmed by TLC analysis.

Determination of saccharides

The content of neutral saccharides (rhamnose, galactose, arabinose, xylose, mannose and glucose) in the original apple sample is presented in Fig. 3. The content of the total neutral saccharides was also determined in the saturated and unsaturated fractions of oligogalacturonic acids and expressed relative to the content of dry mass (Tables II and III), as well as the content of individual saccharides (data not presented).

The total content of neutral saccharides in the original Budimka apple pectin amounted to 5.31 % (Fig. 3). Galactose was present in the greatest amount (1.16 %)

in the original sample, while the content of mannose was the lowest (0.63 %). The contents of rhamnose, arabinose, xylose and glucose were similar and below 1 % in the Budimka apple pectin. The presented results of the neutral saccharides indicated a heteropolysaccharide content of Budimka apple pectin compounds, since a larger content of different saccharides in the isolated fractions of oligogalacturonic acids was detected. These sugars are integral constituent of pectic polysaccharides, which was indicated by the failure of fractionations by ion-exchange chromatography. Generally, the neutral sugar side chains are linked to rhamnogalacturonan segments within the pectin molecule.[16] The presence of xylose and glucose could be explained, in part, by the presence of xyloglucans, which have been shown to be associated with pectic material,[17] or by xylogalacturonan.[18]
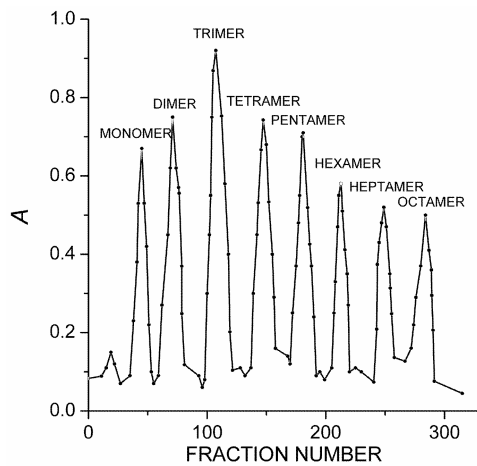

Fig. 1. The elution profile for saturated oligogalacturonic acids (*DP* 2–8) isolated by ion-exchange chromatography on a Dowex 1X-8 column (200–400 mesh), acetate form, in a step-wise gradient using 0.2–0.8 M Na-acetate buffer at pH 6.0.
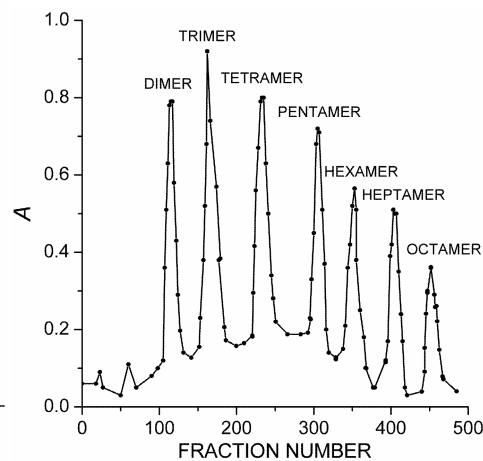
Fig. 2. The elution profile of unsaturated oligogalacturonic acids (*DP* 2–8) separated by ion-exchange chromatography on a Dowex 1X-8 column (200–400 mesh), formate form, in a step-wise gradient using 0.2–0.8 M sodium formate buffer (pH 4.7).
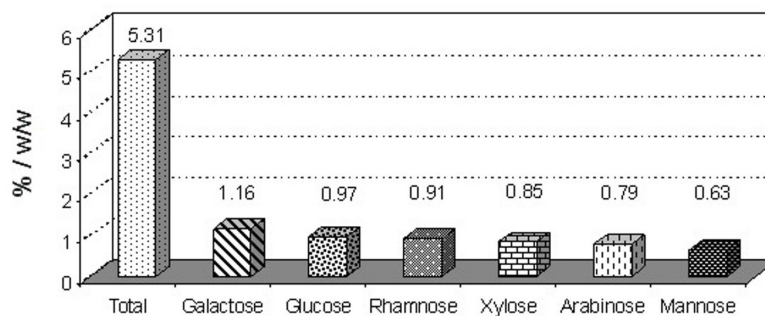

Fig.3. Total and individual content of individual neutral saccharides (galactose, glucose, rhamnose, xylose, arabinose and mannose) determined in Budimka apple pectin.

TABLE II. Composition of the original Budimka apple pectin sample and of saturated oligo-galacturonic acids fractions (*DP* 2–8) of the pectin separated on a Dowex 1X-8 (acetate) column

Quantities in %		Original sample	Oligogalacturonic acid						
			Di-	Tri-	Tetra-	Penta-	Hexa-	Hepta-	Octa-
Degree of esterification		75	68.2	63.5	59.4	58.6	56.9	56.8	53.0
Degree of polymerization		134	2.07	2.95	3.01	4.97	6.03	7.02	8.09
M_r (end groups) / g mol^{-1}		17400	9500	17300	29300	27890	31090	37800	40780
Galacturonic acid content		93	94.7	95.2	93.9	89.4	88.7	86.0	83.7
Neutral sacch. content		5.31	3.41	3.24	4.63	5.47	5.33	6.28	7.91
Methanol content		0.070	0.070	0.050	0.050	0.050	0.050	0.040	0.040
Content of phenolics	VSR	0.15	0.02	0.02	0.02	0.03	0.06	0.07	0.6
	FCR	3.25	1.53	1.32	0.62	0.64	0.69	2.77	2.43
	VSR/FCR	4.6	6.5	4.6	4.4	3.9	3.3	2.5	2.4
Humidity		5.7	8.6	8.2	6.5	7.3	8.9	9.50	9.65
Ash		0.37	0.68	0.79	0.78	0.92	0.64	0.50	0.65

TABLE III. Composition of the unsaturated oligogalacturonic acids fractions (*DP* 2–8) of Budimka apple pectine separated on a Dowex-1X8 (formate) column

Quantities in %		Oligogalacturonic acid						
		Di-	Tri-	Tetra-	Penta-	Hexa-	Hepta-	Octa-
Degree of esterification		72.5	70.8	64.7	61.9	58.7	57.3	54.8
Degree of polymerization		2.07	3.05	4.09	5.00	6.09	7.05	8.00
M_r (end groups) / g mol^{-1}		19500	27480	32720	39650	46900	53000	58390
Galacturonic acid content		91.7	88.4	85.3	81.9	78.9	75.3	72.8
Neutral sacch. content		3.43	3.65	4.40	4.75	5.05	5.55	5.65
Methanol content		0.03	0.09	0.08	0.05	0.07	0.05	0.06
Content of phenolics	VSR	1.97	1.36	0.64	0.86	0.46	0.58	0.58
	FCR	6.35	5.92	4.79	4.53	4.28	4.19	2.43
	VSR/FCR	4.13	4.72	5.78	7.05	8.03	10.68	10.84
Humidity		7.5	7.0	6.8	6.4	6.9	6.3	7.2
Ash		0.63	0.69	0.75	0.81	0.75	0.68	0.55

The amounts of neutral sugars found in the pectin preparations corresponded to those presented in a study on a model of cell wall structure by Talmadge *et al.*[19] Smaller amounts of rhamnose and galactose were observed in fractions containing compounds of lower M_r, confirming that these two sugars might be incurporated into the main chain of the pectin molecule. The successive increase of the content of rhamnose and galactose in the oligogalacturonides with longer chains suggested (data not presented) the assumption that these sugars are included in the main chain of the pectin molecules. Direct evidence for L-rhamnose as an integral constituent in several pectic acids, for example, that from alfa (lucerne) has been obtained by the isolation of aldobiouronic acid 2-*O*-(*α*-D-galactopyranosyluronic acid)-L-rhamnose, as a product of partial acid hydrolysis.[20] Other neutral residues in pectins are probably attached as side chains as depicted on the partial structure shown in Fig. 4.
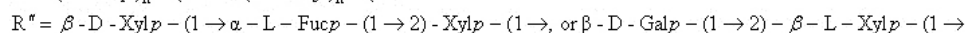
4) - α - D - Gal*p*A - (1 → 2) - β - L - Rha*p* - (1 → 4) - α - (1 → 4) – α – D – Gal*p*A - (1 →
$\qquad$ ↑ – – – – – – – – – – ↑ $\qquad\qquad\qquad\qquad$ ↑
$\qquad\qquad$ R′ $\qquad\qquad\qquad\qquad\qquad\qquad\qquad$ R″

R′ = (D - Gal*p*)$_n$ – (1 → or (L – Ara*f*)$_n$ – (1 →
R″ = β - D - Xyl*p* – (1 → α – L – Fuc*p* – (1 → 2) - Xyl*p* – (1 →, or β - D - Gal*p* – (1 → 2) – β – L – Xyl*p* – (1 →
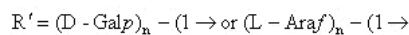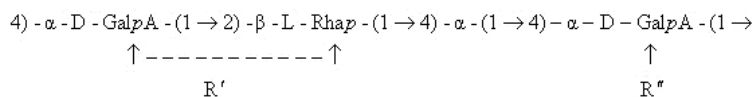
Fig. 4. Model of the partial structure of the neutral residues of pectin compounds.

Chemical composition of the fractions of oligogalacturonic acids

The overall chemical composition of the fractions of saturated and unsaturated oligogalacturonic acids fractions (*DP* 2–8) of Budimka apple pectin are presented in Tables II and III, respectively. It can be seen from these Tables that the degrees of esterification of both the saturated and unsaturated oligogalacturonic acids decreased with increasing chain length of the oligogalacturonic acid. The elution of poorly methylated pectin compounds was possible only at higher buffer concentrations. This leads to the conclusion that pectin binding to ion-exchange resins depends on both the number of free carboxyl groups and the strength of the negative charges in the macromolecule. The extent of esterification in the isolated saturated oligogalacturonic fractions ranged from 68.2 to 53.0 (Table II), while in the unsaturated oligogalacturonic acids, it ranged from 72.5 to 54.8 (Table III). The decrease of the degree of esterification in the isolated oligomers from di- to octamer was accompanied by an increase in the total content of phenolics. It is supposed that the presence of phenolics in pectin compounds amplifies the negative charge of the molecule, which influences the strength of the bonds formed with ion-exchange resins. Most of the phenolics contained in the chain of pectin compounds are not directly bound to pectin since, under the employed experimental conditions for the separation of oligogalacturonides by ion-exchange chromatography, the eluates contained some 96 % of the starting uronides and about 10 % of the starting amount of phenolics in the analyzed pectin preparations.

Oligomers of increasing M_r were eluted with increasing buffer concentration, for both the saturated and unsaturated oligogalacturonic acid fractions (Tables II and III). The gel-filtration effect on the ion-exchange material used for the isolation of oligogalacturonides cannot be the reason for this trend because the sequence of M_r values in the eluted fractions would be reversed in such a case. It is assumed that other bonds are formed, besides the ionic ones (*e.g.*, van der Waals bonds), which are independent of the degree of esterification. Their number and strength would increase with increasing pectin chain length. Moreover, the presence of phenolic hydroxyl groups may lead to an amplification of this effect.

CONCLUSIONS

The isolation of pure homologous fractions, according to the degree of polymerization, of saturated and unsaturated oligogalacturonic acids obtained by

controlled enzymatic hydrolysis of the Budimka variety of apple could be simply and efficiently performed by ion exchange chromatography on a Dowex 1X-8 column.

The saturated oligogalacturonic acids, obtained by controlled hydrolysis with FPG, were eluted with a gradient of Na acetate buffer, pH 6.0, while the unsaturated oligogalacturonic acids (4,5-dehydrogalacturonosyl unit on the non-reducing end), obtained by controlled hydrolysis with PL, were separated on the same resin but using a gradient of Na formate buffer, pH 4.7, as the eluent. The total yields of oligogalacturonic acids recovered from the hydrolysate of polygalacturonic acid were 60.13 % for the saturated (*DP* 2–8) and 53.65 % for the unsaturated (*DP* 2–8) forms. It was confirmed that the separated oligogalacturonides were very pure (≈ 99 % of AGA).

The presence of neutral saccharides, *i.e.*, rhamnose, galactose, arabinose, xylose, mannose and glucose, in the original Budimka apple pectin and in the obtained fractions was confirmed by HPLC analysis of 4-nitrobenzoyl sugar derivatives. The total content of neutral saccharides in the original Budimka apple pectin amounted to 5.31 %. The contents of rhamnose, arabinose, xylose and glucose were similar and below 1 % in the Budimka apple pectin.

The degree of esterification of the isolated oligogalacturonic acids decreased with increasing chain length.

И З В О Д

ДЕГРАДАЦИЈА И КАРАКТЕРИЗАЦИЈА ПЕКТИНА ЈАБУКЕ СОРТЕ БУДИМКА

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У овом раду је испитиван хемијски састав и карактеристике пектина јабуке аутохтоне сорте будимка, која је карактеристична за регион централне Србије. Након екстракције пектина из јабуке, извршена је контролисана ензимска хидролиза помоћу ензима полигалактуроназе (PG) и пектин-лијазе (PL) из *Aspergillus niger* и добијени хидролизати су фракционисани помоћу јоноизмењивачке колоне Dowex 1X-8. Сепарација засићених олигогалактуронских киселина, добијених контролисаном хидролизом помоћу полигалактуроназе је ефикасно извршена градијентном елуцијом са Na-ацетатним пуфером, pH 6,0, док је сепарација незасићених олигогалактуронских киселина добијених контролисаном хидролизом са пектин-лијазом извршена на истој јоноизмењивачкој колони, али коришћењем Na-формијатног пуфера pH 4,7. Такође је утврђен принос индивидуалних фракција различитог степена полимеризације. Укупан садржај неутралних сахарида, који је утврђен помоћу HPLC је износио 5,31 %. Неутралне шећере пектина јабуке будимка чине рамноза, арабиноза, ксилоза, маноза и глукоза.

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