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Characterization of residual pectins from raspberry juices

Frank Will & Helmut Dietrich

Forschungsanstalt Geisenheim, Fachgebiet Weinanalytik und Getrankeforschung, PO Box 1154, D-65366 Geisenheim, Germany

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The influence of different manufacturing steps, like enzymic mash treatment, water extraction of pomace and juice enzymation, on the amount and composition of colloids of clear raspberry juices is investigated. The colloid concentrations were in a range between 1·09 and 4·20 g/l. The kinetics of the enzymic degradation of the raspberry pectins with two commercial pectinases and a mixture of purified pectinesterase and polygalacturonase were monitored. The residual pectins of raspberry juice produced with enzymic mash and juice treatment were further characterized. An arabinogalactan type II with a Ara/Gal ratio of 0·59 and an apparent molecular mass of 40 000 Da was found as well as several populations of rhamnogalacturonans with a broad range of apparent molecular masses. Compared with apple juice, the residual rhamnogalacturonans from raspberry juice contain higher moieties of arabinose as highly branched arabinans and lower amounts of galactose. Also, 1,4-linked galactan chains were found in small amounts. The isolated polymers are involved in clarification and filtration problems of raspberry juices and concentrates.

INTRODUCTION

In the fruit juice industry, raspberries are fruits with a rather high economic value. Besides a reasonable Brix/acid ratio and pleasant sensory properties, colour and stability are the main quality factors of the juices. The liqueur industry is a particularly important customer of raspberry concentrates and demands stable products with intense colour. Raspberries are soft fruits containing high quantities of pectin. The normal way of producing clear raspberry juice concentrates is milling, mash treatment with pectolytic enzymes, dejuicing using a press or decanter followed by subsequent water extraction, juice treatment with pectolytic enzymes, aroma stripping, clarification–filtration and concentration. During juice manufacturing, high amounts of pectic substances pass into the juice.

Pectic substances of clear fruit juices represent a mixture of mainly neutral polysaccharides with molecular masses ranging from 10 000 to 10^6 Da, originating from the 'hairy' regions of the native pectin molecule (DeVries *et al.*, 1983; Saulnier, 1988). Commercial pectolytic enzyme preparations are not able to hydrolyse them completely during juice clarification. They remain as colloidally dissolved polymers in the juice (Schols *et al.*, 1990*b*, 1991).

In practice, high amounts of colloidally dissolved substances lead to problems during filtration and clarification. In particular, the ultrafiltration technique is strongly affected by juice colloids. In the present work, polysaccharides from raspberry juices are characterized. Additionally, the influence of different manufacturing steps on the amount and composition of colloids of the resulting clear juices is investigated.

MATERIAL

Mature raspberries (1992 harvest, 200 kg) were harvested in late June from the research station of Geisenheim (Department of Fruit Growing). The fruits were healthy and in good condition without any mould. They were divided into two batches of 100 kg each. After milling, the first batch was pressed directly on a rack and cloth press, yielding 75 l in 45 min. The pomace was extracted with 30 l of water (40°C, 30 min) and pressed again, yielding 30 l of extraction juice. The same procedure

was carried out with the second batch. In this case, however, prior to pressing, the mash was treated with 20 ml/t Panzym BE (Boehringer Ingelheim, Germany) for 1 h at 50°C. The yield was 82 l in 5 min for the directly pressed, enzyme treated juice. Again, 30 l of water extracted juice was recovered. One part of the juice, coming from the enzyme treated mash, was again treated with the same enzyme preparation for 1 h at 50°C. Before isolating the pectins, the juices were separated (Westfalia SA-1), pad filtered and labelled as follows:

Juice 1.0: single strength (11·0°Brix), directly pressed juice without enzymic mash treatment;
Juice 1.1: mash extracted juice from 1.0 (4·6°Brix);
Juice 2.0: single strength juice after mash treatment with Panzym BE (11·0°Brix);
Juice 2.1: mash extracted juice from 2.0 (3·5°Brix);
Juice 2.2: Juice 2.0 after subsequent enzymic treatment in the juice stage (11·0°Brix).

METHODS

Isolation

Pectic substances were isolated by ultrafiltration (UF) (MWCO 10 000 Da) and subsequent diafiltration of the UF retentate with citrate buffer and water. Finally, the diafiltered retentate was freeze-dried and further dried under vacuum before weighing (Wucherpfennig & Dietrich, 1983).

Monomer sugar composition/degrees of esterification

Saeman hydrolysis of the samples (5–10 mg) with sulphuric acid was performed for 1 h at 130°C. The neutral sugars of the pectins were analysed by GLC of the corresponding alditol acetates; the derivation and separation were performed as described at Blakeney *et al.* (1983). Uronic acids in the hydrolysates were separated with a Dionex Bio-LC system (HPAEC) combined with a Spectra-Physics autosampler AS 3500 using a PA1-column with 0·5 mol/l NaOH as eluent. Pulsed amperometric detection was used. Determination of neutral sugars and uronic acids from enzymic release was carried out with the above equipment in one run, as previously described (Patz *et al.*, 1993). The degrees of methylation and acetylation were analysed following Voragen *et al.* (1986).

Anion exchange chromatography

Quantities (200–1000 mg) of depectinized material from 2.2 were separated on DEAE Sepharose fast flow (Pharmacia) as described earlier (Will *et al.*, 1992).

Size exclusion chromatography

The apparent molecular weights were determined by size exclusion chromatography on Superose 12 (Pharmacia), calibrated with the Pharmacia standard dextrans (Will & Dietrich, 1992*a*).

Enzymic degradation

Native raspberry pectin from juice 1.0 (10 g/l in 0·05 M acetate buffer, pH 5·0) was treated with two desalted commercial enzyme preparations (Novozym 52, Novo Nordisk, Switzerland; Panzym BE, Boehringer Ingelheim, Germany) and with a combination of purified pectinmethylesterase and endopolygalacturonase. Liberation of sugars from enzymic digest was monitored by HPAEC.

Permethylation

Methylation analysis was performed with butyllithium and methyl iodide in dimethyl sulphoxide (Kvernheim, 1987). Hydrolysis of the methylated polysaccharides was done with 2 M trifluoroacetic acid. The partially methylated sugars were converted into their alditol acetates (Harris *et al.*, 1984) and separated by GLC on DB-225 (Will & Dietrich, 1992*a*). Peak areas were corrected using the appropriate response factors (Sweet *et al.*, 1975).

RESULTS

Colloid amounts and sugar composition

The colloid concentrations of the different juices are shown in Table 1. The values are high in comparison with, e.g. apple juice ranging from 1·09 to 4·20 g/l. Raspberries are normally treated with pectolytic enzymes after milling to reduce mash viscosity and the water binding capacity of the native pectin. This leads to higher pressing rates and shorter pressing times combined with higher juice and colour yields. It is obvious that both water extracted juices show higher colloid amounts than the corresponding straight pressed juices. Additional juice treatment with pectolases resulted in a nearly 50% reduction of colloids (juice 2.2).

Table 1 also shows the monomer composition of the different juice colloids. The pectins show different moieties of neutral and acidic sugars, the yields of total saccharides ranging 77·7 to 96·3 g/100 g dry weight. The juices 1.0 and 1.1 show the typical composition of native, untreated and water-soluble raspberry pectin. The galacturonic acid content of more than 70% is very high compared with the amount of neutral sugars. Arabinose and galactose are the dominating neutral

Table 1. Concentrations, degrees of esterification and sugar compositions (mol%) of raspberry pectins from the different juices. DM and DA: degrees of methoxylation and acetylation (%)

	Juice 1.0	Juice 1.1	Juice 2.0	Juice 2.1	Juice 2.2
Colloid concentration (g/l) (referring to 11·0°Brix juice strength)	3·82	4·20	1·97	2·74	1·09
Total saccharides (g/100 g dry wt)	89·1	96·3	92·2	90·6	77·7
DM	46·0	55·1	23·2	53·2	43·2
DA	1·2	3·9	11·9	8·8	18·7
Rhamnose	1·3	1·8	5·3	3·4	7·6
Arabinose	10·2	16·0	30·9	34·4	41·4
Xylose	1·7	1·6	2·0	2·9	3·1
Galactose	8·5	5·9	10·2	11·8	21·6
Glucose	2·0	1·9	1·0	1·4	1·3
Galacturonic acid	76·2	72·9	50·7	46·0	25·0

sugars, followed by rhamnose, xylose and glucose. Fucose, mannose and glucuronic acid are not listed in the composition tables because they occurred only in traces (below 0·1%). During mash treatment with pectolytic enzymes, galacturonic acid is diminished to 50·7% rsp. 46·0%, while neutral sugars are relatively increasing. Additional enzymic treatment of juice 2.0 resulted again in lower amounts of galacturonic acid (25·0%) and higher amounts of neutral sugars (see juice 2.2).

The native raspberry pectins (juices 1.0 and 1.1) show medium (46·0 and 55·1%, respectively) degrees of methoxylation (DM) and low (1·2 and 3·9%, respectively) degrees of acetylation (DA). Pretreatment of the fruit mash with enzymes produces soluble pectins with low DM and high DA (23·2 and 12·0% in juice 2.0). Water-extraction of the pretreated mash, extracts soluble pectins with medium DM and higher DA (53·2 and 8·8 in juice 2.1) from the cell wall. When the low content of galacturonic acid is taken into account, the pectins remaining after enzyme treatment in the juice stage are highly acetylated.

Degradation of raspberry pectin with pectolases

The liberation of neutral and acidic sugars during the action of pectolytic enzymes is of great interest. To study the kinetics of pectin degradation, native raspberry pectin isolated from juice 1.0 was treated with two commercial enzyme preparations under laboratory conditions. Additionally, a third sample was treated with a mixture of purified pectinmethylesterase (PME) and endopolygalacturonase (PG). The treatment was monitored for 8 h. The liberation of neutral sugars over this period is displayed in Fig. 1. During the action of the commercial preparations only arabinose and galactose are liberated. The major release is reached within 3 h. Novozym 52 liberates more neutral sugars than does Panzym BE. Depending on the strong arabinan-degrading activities of Novozym 52, the maximal

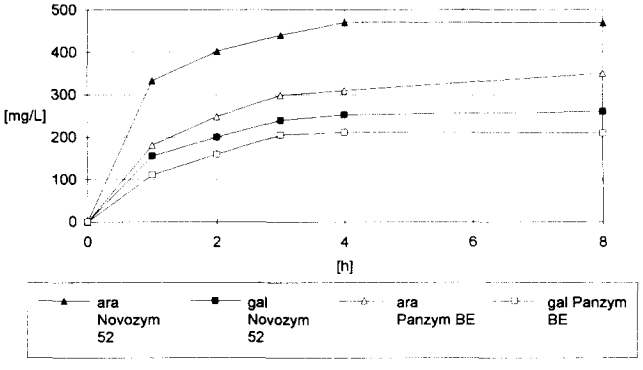

Fig. 1. Enzymic release of neutral sugars from raspberry pectin with Novozym 52 and Panzym BE (pectin concentration 10 g/l).

liberation of arabinose is reached after 2 h. Purified PME/PG liberated only minor amounts of neutral sugars, indicating still some side activities on neutral polysaccharides (data not listed).

The release of galacturonic acid is demonstrated in Fig. 2. The rates obtained follow those for the neutral sugars. Novozym 52 was more active on the raspberry pectin than Panzym BE. Both preparations liberated about 2,500 mg/l monomeric galacturonic acid after 8 h. Again, the major release was achieved in the first 3 h.

After denaturation of the digesting enzymes the clear supernatants were desalted by gel filtration on Sephadex G25. The high molecular mass peaks (>5000 Da) were collected, freeze-dried and analysed for their sugar composition. The percentage recovery of residual pectins after the action of Novozym 52, Panzym BE and purified PME/PG was 10, 8 17%, respectively. The commercial enzymes degrade the pectin to a larger extent than the purified pectolases. Comparing the pectin input (10 g/l) on the one hand with the amount of liberated sugars and the recoveries after enzymic treatment, denaturation, desalting and lyophilization on the other, the balance is not equal. The 'missing' mate-

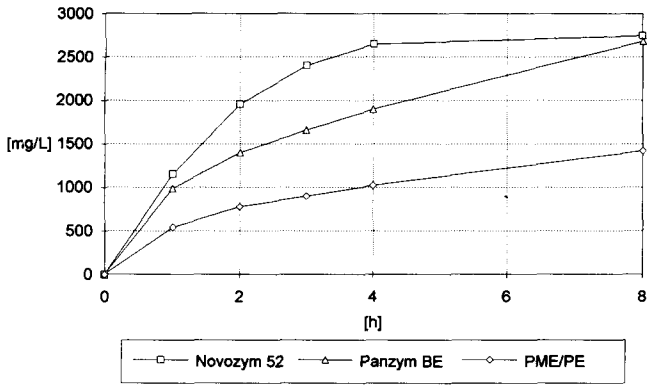

Fig. 2. Enzymic release of galacturonic acid from raspberry pectin with Novozym 52, Panzym BE and purified PME/PG (pectin concentration 10 g/l).

Table 2. Sugar composition of the residual pectins after enzymic digest of native raspberry pectin with different enzymes (mol%). DM and DA: degrees of methoxylation and acetylation (%)

	Novozym 52	Panzym BE	PME/PG
Total saccharides (g/100 g dried material)	39·9	45·6	64·4
DM	39·6	20·6	n.d.
DA	23·6	13·7	n.d.
Rhamnose	14·8	6·6	6·5
Arabinose	10·5	14·7	27·2
Xylose	2·5	6·1	3·3
Galactose	35·3	29·2	31·2
Glucose	0·0	0·0	1·1
Galacturonic acid	36·1	43·4	30·1

rial were oligosaccharides, which were not analysed and also general losses during desalting.

As demonstrated in Table 2, there were also differences in the sugar compositions. The total saccharide recovery of the enzyme treated pectins was poor (39·9–64·4 g/100 g). Like their parent polymers they consisted mainly of rhamnose, arabinose, galactose and galacturonic acid. Xylose appeared in amounts of 2·5–6·1%. The Novozym-degraded polymer contains less arabinose than the Panzym-treated polymer (10·5 and 14·7%, respectively). This confirms the results of the enzymic release studies. The significant difference between the samples treated with commercial enzymes and the one treated with purified PME/PG is the higher saccharide recovery and the two- to three-fold higher arabinose content in the latter, indicating significantly lower activities on arabinose-containing pectic side chains.

The enzyme treated fractions (Novozym 52, Panzym BE) show relatively low degrees of methoxylation, but high degrees of acetylation (see Table 2). The presence of so many acetyl groups in the rhamnogalactutonan backbone probably prevents a further enzymic degradation. During purification of a rhamnogalacturonase, Schols *et al.* (1990*a*) found that the enzyme was only able to degrade the modified hair regions prepared from apple cell wall after removal of the methoxyl and acetyl groups by saponification. Searle-van Leeuwen *et al.* (1992) described the enzymic removal of the acetyl groups by the action of a rhamnogalacturonan acetylesterase from *A. aculeatus*. No further data about the degrees of esterification of berry pectins were found in the literature.

Anion exchange chromatography

In practice the normal way of juice production is juice 2.2, originating from enzymic mash and juice treatment. For better characterization of the hairy regions the residual pectins of juice 2.2 were separated on DEAE-Sepharose fast flow (Pharmacia) using step gradients of water, 0·05, 0·15 and 0·5 M NaCl. The percentage distribution after DEAE-separation was 29·8, 0·0, 12·0 and 58·0%, respectively. No 0·05 M fraction could be collected. The largest moieties were eluted with the highest ionic strength. Table 3 shows the different monomer compositions after anion exchange chromatography. With 35·5%, the neutral, water eluted fraction showed an unexpected high content of galacturonic acid indicating a high degree of esterification. Actually, with 49·7% the DM was similar to the native juice pectin. Therefore, this fraction was not bound to the weak anion-exchange material. The neutral, water eluted polymer and the acidic fraction (0·5 M) show two groups of rhamnogalacturonans, whereas the 0·15 M fraction consisted almost exclusively of arabinogalactans.

Size exclusion chromatography

Using size exclusion chromatography on Superpose 12 (Fig. 3) the DEAE fraction 0·15 M was eluted in two

Table 3. Sugar composition of the residual pectins after enzymic treatment with Panzym BE followed by anion exchange chromatography (mol%). DM and DA: degrees of methoxylation and acetylation (%)

	Neutral	Fraction 0·15 M	Fraction 0·5 M
Total saccharides (g/100 g dried material)	68·7	69·0	88·6
DM	49·7	—	29·1
DA	15·3	—	17·4
Rhamnose	8·7	1·3	7·9
Arabinose	31·9	34·5	55·8
Xylose	8·0	0·9	2·4
Galactose	13·1	58·4	12·6
Glucose	2·8	3·5	1·4
Galacturonic acid	35·5	1·4	20·0

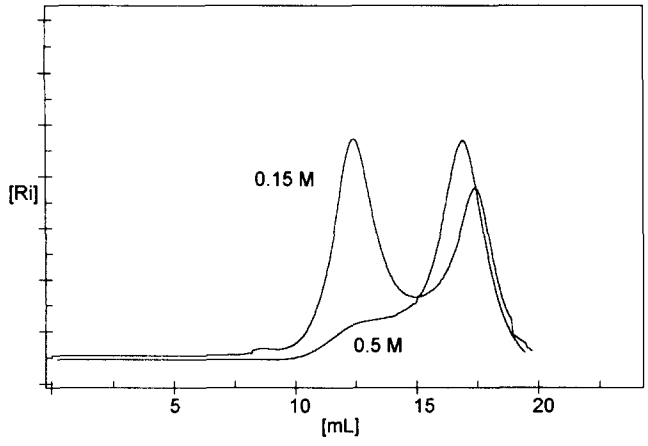

Fig. 3. Size exclusion chromatography of DEAE fractions 0·15 and 0·5 M on Superose 12.

main peaks and a small shoulder in the void volume of the column (7·5 ml). Following the Pharmacia dextran calibration standards, the first peak has an apparent molecular mass of 40 000 Da. The second peak is in the range of about 5000 Da. The 0·5 M fraction showed a broad distribution from 10 18 ml. Enzymic treatment in the mash and the juice stage results in large amounts of small pectin fragments in the low molecular weight range 5000–7000 Da.

Permethylation

Samples 0·15 and 0·5 M resulting from DEAE-separation were per-*O*-methylated. The results (Table 4) confirm that fraction 0·15 M is a highly branched type II arabinogalactan. Arabinose was mainly found at the terminal positions, and galactose in the 3-linked, 6-linked and predominantly 3,6-linked position. Sugar composition and methylation data confirm fraction 0·5 M as rhamnogalacturonans carrying highly branched arabinans. Here again type II arabinogalactans were present. Also, minor amounts of the methyl ether 2,3,6-Gal were found, indicating 1,4-linked galactan chains.

DISCUSSION

The juices examined in this work originate from a rack and cloth press. In the fruit juice industry the solid–liquid separation is performed with horizontal presses, belt presses or decanters. Here, the mechanical action on the fruit cell wall is much higher. High pressure and shear stress inside the dejuicing machines increase the release of pectic polysaccharides from the cell wall. Normally, enzymic mash treatment enhances the colloidal amounts in the resulting juices. This was not the case with the gentle working press used here. The colloid concentrations were in a range between 1·09 and 4·20 g/l.

In practice, the time of enzymic mash treatment has a great influence on the amount of colloidal material obtained. There seem to be no clear distinctions between the action of different enzyme preparations (Will, 1993). About 2–7 g/l is the typical concentration range of residual pectins of industrial raspberry juices. The amounts are enormously high compared with clear apple juices from industrial production. There, the colloid concentrations are in a range between 0·4 and 0·9 g/l (Will & Dietrich, 1992*b*). Under the optimized conditions (pH, temperature, ionic strength, extensive incubation times) chosen here for the enzymic degradation experiments, the raspberry pectin could be degraded to a residual fraction of about 10%. The high colloid contents of commercial industrial juices underline that this is not possible under practical conditions.

Table 4. Methylation analysis of fractions 0·15 and 0·5 M (mol%)

Methyl ether	Linkage	Fraction 0·15 M	Fraction 0·5 M
2,3,5-Ara	Terminal	21·2	38·9
3,5 + 2,5-Ara	1 → 2, 1 → 3	1·5	4·6
2,3-Ara	1 → 5	3·5	8·9
2-Ara	1 → 3,5		12·7
3-Rha	1 → 2,4		4·5
2,3,4,5-Gal	Terminal	1·8	3·9
2,4,6-Gal	1 → 3	14·6	7·8
2,3,6-Gal	1 → 4		5·6
2,3,4-Gal	1 → 6	7·7	4·4
2,4-Gal	1 → 3,6	49·7	8·7

In general, the composition and structure of the residual pectins are similar to those isolated from other fruits. The arabinogalactan from DEAE fraction 0·15 M consisted of 34·5% arabinose, 58·4% galactose (Ara/Gal ratio 0·59) and minor amounts of rhamnose, glucose and galacturonic acid. The apparent molecular mass was 40 000 Da. Will & Dietrich (1992*a*) isolated a comparable polymer from apple juice with an Ara/Gal ratio of 0·37, and Cartier *et al.* (1987) characterized an arabinogalactan from a blackberry cell culture with a ratio of 0·66. Saulnier *et al.* (1992) described a grape arabinogalactan protein with an Ara/Gal ratio of 0·57. The common property was the arabinogalactan type II structure.

The anion exchange chromatography of the residual pectins, which were released after enzymic treatment in the mash and the juice stage, revealed the presence of several populations of rhamnogalacturonans (Table 3) with a broad range of apparent molecular masses. The rhamnogalacturonan from DEAE fraction 0·5 M consisted of 7·9% rhamnose, 55·8% arabinose, 2·4% xylose, 12·5% galactose, 1·4% glucose and 20·0% galacturonic acid. The sugar composition differs from the analogous polymer isolated in the same manner from apple juice with regard to higher amounts of arabinose and galacturonic acid and lower amounts of

galactose. The Ara/Gal ratio of raspberry rhamnogalacuronan was 4·4 compared with 1·13 for the apple juice polymer (Will & Dietrich, 1992*a*). The linkage type analysis revealed the same structural features as found in other fruits (Saulnier *et al.*, 1988; Schols *et al.*, 1990*b*, 1991; Will & Dietrich, 1992*a*). A remarkable difference is the presence of 1,4-linked galactan chains (Table 4). Raspberry juice rhamnogalacturonans show far lower molecular masses compared with fractions extracted from apple (Will & Dietrich, 1992*a*).

The use of UF for the production of clear fruit juices is still increasing worldwide. Residual pectins affect strongly the flux rates in UF-systems. The structure and high molecular weight of these materials are responsible for a layer formation on the membranes. This has been found for apple juice (Will *et al.*, 1992) and is also the case for raspberry juice. In earlier laboratory-scale experiments with hollow fibre UF modules (MWCO 50 000 Da) the colloid-free juice (UF permeate MWCO 10 000 Da) showed an average fluxrate of 90 l/m^2 h. The addition of 300 mg/l of raspberry colloids reduced the performance by 10%, an addition of 900 mg/l by 30% (Will, 1993).

In spite of the fact that the amounts of colloids can be reduced by gentle processing, the release of residual pectins during juice processing cannot be prevented completely. With modern dejuicing systems the industrial practice of gentle processing is nearly impossible because of economics, capacity and yield demands. A more realistic approach is the development of special pectolytic enzymes for fruit processing. In the case of raspberries, this means the supplementation of the preparations with arabinogalactanases and mainly rhamnogalacturonases in combination with the corresponding acetylesterases.

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