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Structural characteristics of pectic polysaccharides from olive fruit (*Olea europaea* cv moraiolo) in relation to processing for oil extraction

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Abstract

In the olive oil industry technical enzyme preparations are used as processing aids to improve the extraction yield and product quality. In order to obtain more insight in the mechanisms by which these enzyme preparations lead to a higher yield and a better quality, the effect of these preparations on the structure of the pectic polysaccharides present in the cell wall of olive fruit was investigated. Four pectin-rich fractions were isolated from the cell wall material of non-enzyme treated and enzyme treated olive fruit by successive extractions with cold buffer, hot buffer, chelating agents and diluted alkali and analysed. The results revealed that the use of technical enzyme preparations during processing mainly affected the pectic material present in the cold and hot buffer fractions. The structures of the arabinose-rich pectic polysaccharides solubilised by extraction with chelating agents and diluted alkali were barely affected by the use of enzyme preparations. The changes of the buffer soluble pectic material were reflected by a decrease in methyl esterification, a change in molecular weight distribution and a degradation of the (1 → 4)-linked galactan chains. No differences were observed in the composition of the arabinan chains. Also, the structural features of the polysaccharides present in the vegetation waters, the liquid by-product of olive processing, were studied. The sugar composition indicated the presence of mainly material of pectic origin in the vegetation waters. As a result of enzymatic processing the degree of methyl esterification decreased, the profile of the molecular weight distribution changed and the (1 → 4)-linked galactan chains were degraded as was also shown for the buffer soluble pectic polysaccharides.

Keywords: Olive oil; Pectic polysaccharides; Enzyme preparations; Processing

1. Introduction

In the processing of fruits and vegetables technical enzyme preparations are used as aids to improve the yield and quality of the product (Voragen, 1990). Also in the olive oil industry technical enzyme preparations are used as processing aids and several studies have been carried out on the effect of enzymatic treatment on the yield and quality characteristics of the oil (Domínguez, Núñez, & Lema, 1994; Montedoro, Begliomini, Servili, Petruccioli, & Federici, 1993; Ranalli & De Mattia, 1997; Ranalli & Serraiocco, 1995; Servili, Begliomini, Montedoro, Petruccioli, & Federici, 1992). However, still very little is known about the specific role of the various constituent enzymes present in the enzyme preparations.

One of the commercial enzyme preparations used in the olive oil industry is the pectinolytic enzyme preparation Olivex, which is known to significantly increase the oil extraction output (Ranalli & Serraiocco, 1995) and to improve certain olive oil quality indicators (Christensen, 1991; Vierhuis, Servili, Baldioli, Schols, Voragen & Montedoro, 2001b). Since Olivex has a prevalent pectinolytic activity the primary substrates for the enzymes are expected to be pectic polysaccharides in the cell wall material. A previous study of our group on pectins present in olive paste has revealed that the yield of buffer extractable pectins increases when the pectinolytic enzyme preparation Olivex is used and that the buffer extractable pectins are enriched in uronic acid (Vierhuis et al., 2001b). A more detailed study of the effect of the enzymes present in the technical enzyme preparation Olivex on the structure of the pectic polysaccharides present in olive fruit is reported in this paper. In Sections 3.1, 3.2 and 3.3, the effect of the addition of commercial enzyme preparations during processing on the structural features of the polysaccharides present in the vegetation waters, the liquid by-product of olive processing was studied. In Sections 3.4, 3.5 and 3.6, the effect of the addition of enzyme preparations on the

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structure of the cell wall polysaccharides in the olive paste was investigated.

2. Experimental

2.1. Materials

2.1.1. Substrates

Processed olive fruit (*Olea europaea* cv moraiolo) from pilot plant experiments was kindly supplied by the Department of Food Sciences of the University of Perugia, Italy. The olive fruit (90 kg) was ground with a hammer crusher and the oily paste was slowly mixed (malaxed) for 60 min at 30 °C. The extraction of the oil from the malaxed paste was performed with a decanter with a working capacity of 1.00 ton/h (paste/water ratio 1:0.15 (w/v)). Technical enzyme preparations were added to the malaxer when the olive fruit was ground (Olivex in combination with Novoferm 12, 500 mg/kg of paste) and analysis of the olive oil revealed that the quality of the oil was improved when the enzyme preparations were added. The results of the analysis of the oil have been described in a previous paper of our group (Vierhuis et al., 2001b). Directly after malaxation samples of the paste were taken for analyses (MP^-: Malaxed paste blank; MP^+: Malaxed paste + Olivex/Novoferm 12). Also samples of the vegetation water, the liquid by-product, which is produced during the extraction of the oil were taken for analyses (VW^-: Vegetation water blank; VW^+: Vegetation water + Olivex/Novoferm 12). Alcohol insoluble solids (AISs) were isolated from the malaxed pastes and vegetation waters as described (Vierhuis et al., 2001b).

2.1.2. Enzymes

Olivex is an enzyme preparation with pectolytic, hemicellulolytic and cellulolytic activities produced by *Aspergillus aculeatus* and adapted for the extraction of olive oil. Novoferm 12 is an enzyme preparation of *Aspergillus niger* origin, which contains in addition to pectolytic enzyme activities also a β-glucosidase activity. Both preparations were kindly provided by Novo Nordisk Ferment Ltd (Dittingen, Switzerland).

Endo-polygalacturonase originated from *Kluyveromyces fragiles* and pectin methyl esterase from *A. niger* (Schols, Posthumus, & Voragen, 1990). Pectin lyase was purified from a commercial enzyme preparation of *A. niger* (Van Houdenhoven, 1975). Arabinofuranosidase B was isolated from a preparation derived from *A. niger* (Rombouts, Voragen, Searle-van Leeuwen, Geraeds, Schols & Pilnik, 1988). Endo-arabinanase was purified from a cloned enzyme preparation of arabinanases produced by *A. aculeatus* from Novo Nordisk A/S (Copenhagen, Denmark). Exo-galactanase was purified from an *A. niger* preparation (Van de Vis, 1994). Endo-galactanase was purified from a commercial enzyme preparation from *A. aculeatus* (Van de Vis, Searle- van Leeuwen, Siliha, Kormelink, & Voragen, 1991).

2.2. Sequential extraction of AIS

Pectins present in the AISs prepared from differently treated olive pastes (MP^- and MP^+) were sequentially extracted with various solvents as described by Schols, Vierhuis, Bakx, and Voragen (1995). The extraction procedure was extended with an extraction with 4 M KOH to study also the hemicellulosic polysaccharides present in the olive pastes. AIS (15 g) was sequentially extracted with 0.05 M NaOAc buffer, pH 5.2 (three times 600 ml) at room temperature for 60 min (Cold Buffer Soluble Solids, CBSS); 0.05 M NaOAc buffer, pH 5.2 (three times 550 ml) at 70 °C for 30 min (Hot Buffer Soluble Solids, HBSS); 0.05 M EDTA and 0.05 M NH_4-oxalate in 0.05 M NaOAc-buffer, pH 5.2 (two times 450 ml) at 70 °C for 30 min (Chelating agent Soluble Solids, ChSS); washed with distilled water; extracted with 0.05 M NaOH (two times 450 ml) at 4 °C for 30 min (0.05 M NaOH fraction); 4.0 M KOH + 20 mM $NaBH_4$ (two times 450 ml) at room temperature for 2 h (4 M KOH fraction). After each extraction, solubilised polymers were separated from the insoluble residue by centrifugation. The supernatants were filtered through a G3 glass sinter (those containing alkali were neutralised by adding glacial acetic acid), dialysed and freeze dried. The final residue was also dialysed and freeze dried.

2.3. Enzyme incubations

The pectin-rich pools were dissolved in a 50 mM NaOAc buffer of pH 5.0 containing 0.01% (w/v) NaN_3 and incubated with purified enzyme. The amount of enzyme used was 1.0 μg protein/ml for endo-polygalacturonase, pectin methyl esterase and pectin lyase and 2.0 μg protein/ml and 10.0 μg protein/ml for endo-arabinanase and arabinofuranosidase B, respectively. For the incubations with galactanases the pectin-rich pools were dissolved in a 100 mM NaOAC buffer of pH 5.0 containing 0.01% (w/v) NaN_3. The amount of enzyme used was 0.5 μg protein/ml for exo-galactanase and 2.0 μg protein/ml for endo-galactanase. All incubations were performed at 40 °C for 24 h with a substrate concentration of 1 mg/ml. The resulting digests were heated for 15 min at 100 °C to inactivate the enzymes. The change in molecular weight distribution and the release of monomeric and oligomeric end products were studied by high-performance size-exclusion chromatography (HPSEC) and high-performance anion-exchange chromatography (HPAEC), respectively. For each enzyme it was checked that the limit of digestion was reached after 24 h for the concentration of the enzyme used. For that, the samples were re-incubated with a fresh batch of enzyme and analysed by HPSEC and HPAEC.

The incubations with endo-polygalacturonase were also performed on chemically saponified pectin material. The

pectins were saponified in a 0.05 M NaOH solution for 4 h at 0 °C followed by neutralisation with 0.5 M HAc. The solutions were adjusted to the desired pH and molarity with a 0.5 M NaOAc buffer of pH 5.0 and used for enzymatic degradation studies with endo-polygalacturonase.

2.4. Analytical methods

2.4.1. Sugar (linkage) composition

The neutral sugar composition was determined by gas chromatography according to Englyst and Cummings (1984) using inositol as internal standard. The AISs and residues of the sequential extraction of the AISs were treated with 72% (w/w) sulphuric acid for 1 h at 30 °C prior to hydrolyses with 1 M sulphuric acid for 3 h at 100 °C. The released constituent sugars were analysed as their alditol acetates. Cellulosic glucose in the AISs and the residues was calculated as the difference between the glucose contents determined with and without pre-treatment with 72% (w/w) sulphuric acid. The sugar composition of the pectin-rich fractions and the 4 M KOH fraction was determined by a direct hydrolyses without a pre-treatment with 72% (w/w) sulphuric acid, because no cellulose was expected in these samples. The uronic acid content was determined colorometrically by the automated *m*-hydroxydiphenyl assay (Thibault, 1979). Galacturonic acid was used as a standard. Corrections were made for the interference of neutral sugars in the samples. The sugar linkage composition was determined as described previously (Vierhuis, Schols, Beldman, & Voragen, 2001a).

2.4.2. Determination of the methoxyl and acetyl content

The amount of methyl esters and *O*-acetyl groups was determined by HPLC after saponification with 0.4 M NaOH according to Voragen, Schols, and Pilnik (1986) and expressed as mol methyl esters or *O*-acetyl groups per 100 mol uronic acid.

2.4.3. High-performance anion-exchange chromatography

The pectin digests obtained after endo-polygalacturonase treatment were analysed by HPAEC at pH 5 to detect the partially methyl esterified galacturonic acid oligomers (Daas, Arisz, Schols, Ruiter, & Voragen, 1998). Post-column sodium hydroxide addition allowed pulsed amplified detection. Calibration was performed with a standard mixture of partially methyl esterified galacturonic acid oligomers.

HPAEC at high pH was performed as described previously (Vierhuis et al., 2001a). The release of monomeric and oligomeric end products was analysed with the following NaOAc gradient in 100 mM NaOH: 0 → 50 min, linear gradient of 0 → 0.5 M NaOAc; 50 → 60 min, linear gradient of 0.5 → 1 M NaOAc. In addition, the galacturonic acid oligomers were analysed using the following gradient resulting in a better separation of the larger oligomers: 0 → 40 min, linear gradient of 0.2 → 0.7 M NaOAc; 40 → 45 min, linear gradient of 0.7 → 1 M NaOAc. After each run the column was washed for 5 min with 1 M NaOAc in 100 mM NaOH and subsequently equilibrated for 15 min with the starting eluent.

2.4.4. High-performance size-exclusion chromatography

HPSEC was performed on a SP8810 HPLC (Spectra Physics) equipped with three Bio-Gel TSK columns (each 300 × 7.5 mm) in series (40XL, 30XL and 20XL; Bio-Rad Labs) in combination with a TSK XL guard column (40 × 6 mm). Elution took place at 30 °C with 0.4 M NaOAc (pH 3.0) at a flow rate of 0.8 ml/min for the AIS fractions of the pastes. For the analysis of the polymeric material in the vegetation waters 0.2 M $NaNO_3$ was used as eluent instead of 0.4 M NaOAc (pH 3.0). This new eluent gave elution profiles comparable with the former elution profiles of the vegetation waters presented in a previous study (Vierhuis et al., 2000b). The column effluent was monitored using a refractive index detector (Shodex RI SE-61). Calibration was performed using dextrans (M_w 4–500 kDa).

2.4.5. Mass spectrometry

Matrix-assisted laser-induced desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) was performed as described (Vierhuis et al., 2001a)

3. Results and discussion

3.1. Composition of the polysaccharides present in the vegetation waters

The polysaccharides present in the vegetation waters of the non-enzyme treated sample (VW^-) and the enzyme treated sample (VW^+) were isolated to determine the effect of the addition of commercial enzyme preparations during processing on the water soluble polysaccharides. About 5–10% of the polysaccharides present in olive fruit were soluble in water and turned up in the vegetation waters (VW^- and VW^+) when the oil was extracted from the malaxed paste with a decanter. Unfortunately, the exact amount of the polysaccharides present in VW^- and VW^+ and the amount of additional polysaccharides released by the enzyme preparations from the olive fruit cannot be given, because too many assumptions had to be made with regard to the mass balance. However, based on the results of a previous study by our group (Vierhuis et al., 2001b) and the results described in Section 3.4 (Tables 2 and 3) we expect that the use of enzyme preparations during processing increased the amount of polysaccharides in the vegetation waters with about 30–40%.

The sugar composition of the polysaccharides indicated the presence of material mainly of pectic origin in VW^- and VW^+ (Table 1). Arabinose and galactose were the major neutral sugars in both samples. Arabinose was mainly

Table 1
Neutral sugar linkage composition (mol%) of the AIS isolated from the vegetation waters of non-enzyme treated paste (VW^-) and enzyme treated paste (VW^+); within brackets the neutral sugar composition determined by alditol acetates and the uronic acid content determined by the *m*-hydroxydiphenyl assay is given (mol per 100 mol neutral sugars)

Sugar linkage	Vegetation water$^-$ (VW^-)	Vegetation water$^+$ (VW^+)
Rhamnose		
T-Rha*p*	1.2	1.0
1,2-Rha*p*	0.1	0.1
1,2,4-Rha*p*	0.1	0.2
1,2,3,4-Rha*p*	0.4	0.2
Total	1.8(6)	1.5(7)
Fucose		
T-Fuc*p*	0.2	0.2
Total	0.2(–)	0.2(–)
Arabinose		
T-Ara*f*	20.1	22.8
1,2-Ara*f*	0.4	0.2
1,3-Ara*f*	1.4	2.3
1,5-Ara*f*	20.8	26.7
1,2,5-Ara*f*	1.1	1.1
1,3,5-Ara*f*	5.4	9.3
1,2,3,5-Ara*f*	1.6	1.0
Total	50.8(39)	63.4(53)
Xylose		
T-Xyl*p*	2.9	1.7
1,2-Xyl*p*	3.5	2.1
1,2,3,4-Xyl*p*	0.3	0.6
Total	6.7(5)	4.4(3)
Mannose		
1,4-Man*p*	0.3	0.3
1,2,3,4,6-Man*p*	0.4	0.5
Total	0.7(2)	0.8(3)
Galactose		
T-Gal*p*	2.0	2.4
1,3-Gal*p*	4.3	4.3
1,4-Gal*p*	5.4	0.1
1,6-Gal*p*	2.1	4.6
1,3,4-Gal*p*	0.2	0.2
1,3,6-Gal*p*	12.3	13.1
1,2,3,4,6-Gal*p*	0.1	0.2
Total	26.4(35)	24.9(28)
Glucose		
1,4-Glc*p*	7.6	4.2
1,4,6-Glc*p*	3.2	0.2
1,2,3,4,6-Glc*p*	2.6	0.4
Total	13.4(13)	4.8(6)
Uronic acid		
Total	–[a](64)	–(167)
Ratio terminal/branching	0.73	0.90
Carbohydrate content[b]	32	47

[a] Not included in the sugar linkage analysis.
[b] Expressed as % (w/w).

present as terminal and 1,5-linked residues in the non-enzyme treated sample VW^-. About 13% of the arabinose residues were mono substituted with branch points at C-2 or C-3. In addition, a small amount of double substituted arabinose residues could be detected. The low proportion of branched arabinose residues indicated a relatively low degree of branching of the arabinans present in VW^- compared to arabinans isolated from other sources (Beldman, Schols, Pitson, Searle- van Leeuwen, & Voragen, 1997). Most of the galactose residues present in the VW^- appeared to be present as (1 → 3)/(1 → 6)-linked galactans with a high degree of branching. Because of the high degree of branching of the (1 → 3)/(1 → 6)-linked galactans in VW^- and the low degree of branching of the arabinans it is very likely that a relative large proportion of the terminal arabinose residues originated from the side chains of (1 → 3)/(1 → 6)-linked galactans. (1 → 3)/(1 → 6)-Linked galactans are often found to be associated with hydroxyproline-containing proteins (O'Neill, Albersheim, & Darvill, 1990). However, they can also be present as covalently linked side chains of rhamnogalacturonan I or as separate polysaccharides (Voragen, Pilnik, Thibault, Axelos, & Renard, 1995). About 20% of the galactose residues was present as (1 → 4)-linked galactan in VW^-.

The glycosidic linkage composition showed that the use of enzymes during processing resulted in an almost complete degradation of the (1 → 4)-linked galactan chains. No major changes of the composition of the other neutral sugar linkages were noticed due to the use of enzyme preparations. The various arabinose linkages were present in almost similar ratios in both vegetation waters. Furthermore, the sugar composition showed that the galacturonic acid content of the polysaccharides present in VW^+ was relatively high compared to polysaccharides present in VW^-. The use of commercial enzyme preparations might have solubilised additional pectic material with a high galacturonic acid content or part of the pectic material may have been partly debranched. The level of methyl esters of the pectic material present in the vegetation waters decreased due to the use of commercial enzyme preparations. The degree of methyl esterification was 75 and 39 for VW^- and VW^+, respectively. The degree of acetylation was about 20 for both samples.

The HPSEC elution patterns of the pectic material present in the vegetation waters are shown in Fig. 1. Both samples contained material with a broad molecular weight distribution. The use of enzyme preparations changed the profile and shifted the molecular weight distribution of the material towards the lower molecular weight ranges, but most pectic material in VW^+ remained present as material with a high molecular mass.

3.2. Treatment of the polysaccharides from the vegetation waters with pectin lyase, endo-polygalacturonase and pectin methyl esterase

To obtain more information on the structure of the galacturonic acid backbone, the pectic polymers present in the vegetation waters were studied by incubation with pectin

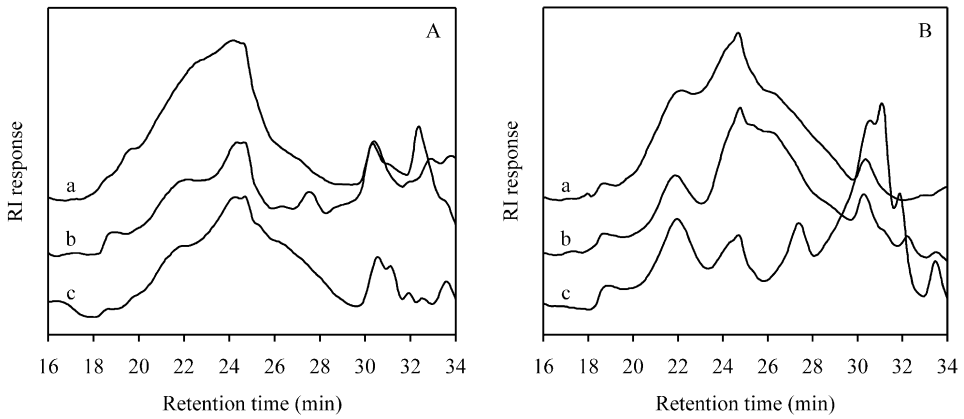

Fig. 1. HPSEC-patterns of the polysaccharides isolated from the vegetation waters of (A) non-enzyme treated paste, VW^- and (B) enzyme treated paste, VW^+ (a) before incubation and after incubation with (b) pectin lyase and (c) endo-polygalacturonase.

lyase and endo-polygalacturonase. Pectin lyases split glycosidic linkages between methyl esterified galacturonide residues by a trans-elimination reaction, and therefore, have a preference for high-methyl esterified pectins. Endo-polygalacturonases preferentially hydrolyse low-methyl esterified pectins or pectic acid because these enzymes can only split glycosidic linkages adjacent to galacturonic acid residues with free carboxyl groups (Voragen et al., 1995).

Analysis of the pectin lyase digests by HPSEC showed that pectin lyase was able to degrade the polymers in VW^- to a larger extend than those present in VW^+ (Fig. 1). This agreed with the higher degree of methyl esterification of the former substrate. Analysis of the digests by HPAEC showed the presence of mainly unsaturated galacturonic acid oligomers with a degree of polymerisation (DP) of 2–10. Also small quantities of saturated oligomers were detected. The saturated oligogalacturonic acids were most certainly released by an endo-polygalacturonase impurity in the pectin lyase preparation. Pectin lyase was able to release ten times higher amounts of oligosaccharides from VW^- compared to VW^+ as calculated from the release of oligomers analysed by HPAEC expressed as percentage of the total amount of galacturonic acid present. However, despite of the large decrease in molecular mass on HPSEC, the amount of oligomeric degradation products in the digest of VW^- was rather low. Apparently, relatively large degradation products remained after incubation with pectin lyase, which could not be further degraded to smaller oligosaccharides to be analysed on HPAEC.

Analysis of the endo-polygalacturonase digests by HPSEC showed that the pectic material present in VW^- was rather resistant to the pectic enzyme, which agreed with the degree of methyl esterification of 75 (Fig. 1). The incubation of the low methyl esterified VW^+ with endo-polygalacturonase resulted in a drastic shift in retention times on HPSEC. Analysis of the endo-polygalacturonase digests on HPAEC showed that the degradation products were monogalacturonic acid and galacturonic acid oligomers in the range of DP 2–10. The analysis on HPAEC confirmed that endo-polygalacturonase was more active towards VW^+ than VW^- based on the amount of oligomers released expressed as percentage of the total amount galacturonic acid present in the sample. About three times higher amounts of oligosaccharides were released from VW^+ compared to VW^-. An exact quantification of the oligomers was not possible, because the PAD-response factors were not known for all galacturono-oligosaccharides. The pectic material was also incubated with endo-polygalacturonase in combination with pectin methyl esterase. Pectin methyl esterase is able to remove ester-linked methoxyl groups from the pectins, which form structural barriers for endo-polygalacturonase (Voragen, 1990). When endo-polygalacturonase was combined with pectin methyl esterase, the amount of oligomers present in the digests increased. The amount of galacturono-oligosaccharides released was about equal for VW^- and VW^+ and estimated to be 10–15% of the total amount of galacturonic acid present in the sample based on the peak areas. After removal of the methyl esters and *O*-acetyl groups by chemical saponification the substrates were degraded even further by endo-polygalacturonase to resistant polymeric fractions and mono-, di- and trigalacturonic acid oligomers. Also in this case the amounts of galacturono-oligosaccharides released were about equal for VW^- and VW^+ and estimated to be 15–20% of the total amount of galacturonic acid present in the sample.

3.3. Treatment of the polysaccharides from the vegetation waters with endo-arabinanase, arabinofuranosidase B, endo-galactanase and exo-galactanase

The sugar composition revealed that a considerable part of the pectic material present in VW^- and VW^+ consisted of arabinans and galactans. More information about these structures was obtained by incubation with specific and well-characterised enzymes. The digests were analysed by HPSEC and HPAEC.

HPAEC analysis of the VW^- digest showed that endo-arabinanase was hardly able to degrade the pectic arabinan side chains present. About 0.5% of the total amount of arabinose residues present was released as monomeric arabinose and very few oligomeric degradation products were detected in the digest. The endo-arabinanase used in this experiment is known to be especially active towards linear (1 → 5)-α-L-arabinan and shows little activity towards highly branched arabinans like beet arabinan. The results of the sugar linkage analyses revealed that the arabinans present in olive fruit were less branched than beet arabinan, but apparently the degree of branching was still too high and restricted the activity of endo-arabinanase. Arabinofuranosidase B is able to linearise branched arabinans to enhance the degradation of the arabinan by endo-arabinanase (Beldman et al., 1997). Incubation with arabinofuranosidase B showed that this enzyme was able to release about 30% of the arabinose present as monomeric arabinose. Endo-arabinanase in combination with arabinofuranosidase B enhanced the degradation of the polymer and about 40–50% of the arabinose present was analysed as monomeric arabinose on HPAEC. The removal of the arabinofuranose side chains by arabinofuranosidase B improved the degradability of the substrate by endo-arabinanase. Although arabinofuranosidase B is able to split besides (1 → 5)- also (1 → 2)- and (1 → 3)-α-L-linked arabinose (Rombouts et al., 1988) the enzyme was not able to completely degrade the arabinan. A time course study of the degradation of the arabinan present in the vegetation water by arabinofuranosidase B as analysed by HPAEC revealed that after 4 h already half of the maximum amount of arabinan that could be degraded had been released as monomeric arabinose. Incubation with a 10-fold higher amount of enzyme hardly affected the degradation of the polymer. So, apparently the amount of enzyme was not limiting and probably the structure of the substrate restricted the activity of arabinofuranosidase B. No significant differences were noticed in the degradability of the arabinans present in VW^+ compared to VW^- for the arabinan degrading enzymes used.

The pectic material present in the vegetation waters was also incubated with endo-galactanase and exo-galactanase. Both enzymes are only active towards (arabino)galactans with a backbone consisting of (1 → 4)-linked galacto-pyranose residues (Van de Vis et al., 1991). Unfortunately, no 'purified' enzymes having activity towards (1 → 3)/(1 → 6) galactans were available to study arabinogalactan type II, which was also present in the vegetation waters. HPAEC analysis of the reaction products present in the VW^- digest after incubation with endo-galactanase showed that galactose and galactobiose were released. Based on the peak areas it was estimated that about 50% of the (1 → 4)-linked galactan present had been degraded. Exo-galactanase showed very little activity towards the pectic material present in VW^- and released only monomeric galactose. About 5% of the (1 → 4)-linked galactan present was degraded. The combination of endo- and exo-galactanase did not enhance the degradation of the (1 → 4)-linked galactan present in VW^-. Analysis of the digest of VW^+ incubated with endo-galactanase on HPAEC revealed that hardly any degradation products were present. Only 0.4% of the galactose residues present in VW^+ was released as monomeric galactose. Also, exo-galactanase showed very little activity towards the pectic material present in VW^+. These experiments confirmed the data of the sugar linkage analysis, which showed that less than 1% of the galactose residues present in VW^+ originated from a (1 → 4)-linked galactan.

The interaction between galactanases and arabinanases was also studied. The degradation of the (1 → 4)-linked (arabino)galactan was not enhanced when arabinofuranosidase B was combined with endo-galactanase and confirmed the results of the sugar linkage analysis, which showed that only a very small part of the (1 → 4)-linked galactose residues was substituted at C-3.

The digests were also analysed on HPSEC for changes in the molecular weight distribution. Treatment of the material with the enzymes resulted only in very slight shifts towards lower molecular weights compared to the blank (not shown). Apparently, the arabinan and galactan chains present in the vegetation water hardly affected the hydrodynamic volume of the pectic polymers.

3.4. Composition of the polysaccharides present in the malaxed pastes

In addition to the effect of the use of commercial enzyme preparations during processing on the water soluble polysaccharides also the effect on the structure of the other cell wall polysaccharides present in the olive paste was investigated. Therefore, the cell wall material present in the non-enzyme treated (MP^-) and the enzyme treated malaxed paste (MP^+) was sequential extracted with different solvents to characterise the polysaccharides. Pectic material was solubilised by extraction with cold buffer, hot buffer, chelating agent and diluted alkali. Subsequently, a fractionation with 4 M alkali was performed to solubilise the hemicelluloses. When olive oil is extracted from the malaxed paste with a decanter, the water soluble polysaccharides in olive fruit turn up in the vegetation waters. Consequently, the pectic material solubilised by the extraction with cold buffer from the malaxed paste is expected to show the same characteristics as the pectic material in the vegetation waters. So, especially the pectin-rich fractions extracted with hot buffer, chelating agent and diluted alkali are of interest in this Section and Sections 3.5 and 3.6, because these fractions will give additional information on the effect of the enzyme preparations towards pectins present in the cell wall material, not soluble in the vegetation waters. The sugar compositions of the AISs and the obtained fractions are presented in Tables 2 and 3 for MP^- and MP^+, respectively. The yield of the AIS

Table 2
Yield on sugar basis (%) and sugar composition (mol%) of the fractions and the residue of AIS isolated from the non-enzyme treated malaxed paste, MP^- of olive fruit (tr = trace amount)

Sample	Yield	Rha	Ara	Xyl	Man	Gal	Glc	Uronic acids	OMe/OAc[a]	Carbohydrate content[b]
AIS	100	2	13	30	1	4	36	14	79/114	46
$CBSS^-$	6.6	2	15	3	1	14	3	62	56/17	53
$HBSS^-$	1.3	4	27	3	1	11	4	50	65/32	34
$ChSS^-$	2.2	4	30	1	tr	6	1	58	62/27	34
0.05 M $NaOH^-$	2.5	6	57	3	tr	9	3	22	0/0	33
4 M KOH^-	9.6	2	10	48	5	7	16	12	0/0	41
RES^-	72.3	2	8	34	1	2	46	8	0/0	50

[a] Expressed as mol methyl esters or *O*-acetyl groups per 100 mol uronic acid.
[b] Expressed as % (w/w).

expressed as percentage of the fresh weight of the malaxed paste was about 4–5% for MP^- as well as MP^+.

Arabinose and uronic acid accounted for more than 75% of the sugars present in the pectin-rich fractions from MP^-. Especially, the 0.05 M NaOH fraction contained a large amount of arabinose, which indicated the presence of pectins with a considerable amount of arabinan or arabinogalactan side chains in this fraction. The galactose content was relatively low for all pectin-rich fractions (6–14%). The 4 M KOH fraction consisted mainly of xylose, which accounted for 48 mol% of the sugars present. The residue consisted for about 46–47 mol% of glucose of which the main part (95%) could be designated as cellulose. Besides glucose the residue still contained a considerable proportion of xylose, and small amounts of arabinose and uronic acids. Apparently, the solvents used to extract the AIS were not able to solubilise all pectic and hemicellulosic substances as was observed before (Coimbra, Waldron, & Selvendran, 1994; Vierhuis, Schols, Beldman & Voragen, 2000). The sugar composition of the pectin-rich fractions CBSS, HBSS and ChSS from MP^- and MP^+ were quite similar. Some differences were found for the amounts of uronic acid, arabinose and galactose present in the samples but no clear trends were noticed. The composition of the 0.05 M NaOH and the 4 M KOH fraction from MP^- and MP^+ were practically identical. Also the sugar composition of the residues from both pastes appeared to be very similar.

More information about the structure of the pectic polysaccharides can be obtained by sugar linkage analysis. However, the results of the sugar linkage analysis of the pectin-rich fractions revealed high proportions of unmethylated sugar residues for almost all of the fractions (not shown). Calculation of the ratio between terminal and branched residues suggested that under-methylation had occurred, even though the methylation procedure was repeated to improve the completeness of the methylation of the free hydroxyl groups. Nevertheless, further study of the results of the sugar linkage analysis indicated that the relative proportions of the individual partially methylated sugars in an under-methylated sample were quite similar to those in a comparable sample with negligible under-methylation as was also shown by Düsterhöft, Posthumus, and Voragen (1992). So, although no exact data could be obtained from the methylation analysis, it was possible to give some trends. In all pectin-rich fractions from MP^- arabinose was essentially present as 1,5-linked and terminal residues. The relative amount of 1,3,5-linked arabinose increased when stronger solvents were used to extract the pectic polymers. Since several samples were under-methylated the amount of disubstituted arabinose was difficult to determine but is expected to be negligible as was also shown for the pectins present in the vegetation waters and by Coimbra et al. (1994) for a CDTA and a Na_2CO_3 fraction from olive fruit cell wall material. The major part of the galactose residues in the CBSS and HBSS fractions was present as (1 → 3)/(1 → 6)-linked galactans.

Table 3
Yield on sugar basis (%) and sugar composition (mol%) of the fractions and the residue of AIS isolated from the enzyme treated malaxed paste, MP^+ of olive fruit (tr = trace amount)

Sample	Yield	Rha	Ara	Xyl	Man	Gal	Glc	Uronic acids	OMe/OAc[a]	Carbohydrate content[b]
AIS	100	2	12	30	2	3	37	15	76/123	42
$CBSS^+$	9.0	3	19	2	1	8	1	66	35/20	58
$HBSS^+$	1.1	5	36	3	2	11	4	39	49/20	44
$ChSS^+$	1.3	5	34	1	1	6	1	52	49/22	40
0.05 M $NaOH^+$	2.2	6	55	4	tr	9	3	23	0/0	38
4 M KOH^+	7.4	2	10	50	5	6	16	11	0/0	53
RES^+	78.5	2	5	36	1	1	47	8	0/0	49

[a] Expressed as mol methyl esters or *O*-acetyl groups per 100 mol uronic acid.
[b] Expressed as % (w/w).

About 20–30% of the galactose arose from (1 → 4)-linked galactan. The ChSS and the 0.05 M NaOH fractions contained both relatively small amounts of galactose residues, which were also for the major part present as (1 → 3)/(1 → 6)-linked galactans. The use of commercial enzyme preparations during processing considerably decreased the relative amount of linkages associated with (1 → 4)-linked galactan for CBSS and HBSS, which agreed with the results of the analysis of the pectic polysaccharides present in the vegetation waters. The addition of commercial enzyme preparations did not affect the composition of the galactan polysaccharides present in the ChSS and 0.05 M NaOH fractions. Also, no significant degradative effect was noticed for the arabinans present in the various pectin-rich fractions from MP^+.

The CBSS, HBSS and ChSS fractions from MP^- were all highly methyl esterified (DM 56–65) and the degree of acetylation varied from 17 to 32. The polymers solubilised by (diluted) alkali were de-esterified by the conditions of their extraction. However, based on the high content of methyl esters and *O*-acetyl groups in the cell wall material (AIS) from MP^- it is likely that the polymers present in the 0.05 M NaOH and 4 M KOH fractions and the residue were highly esterified as well. In the CBSS, HBSS and ChSS fractions only about 30% of the methyl esters and about 10% of the *O*-acetyl groups were recovered. The remainder of the methyl esters were most likely linked to galacturonic acid residues of the pectic material present in the 0.05 M NaOH and 4 M KOH fractions or the pectic material which remained in the residue. The remainder of the *O*-acetyl groups could have been located on the pectic material as well as on the xyloglucans present in olive fruit (Kiefer, York, Darvill, & Albersheim, 1989; Sims, Munro, Currie, Craik, & Bacic, 1996; Vierhuis, York, Kolli, Vincken, Schols, Van Alebeek & Voragen, 2001c; York, Kolli, Orlando, Albersheim, & Darvill, 1996). Xylans can also carry *O*-acetyl groups but the presence of these groups has mainly been described for hardwood xylans (Bardet, Emsley, & Vincedon, 1997; Ishii, 1991; Puls, Tenkanen, Korte, & Poutanen, 1991; Ross, Johnson, Braun, MacKenzie, & Schneider, 1992; Van Hazendonk, Reinerink, De Waard, & Van Dam, 1996). The level of methyl esters appeared to be lower for the pectin-rich fractions isolated from MP^+ than the fractions from MP^-. The degree of methyl esterification of the CBSS, HBSS and ChSS fractions from MP^+ varied from 35 to 49. The lower contents of methyl esters present in the pectin-rich fractions from MP^+ were consistent with the lower content of methyl esters present in the pectic material isolated from VW^+ compared to VW^-. The degree of acetylation of the pectin-rich fractions from MP^+ was about 20 and in general lower than the degree of acetylation of the MP^- fractions, but no clear trend could be ascertained.

The HPSEC elution patterns of the pectin fractions are shown in Figs. 2 and 3. The pectin-rich fractions consisted of high molecular weight material with a broad molecular weight range. The use of enzyme preparations during the olive oil extraction procedure changed the profile of the pectins present in the CBSS and HBSS fractions. The CBSS and HBSS fractions from MP^- seemed to be one population, while in the elution pattern of the CBSS and HBSS fractions from MP^+ two populations could be distinguished. A shift of the molecular weight distribution to lower molecular weight ranges as was seen for the pectins present in the vegetation waters was not noticed. The pectins extracted with chelating agents from MP^- and MP^+ showed a similar HPSEC elution behaviour, which was also the case for the 0.05 M NaOH fractions.

3.5. Treatment of the various pectin fractions from the malaxed pastes with pectin lyase, endo-polygalacturonase and pectin methyl esterase

A more detailed study was performed for the pectin-rich fractions from MP^- and MP^+. The galacturonic acid backbone of the pectin fractions from MP^- and MP^+ was studied by incubation with pectic enzymes specific for high- and low-methyl esterified pectins and the digests were analysed by HPSEC and HPAEC. The HPSEC elution patterns before and after enzymatic treatment with pectin lyase are shown in Figs. 2 and 3. Incubation of the CBSS fraction from MP^- with pectin lyase showed that this enzyme degraded the polymers to a large extend and the molecular weight distribution shifted towards lower molecular weight ranges. By contrast, the pectic material present in the CBSS fraction from MP^+ was rather resistant to pectin lyase and only a slight decrease of the molecular mass was observed. The limited action of this pectic enzyme towards the CBSS fraction from MP^+ agreed with the degree of methyl esterification of the fraction of only 35, which is in general too low for pectin lyase activity (Voragen et al., 1995). The degree of methyl esterification of the HBSS fraction from MP^- and MP^+ were 65 and 49, respectively, and high enough for pectin lyase to act upon the galacturonic backbone. Also the pectic polymers present in both ChSS fractions were degraded to a large extent by pectin lyase. The saponified pectins present in the 0.05 M NaOH fractions were expected to be very resistant to degradation with pectin lyase. Still, a small shift in the molecular weight distribution was noticed which could not be explained.

Analysis of the endo-polygalacturonase digests by HPSEC revealed that the incubation of the $CBSS^+$ fraction (DM of 35) with endo-polygalacturonase resulted in a larger shift in the molecular weight distribution than the $CBSS^-$ fraction (DM of 56) as shown in Figs. 2 and 3. Incubation of the $CBSS^-$ fraction with in addition to endo-polygalacturonase pectin methyl esterase, which is able to remove the methoxyl groups, made the substrate more susceptible for endo-polygalacturonase. The addition of endo-polygalacturonase to the HBSS fraction from MP^- resulted only in slight shifts in retention times and also no obvious shifts in

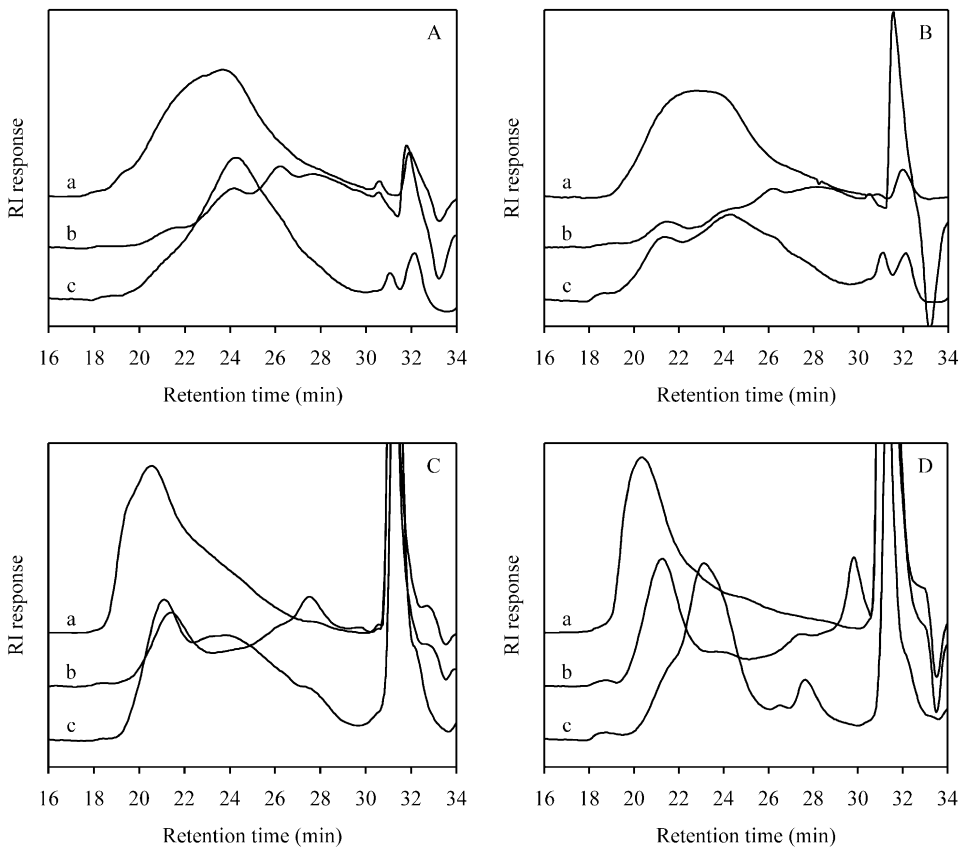

Fig. 2. HPSEC-patterns of the pectin-rich pools obtained from non-enzyme treated malaxed paste, MP^- (a) before incubation and after incubation with (b) pectin lyase and (c) endo-polygalacturonase: (A) CBSS; (B) HBSS; (C) ChSS; and (D) 0.05 M NaOH.

the molecular weight distribution of the HBSS fraction from MP^+ were noticed. Apparently, the degree of methyl esterification of these substrates was too high. Also the pectic polymers present in both ChSS fractions showed no obvious shift in the molecular weight distribution. The polymeric material present in the 0.05 M NaOH fractions also appeared to be very resistant to endo-polygalacturonase even though the degrees of methyl esterification and acetylation were zero due to the extraction procedure. The average molecular mass of the population decreased, but most of the polymeric fraction remained present as a polymeric material. This has also previously been observed for a 0.05 M NaOH fraction isolated from apple cell wall material (Schols et al., 1995) and can be explained by the fact that most of the galacturonic acid residues in the 0.05 M NaOH fractions are present in rhamnogalacturonan-type segments instead of homogalacturonan segments.

Analysis by HPAEC of the endo-polygalacturonase digests showed that the release of oligomers (DP 2–10) was relatively small. The amount of galacturonan oligomers released was estimated to be 5% of the total amount of galacturonic acids present in the sample based on the peak areas. Even the enormous decrease in molecular weight of the CBSS fraction from MP^+ incubated with endo-polygalacturonase did not result in large amounts of oligomeric end products. The combination of endo-polygalacturonase and pectin methyl esterase increased the amounts of galacturonic acid oligomers in the digests of the pectin-rich fractions CBSS, HBSS and ChSS and chemical saponification of the methyl esterified pectin-rich fractions made the substrates even more susceptible. The amount of galacturono-oligosaccharides released after chemical saponification was estimated to be 15–20% of the total amount of galacturonic acid present in the CBSS, HBSS and ChSS fractions. From the 0.05 M NaOH fractions only 3–4% of the total amount of galacturonic acid residues was released as mono-, di- and trigalacturonic acid. No marked differences were noticed between the degradation products and relative amounts of the oligomers for the pectins isolated from the non-enzyme treated paste, MP^- and the enzyme treated paste, MP^+ by HPAEC.

In addition to the analyses on HPAEC at high pH, the endo-polygalacturonase digests of the pectin-rich fractions were also analysed on HPAEC with a sodium gradient at pH 5.0 to be able to distinguish between the non-esterified oligomers and the methyl esterified oligomers. The endo-polygalacturonase digests of VW^- and VW^+, which were described in Section 3.2 were not analysed at HPAEC at pH 5.0, but are expected to show similar characteristics as the HPAEC-patterns of $CBSS^-$ and $CBSS^+$. The HPAEC-pattern of the analysis at pH 5.0 revealed that the endo-polygalacturonase digest of the CBSS fraction from MP^-

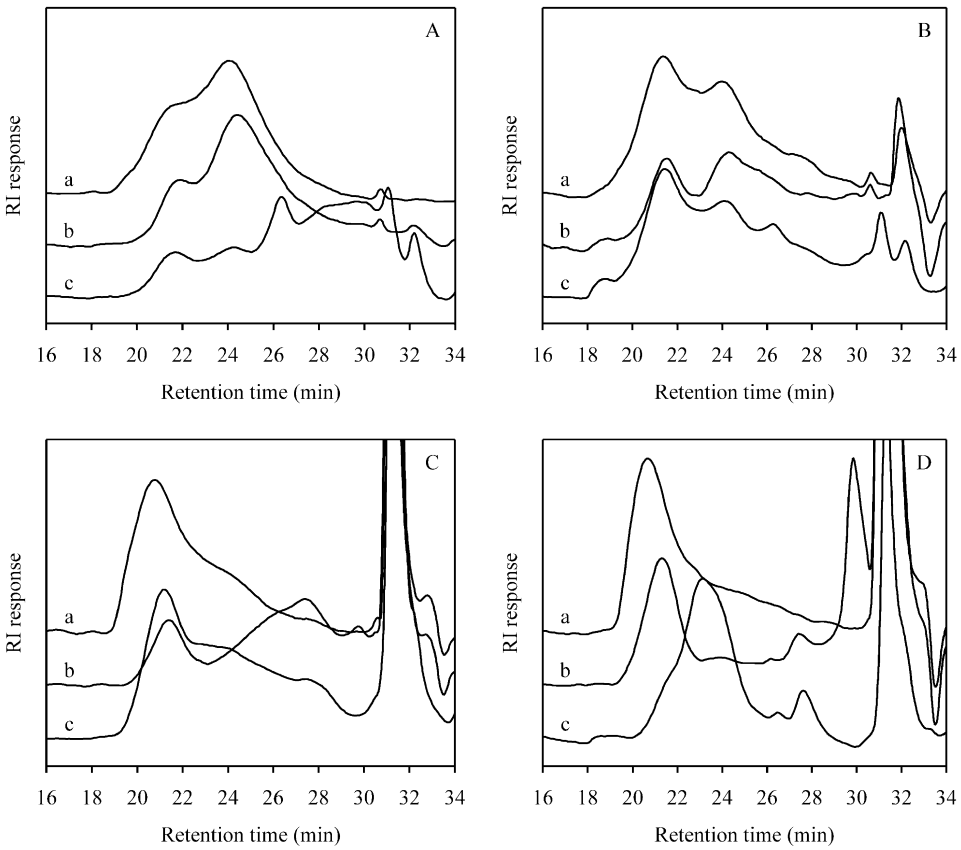

Fig. 3. HPSEC-patterns of the pectin-rich pools obtained from enzyme treated malaxed paste, MP^{+} (a) before incubation and after incubation with (b) pectin lyase and (c) endo-polygalacturonase: (A) CBSS; (B) HBSS; (C) ChSS; and (D) 0.05 M NaOH.

contained mainly non-methyl esterified mono- and digalacturonic acid and a small amount of trigalacturonic acid (Fig. 4). Incubation of endo-polygalacturonase combined with pectin methyl esterase made the CBSS fraction more susceptible for degradation. Predominantly non-methyl esterified galacturonic acids were released, but also partially methyl esterified galacturonic acid oligomers were detected in the digest. Endo-polygalacturonase was shown to release the largest amounts of mono-, di-, and trigalacturonic acid when the pectic material was chemically saponified. Although a complete saponification was expected, the chromatogram showed also small amounts of partially

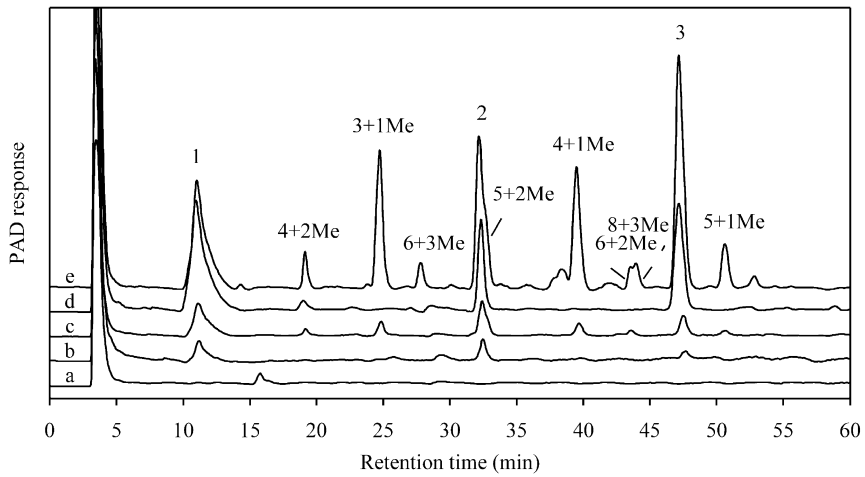

Fig. 4. HPAEC-patterns (pH 5.0) of the CBSS fraction obtained from non-enzyme treated malaxed paste ($CBSS^{-}$; a) before incubation and after incubation with (b) endo-polygalacturonase and (c) endo-polygalacturonase combined with pectin methyl esterase; (d) chemically saponified $CBSS^{-}$ after incubation with endo-polygalacturonase; (e) standard of a pectin polygalacturonase digest. The composition of the galacturonic acid oligomers (for example, 3 + 1Me: a trimer of galacturonic acid having one methyl ester group) are indicated above the corresponding peaks as determined by Daas et al. (1998)..

methyl esterified oligomers. The CBSS fraction from MP^+ incubated with endo-polygalacturonase contained besides non-methyl esterified galacturonic acid oligomers also partially methyl esterified oligomers, this in contrast to the CBSS fraction from MP^-, which contained only non-methyl esterified oligomers. Daas, Schols, and Voragen (2000) have concluded from their study of the methyl ester distribution of pectins that endo-polygalacturonase can only release methyl esterified oligomers from pectin when it contains non-esterified galacturonic acid sequences (so-called blocks) close enough to each other. When the enzyme degradable blocks are located too far from each other relatively large methyl esterified degradation products remain and these cannot be detected by the HPAEC method at pH 5.0. So apparently, the use of enzyme preparations during processing demethylated part of the CBSS present in the malaxed paste in such a way that it contained more clustered endo-polygalacturonase degradable blocks.

The proportion in which the non-esterified mono-, di- and trigalacturonic acid were released were practically identical for both CBSS fractions and appeared not to be affected by the use of commercial enzyme preparations during processing. The endo-polygalacturonase digests of the CBSS fractions from MP^- and MP^+ contained both predominantly mono- and digalacturonic acids, which indicated that the pecins present in the fractions contained mainly relatively small non-esterified blocks (Daas et al., 2000). The endo-polygalacturonase used for our study is 'most likely' able to cleave in non-esterified galacturonic acid sequences of four and more residues (Daas, Meyer--Hansen, Schols, De Ruiter, & Voragen, 1999). As a result, endo-polygalacturonase has to degrade blocks close to the limit of its mode of action and will only release small (mono- and di-) galacturonic acid molecules. The proportion of trigalacturonic acid in the digests increased, as expected, when endo-polygalacturonase was used in combination with pectin methyl esterase and increased even further when the pectins were saponified before incubation with endo-polygalacturonase.

The profile of the endo-polygalacturonase digest on HPAEC of the HBSS fraction from MP^- contained non-methyl esterified and partially methyl esterified galacturonic acid oligomers and the profile was comparable to the endo-polygalacturonase digest of the CBSS fraction from MP^+. The HBSS fraction from MP^+ showed a similar HPAEC pattern as the fraction from MP^-. The pectins present in the ChSS fraction are believed to participate in calcium cross-linking in the cell walls. To be able to participate in calcium cross-linking these pectins have to contain relatively large blocks of non-methyl esterified homogalacturonan compared to the pectins present in the CBSS and HBSS fractions. So, the relative proportion of trigalacturonic acid to mono- and digalacturonic acid should be higher in the ChSS digests compared to the CBSS and HBSS fractions because endo-polygalacturonase is less hindered by methyl esters. However, the relative proportion of trigalacturonic acid hardly increased. Apparently, in addition to methyl esters also other factors were involved in the degradation of the homogalacturonan by endo-polygalacturonase. Renard & Jarvis (1999), for example, have shown that endo-polygalacturonase is inhibited by the presence of *O*-acetyl groups on chemically acetylated homogalacturonan. In general, it is stated that the homogalacturonan regions of pectins are only slightly *O*-acetylated or not at all, and that *O*-acetylation is mostly confined to the rhamnogalacturonan regions (Ishii, 1995; Komalavilas & Mort, 1989; Renard & Jarvis, 1999). However, high amounts of *O*-acetyl groups in the homogalacturonan regions have been described for sugar beet pectin with a degree of acetylation of 35 (Voragen, Beldman, & Schols, 2001). The *O*-acetyl content of the pectins extracted from olive fruit was also high (17–32), and may indicate that the pectins present in olive fruit have also *O*-acetyl groups linked to the galacturonic acid residues in the homogalacturonan regions. MALDI-TOF mass spectrometry was performed to investigate the presence of *O*-acetyl groups on oligomers present in a pectin fraction digested with endo-polygalacturonase in combination with pectin methyl esterase (Fig. 5). The MALDI-TOF mass spectrum showed that part of the partially methyl esterified oligomers released from the homogalacturonan regions were indeed (mono)-*O*-acetylated and indicated that the presence of *O*-acetyl groups in the homogalacturonan regions could have hindered endo-polygalacturonase. The relative proportion of trigalacturonic acid to mono- and digalacturonic acid increased when the pectins of the ChSS fraction were saponified but endo-polygalacturonase could still not produce mono-, di- and trigalacturonic acid in the proportions, determined when a commercial polygalacturonic acid was used as a substrate. When a commercial polygalacturonic acid is used as a substrate endo-polygalacturonase is not hindered by any methyl esters or *O*-acetyl groups and produces mono-, di- and trigalacturonic acid in proportions strictly determined by the mode of action of the enzyme. So, apparently the enzyme was still hindered in the saponified ChSS fraction even though the methyl esters substituents and *O*-acetyl groups were not present anymore. It might be that, for example, the presence of blocks of the more highly substituted rhamnogalacturonan regions, which are hardly present in commercial polygalacturonic acid hindered the enzyme. No differences were distinguished between the ChSS fractions from MP^+ and MP^-. The HPAEC patterns of the ChSS fractions from MP^+ and MP^- showed the same characteristics. The NaOH fractions from MP^- and MP^+ showed also similar elution patterns and contained, as expected, only non-methyl esterified mono-, di- and trigalacturonic acid.

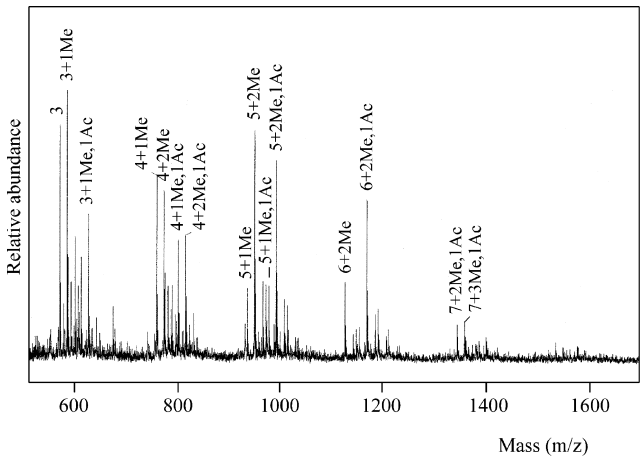

Fig. 5. MALDI-TOF mass spectrum of the CBSS fraction obtained from non-enzyme treated malaxed paste ($CBSS^-$) digested with endo-polygalacturonase combined with pectin methyl esterase. The composition of the galacturonic acid oligomers (for example, 3 + 1Me, 1Ac: a trimer of galacturonic acid having one methyl ester group and one *O*-acetyl group) are indicated above the corresponding peaks.

3.6. Treatment of the various pectin fractions from the malaxed pastes with endo-arabinanase, arabinofuranosidase B, endo-galactanase and exo-galactanase

The arabinan and galactan chains present in the pectin fractions from MP^- and MP^+ were further studied by incubation with specific arabinan and galactan degrading enzymes. The digests were analysed by HPSEC and HPAEC. Incubation of the pectin-rich fractions from MP^- with endo-arabinanase resulted in small amounts of mono- and oligomeric reaction products. Incubation of the CBSS fraction from MP^- with arabinofuranosidase B showed that this enzyme was able to degrade the arabinan chains present in CBSS to a large extent (70%). For the other pectin-rich fractions from MP^- (HBSS, ChSS and 0.05 M NaOH) the degradation of the arabinan side chains by arabinofuranosidase B was limited till 30–40%. Incubation of the CBSS fraction with a combination of arabinofuranosidase B and endo-arabinanase enhanced the degradation and resulted in an almost complete degradation of the arabinan side chains. Arabinose monomer was the major degradation product, but also small amounts of oligomeric products were released. The combination of both arabinan degrading enzymes increased also the degradation of the arabinan side chains present in HBSS and ChSS (till 60–70%) and 0.05 M NaOH (till 40%). Supplementation of the arabinan degrading enzymes with the galactan degrading enzymes endo-galactanase and exo-galactanase showed no additional effect on the release of arabinose. No differences were distinguished between pectin-rich fractions from MP^+ compared to MP^- for the enzymes we used for our research.

The degree of degradation of the galactan side chains differed markedly for the fractions isolated from MP^- compared to MP^+. Exo-galactanase was able to hydrolyse 19% of the total amount of galactose present in the CBSS fraction from MP^-, while incubation of the CBSS fraction from MP^+ resulted in relatively very small amounts of galactose hydrolysed. Only 3% of the galactan present in this fraction was degraded to galactose monomer. The same trends were observed for the HBSS and ChSS fractions (Table 4). The enzymatic degradability of the 0.05 M NaOH fractions with exo-galactanase was practically identical for both processing procedures. Also endo-galactanase degraded the pectin-rich fractions CBSS, HBSS and ChSS from the non-enzyme treated paste, MP^- to a large extent, while very small amounts of degradation products were present in the endo-galactanase digests of CBSS, HBSS and ChSS from MP^+. The results of the incubations with exo- and endo-galactanase confirmed that the use of enzyme preparations during processing fragmented the (1 → 4)-linked galactan chains as shown by the data of the sugar linkage analysis.

The incubation of the pectin-rich fractions with the enzymes described resulted only in rather slight shifts in the molecular weight distribution on HPSEC (not shown). Even when considerable amounts of arabinose were released from the pectic polysaccharides, the effect of this enzyme on the molecular weight distribution of the polysaccharides was negligible. Apparently, the arabinan chains had a rather small effect on the hydrodynamic volume of the polysaccharide.

4. Concluding remarks

Based on the results of this study it can be concluded that the composition of the water soluble pectins present in the vegetation waters and the buffer soluble pectic material isolated from the malaxed paste changed due to the use of enzyme preparations during processing. This means that the enzymes affected only a relatively small part of the cell wall material. Previous research has shown that the action of the enzyme preparations is probably restricted during processing by the high concentration of phenolic compounds present in olive pulp (Vierhuis et al., 2001b). It appeared that re-incubation of the isolated polymeric material present in VW^+ with a fresh batch of Olivex resulted in an almost complete degradation of the polymers to oligosaccharides and monosaccharides. So, apparently the structure of the polysaccharides present in VW^+ or the enzyme preparations used are not limiting for the degradation but other factors like, for example, phenolic compounds affect the activity of the enzymes in the preparations. However, not all enzymes in the enzyme preparations seemed to be restricted in their activity during processing. For example, the use of enzyme preparations during processing resulted in an almost complete degradation of the (1 → 4)-linked galactan chains in VW^+. This suggests, that the enzymes involved in the degradation of the (1 → 4)-linked galactan chains were

Table 4
Release of galactose from the pectin-rich extracts after 24 h incubation with exo-galactanase and endo-galactanase expressed as percentage of total galactose present

	Non-enzyme treated paste (MP^-)		Enzyme treated paste (MP^+)	
	Exo-galactanase	Endo-galactanase	Exo-galactanase	Endo-galactanase
CBSS	19	35	3	5
HBSS	14	37	5	5
ChSS	10	24	6	11
0.05 M NaOH	10	18	11	14

contrary to other enzymes present in the preparations less affected by the phenolic compounds present in the olive pulp.

Although the results of this work gave more insight in the action of the commercial enzyme preparation Olivex towards the pectic polysaccharides present in the cell wall, it is still difficult to conclude how the changes in the structure to the improved quality characteristics of the oil of the pectic polysaccharides are related. Further research will be necessary on this aspect. Especially, because it is known that the use of commercial enzyme preparations in the olive oil industry not always assures a significant increase of the quality aspects or the yield of the oil (Ranalli & Serraiocco, 1995). An adequate strategy to efficiently carry out enzymatic treatment remains difficult to give, because the effect of enzymatic treatment depends on several factors in which not only technological aspects, but also agronomic aspects, like variety, geographical location, etc. are expected to play an important role.

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