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Characterization of hop pectins shows the presence of an arabinogalactan-protein

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Abstract

Hop pectins were extracted from spent hops using acid extraction conditions and were characterized chemically. The acid extraction of spent hops resulted in a yield of 2%, containing 59% of polysaccharides. The hop pectins under investigation had a relatively high molecular weight and an intrinsic viscosity comparable to that of commercially available apple and citrus pectins. The low degree of methyl esterification of these pectins implicates that they are mainly suitable for use in calcium gels. The degree of acetylation and the neutral sugar content were relatively high.

A high molecular weight fraction which contained arabinogalactan-proteins was shown to be present in the hop pectin extract after preparative size-exclusion chromatography. Additionally, a fraction with a lower molecular weight was present containing mainly homogalacturonans. The arabinogalactans in the high molecular weight population consisted of (1 → 3)- and (1 → 3,6)-linked galactans highly branched with arabinose and galactose side-chains. The protein part of the arabinogalactan-protein (13%) was found to be rich in cystein, threonin, serinin, alanin, and hydroxyprolin. The molecular weight distribution of the hop pectin after degradation with the enzymes *endo*polygalacturonase plus pectin methyl esterase suggested that the arabinogalactan-protein present in the hop pectin extract was linked to the pectin and that the arabinogalactan-protein itself had a fairly low molecular weight.

Keywords: Spent hops; Pectin; Arabinogalactan-protein; Humulus lupulus

1. Introduction

Spent hops are an important by-product of the hop processing industries. Currently a major share of these spent hops are used as fertilizer. However, in order to increase the added value of spent hops, hop processing industries have been looking for alternative utilization of spent hops.

Hops are rich in cell-wall polysaccharides (De Clerck, 1957), but relatively little is known about the chemical structure and functional properties of these cell wall polysaccharides. Knowledge about the composition of these polysaccharides is a prerequisite for the valorization of spent hops.

Pectins represent a large part of the polysaccharides in spent hops (De Clerck, 1957). Pectins are widely used as ingredient for the food industry as gelling agent and thickening agent. Most of the pectin used in food originates from citrus or from apple (Rolin & De Vries, 1991). However, recently several other sources of pectin (sugar beet, sun flower, potato) have been investigated for use in the food industry (Oosterveld, Beldman, Searle-van Leeuwen, & Voragen, 2000a; Turmucin, Ungan, & Yilder, 1983; Turquois, Rinaudo, Taravel, & Heyraud, 1999).

Pectins are considered to consist of homogalacturonan regions and ramified regions in which most of the neutral sugars are located (Schols & Voragen, 1996; Thibault, Guillon, & Rombouts, 1991). The homogalacturonan regions consist of a backbone of (1 → 4)-linked galacturonic acid (Thibault, Renard, Axelos, Roger, & Crepeau, 1993). Both methyl esters and acetyl groups can be present in the homogalacturonan. The ramified regions of pectins extracted from apples are believed to consist of three subunits (Schols, Bakx, Schipper, & Voragen, 1995): (1) a rhamnogalacturonan subunit consisting of a backbone of alternating (1 → 4)-linked galacturonic acid and (1 → 2)-linked rhamnose residues, partly substituted with single unit galactose residues (1 → 4)-linked to the rhamnose residues; (2) a rhamnogalacturonan subunit substituted with arabinan and galactan side-chains; (3) a xylogalacturonan subunit, which consists of a galacturonan backbone, with

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single unit xylose residues linked at the C-3 position of galacturonic acid residues. It is suggested that the ramified regions of pectins obtained from other sources are built from similar subunits, although principal differences in the relative amounts of the various subunits may exist (Schols & Voragen, 1996).

Arabinogalactan-proteins (AGPs) are found in most higher plants (Fincher & Stone 1983). Although the physiological functions of AGPs have not yet been identified, some evidence is found that they are involved in plant reproductive development, pattern formation and somatic embryogenesis (Nothnagel, 1997). In general the arabinogalactan moiety of AGPs represent 90% of the weight, while the protein moiety represents 10% of the weight (Nothnagel, 1997). The arabinogalactan moiety is characterized as a type II arabinogalactan consisting of a (1 → 3)-β-D-galactan core branched at O-6 with (1 → 6)-linked galactan outer chains heavily substituted with terminal α-L-arabinofuranosyl residues (Brillouet, Williams, Will, Muller, & Pellerin, 1996). The protein moiety was found to be rich in the amino acids hydroxyprolin, serin, alanin, threonin, and glycin (Clarke, Anderson, & Stone, 1979; Fincher & Stone, 1983; Nothnagel, 1997), although also AGPs have been characterized with different amino acid compositions (Nothnagel, 1997).

In order to explore the possibilities for valorization of spent hops, the chemical structure of acid extracted pectins from spent hops after separation by size-exclusion chromatography (SEC) was studied. The effect which the chemical structure of these hop pectins may have on the physical properties is discussed.

2. Experimental

2.1. Materials

Pectins were extracted from spent hops (variety: Target; crop 1994; obtained after CO_2 extraction of hops) on a pilot plant scale by acid extraction (pH ~ 2, T ~ 80–90 °C, ~2 h) with extraction conditions usually used to extract pectins from apple pomace and citrus peel (Voragen, Pilnik, Thibault, Axelos, & Renard, 1995). The ratio extractant: spent hops was approximately 10:1.

2.2. Analytical methods

The uronic acid content was determined by the automated *m*-hydroxy biphenyl assay (Thibault, 1979). The neutral sugar composition was determined after pretreatment with 72% H_2SO_4 and hydrolysis with 1 M H_2SO_4 as described earlier (Oosterveld, Beldman, Schols, & Voragen, 1996). The sugar linkage composition of the neutral sugars was determined using the methylation analysis as described previously (Oosterveld et al., 1996), using hydrolysis with 90% (v/v) formic acid (5 h, 100 °C). The partially methylated alditol acetates were identified by GC-MS and quantified by GLC. The degrees of methyl esterification (DM) and acetylation (DA) of the pectins were determined as described by Voragen, Schols, and Pilnik (1986). Polyphenols were determined according to the Folin-Ciocalteu procedure (Scalbert, Monties, & Janin, 1989). Protein contents were determined as described by Sedmak and Grossberg (1977). BSA was used as a standard. The amino acid composition of the polysaccharide fractions was determined after oxidation with performic acid to prevent degradation of cystine and methionine (Rudemo, Bech-Andersen, & Mason, 1980). Subsequently the samples were hydrolized with 6N HCl at 100 °C for 21 h under N_2 and analyzed on a Biochrom 20 Amino Acid Analyzer (Amersham Pharmacia Biotech) equipped with a LiHR column. Ash was determined by incineration at 550 °C overnight.

2.3. Gel diffusion reaction with Yariv reagent

Petri dishes containing 1% of agarose gel in 10 mM Tris buffer (pH 7.3) together with 0.9% NaCl and 10 mM $CaCl_2$ were used. Yariv reagent (1 mg/ml) was delivered to a central well and the hop pectin extract as well as gum arabic (which was used as a positive control) was put in a well 1 cm from the center of the plate. Gels were left at room temperature overnight.

2.4. Chromatography

High-performance size-exclusion chromatography (HPSEC) was performed on three Bio-Gel TSK columns in series (60XL-40XL-30XL) as described (Oosterveld, Beldman, Schols, & Voragen, 2000b), using a combined RI detector and viscometer (Viscotek, model 250), a Right Angle Laser Light Scattering detector (RALLS, Viscotek, LD 600) and a UV detector (Kratos, Spectroflow 773). Molecular weights were calculated using the light scattering module of the Trisec software (Viscotek).

Preparative SEC was performed on two columns (length: 50 cm; diameter: 10 cm) of Sephacryl S-500 (separation range for dextrans: 40–20,000 kDa; Amersham Pharmacia Biotech) in series, using a Biopilot system (Amersham Pharmacia Biotech). The sample (1.5 g) was eluted with 0.05 M NaOAc pH 5.0 at a flow rate of 37 ml/min. The fractions obtained by preparative SEC were assayed for total neutral sugar (Tollier & Robin, 1979) and uronic acid (Thibault, 1979) content, using arabinose and galacturonic acid as standards. A correction was made for the response of uronic acids in the neutral sugar test. The presence of protein in the fractions was monitored spectrophotometrically at 280 nm. Pooled fractions were dialyzed and freeze-dried.

2.5. Enzymatic degradation

The acid extracted hop pectin was treated with the following combinations of enzymes: arabinofuranosidase B (AF) plus *endo*-arabinanase (EA) (Rombouts et al., 1988),

Table 1
Sugar composition of spent hops and of an acid extracted hop pectin (AHP) in %w/w

	Spent hops	AHP
Rha	0.8	1.8
Ara	1.9	3.4
Xyl	3.7	0.7
Man	1.4	1.1
Gal	2.2	8.2
Glc	15.3	1.3
UA	12.9	42.9
Total sugar	38.3	59.4

*endo*polygalacturonase (PG) (Pasculli, Gereads, Voragen, & Pilnik, 1991) combined with pectin methyl esterase (PE) (Baron, Rombouts, Drilleau, & Pilnik, 1980), and rhamnogalacturonase (RG) (Schols, Gereads, Searle-van Leeuwen, Kormelink, & Voragen, 1990) in combination with rhamnogalacturonan acetyl esterase (RGAE) (Searle-van Leeuwen, van den Broek, Schols, Beldman, & Voragen, 1992). Measurement of activity towards a broad range of polysaccharides showed that these enzymes had no measurable activity on pectic substrates other than their main activity. The pectin (5 mg/ml) was dissolved in 0.04 M sodium acetate buffer pH 5.0. All enzymes were added to obtain a final concentration of 1 μg of protein/ml, sufficient to obtain the maximum degradation possible. Incubations were carried out at 30 °C for 20 h. The reactions were stopped by heating at 100 °C for 5 min. The digests were analyzed by HPSEC.

3. Results and discussion

3.1. Characterization of hop pectin

Extraction of hop pectin from spent hops using acid extraction conditions yielded 2% of material, containing 59% of polysaccharides. To obtain a first characterization the sugar composition of spent hops and hop pectin was determined (Table 1). The sugar composition of the spent hops shows the presence of high amounts of glucose, uronic acid and xylose, indicating the presence of a type I cell wall (Carpita & Gibeaut, 1993), composed mainly of cellulose and pectins. The pectic sugars, uronic acid, rhamnose, arabinose and galactose, accounted for 46% of the polysaccharides. The arabinose and galactose content was 6.1 and 5.7 mol%, respectively, which is somewhat lower than that in apple cell walls (Renard, Voragen, Thibault, & Pilnik, 1990), and much lower than in sugar beet cell walls (Oosterveld et al., 1996). The relatively high xylose content suggests the presence of xyloglucans. The hop pectin extract consisted for only 60% of sugars. Uronic acid accounted for 75 mol% of these sugars. The neutral sugar content (25 mol%) of the hop pectin was relatively high as compared with apple and citrus pectins (Thibault et al., 1993), but it was comparable with that of sugar beet pectin (Oosterveld et al., 2000a). This indicates the presence of relatively high amounts of rhamnogalacturonan regions in hop pectin, as was also found for beet pectin (Oosterveld et al., 2000a). The degree of methyl esterification (DM) of the hop pectin was approximately 50. This is rather low in comparison with pectins from other sources like sugar beet (Guillon & Thibault, 1990) and apple (Renard et al., 1990). Since a DM from approximately 50–80 is required for the preparation of pectin gels with acid and sugar, and the DM of hop pectins is at the low end of this range, it is expected that these hop pectins will have rather poor gelling properties for this type of gel and will have a high setting time. Additionally, hop pectins can be used for the preparation of calcium gels, for which pectin with a DM lower than 50 is required. The degree of acetylation (DA; 8.4%) is comparable to that of acid extracted apple pectins (Renard et al., 1990). Kravtchenko, Voragen, and Pilnik, (1992) stated that DA values lower than 12.5% do not hinder gelation, but that it is likely that acetylation influences gel properties. Besides polysaccharides relatively high amounts of ash (13%) and protein (8%) were found in the hop pectin extract, as well as a low amount of polyphenols (3%). The amino acid composition of the protein present in the hop pectin extract was comparable to the amino acid composition of lemon and apple (Kravtchenko et al., 1992) and also to that of the initial spent hops (Table 2).

Table 2
Relative amino acid composition (mol%) of spent hops, acid extracted hop pectin (AHP), and SEC pools of hop pectin

	Spent hops	AHP	Pool I	Pool II	Pool III
CYS	2.0	2.6	7.1	6.1	0.0
ASP	10.1	9.7	8.4	8.3	8.8
METH	0.0	0.7	0.0	0.0	0.0
THR	5.9	6.6	10.6	8.8	4.4
SER	9.8	9.0	17.3	15.7	10.1
GLU	11.9	10.7	7.9	7.7	11.9
GLY	8.4	7.3	7.3	7.4	9.5
ALA	9.3	9.4	18.7	17.6	7.5
VAL	5.0	6.0	3.8	4.1	6.3
ILE	3.8	3.6	0.0	0.0	3.2
LEU	6.2	6.0	0.0	3.4	5.5
TYR	3.8	3.1	0.0	0.0	0.0
PHE	2.8	2.7	0.0	0.0	2.7
LYS	7.5	7.5	4.1	4.1	10.1
HIS	2.0	2.1	0.0	0.0	5.3
ARG	3.3	3.6	0.0	0.0	4.0
HYPRO	4.0	5.3	14.8	15.7	7.1
PRO	4.2	4.2	0.0	1.1	3.7
Total	100.0	100.0	100.0	100.0	100.0

The hop pectin extract was further characterized by HPSEC with detection of UV at 280 nm, refractive index, differential viscosity and light scattering. The refractive index and UV detection are shown in Fig. 1(a). Three

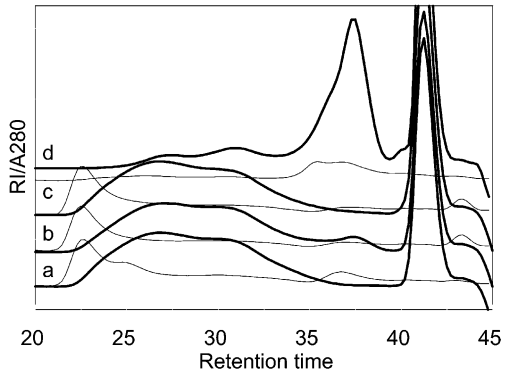

Fig. 1. HPSEC of an acid extracted hop pectin (AHP) before (a) and after degradation with the enzyme combinations rhamnogalacturonase plus rhamnogalacturonan acetyl esterase (b), *endo*-arabinanase and arabinofuranosidase (c), and polygalacturonase plus pectin methylesterase (d). Thin line: UV_{280}; thick line: refractive index.

major populations could be distinguished: a high molecular weight population with a high UV absorption at 280 nm, and two pectic populations with a high refractive index response and low UV absorption. Note that the peak eluting at 42 min originates from the buffer used. In order to investigate the possibility that the high UV_{280} absorption of the population eluting at 22 min resulted from the presence of AGPs, the reactivity of the crude hop pectin extract with Yariv reagent was tested. It was found that the hop pectin extract reacted positively with the Yariv reagent, showing that AGPs were present in the extract. The molecular weight of the parental extract was found to be 504 kDa, which is relatively high as compared with apple and citrus pectins as found by Thibault et al. (1993), while the intrinsic viscosity of this extract was comparable to those of citrus and apple pectins (Table 3). This can be explained by the presence of the relatively high amount of neutral sugars in hop pectin, which may be present as side-chains of the rhamnogalacturonan regions of the pectin. These side-chains contribute to the molecular weight but not that much to the intrinsic viscosity (Hwang, Pyun, & Kokini, 1993).

3.2. Preparative size-exclusion chromatography

In order to study the nature of the populations found by HPSEC, the hop pectin extract was separated by preparative

Table 3
Molecular weights (M_w (kDa)) and intrinsic viscosities ($[\eta]_w$ (dl/g)) of an acid extracted hop pectin (AHP) before and after enzyme treatment with rhamnogalacturonase plus rhamnogalacturonan acetyl esterase (RG + AE), arabinofuranosidase B plus *endo*-arabinanase (EA + AF), and *endo*polygalacturonase plus pectin methyl esterase (PG + PE)

	M_w	$[\eta]_w$
AHP	504.7	2.9
AHP + RG + AE	357.3	2.8
AHP + EA + AF	519.7	3.0
AHP + PG + PE	94.2	0.1

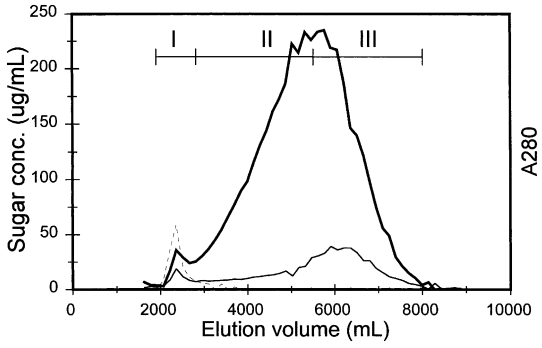

Fig. 2. SEC (Sephacryl S-500) of an acid extracted hop pectin (AHP); Thick line: uronic acid; thin line: neutral sugars; dashed line: UV_{280}.

SEC. The elution pattern is shown in Fig. 2. Only two distinct populations could be recognized: a high molecular weight population with a high UV response containing the AGPs (vide infra), and a lower molecular weight pectin population representing the bulk of the material. The differences between the results found by preparative SEC and HPSEC are probably a result of different separation ranges of the colums used for HPSEC and preparative SEC. The fractions were pooled as indicated in Fig. 2 and analyzed (see Table 4). Pool I contained only 52% of sugars, while populations II and III contained more than 96% of sugars. Pool I was relatively rich in protein when compared to pools II and III. Pool I contained a relatively high amount of arabinose and galactose, indicating that a large proportion of the polysaccharides in this pool consist of arabinogalactans. The ratio carbohydrate: protein in pool I was approximately 8:1, which was also found for AGPs from various sources (Clarke, Gleeson, Jermyn, & Knox, 1978; Jermyn & Yeow, 1975). Pools II and III consisted for 73–83% of uronic acid, showing that these pools contained mainly homogalacturonans.

3.3. Sugar linkage composition

The sugar linkage composition of the SEC pools was investigated in order to study the structural characteristics of the polysaccharides present in hop pectin (Table 5) and to

Table 4
Composition of the pools of an acid extracted hop pectin obtained after preparative SEC in % w/w (between brackets: mol%)

Sample	Pool I	Pool II	Pool III
Rha	1.6	1.8	2.1
Ara	5.1	2.8	5
Xyl	0.9	1.1	0.8
Man	1.9	2.2	2.2
Gal	8.2	5.7	12.4
Glc	2.8	0.9	0.7
GalA	31.9	84.6	73.6
Total sugar	52.4	99.1	96.8
Protein	6.8	1.5	0.4

Table 5
Sugar linkage composition of SEC pools of the acid extracted hop pectin (AHP)

Me-group:	Linkage	Pool I	Pool II	Pool III
234 ara	*t*-fur	9.6	13.8	15.8
35 ara	1 → 2-fur	1.4	0.4	0.7
23 ara	1 → 5-fur	7.1	8.4	6.9
2 ara	1 → 3,5-fur	0.9	2.0	1.2
unme ara	1 → 2,3,5-fur	2.9	0.0	1.4
		21.9	24.6	25.9
234 xyl	*t*-pyr	0.4	0.8	1.0
23 xyl	1 → 4-xyl	2.8	0.4	0.5
		3.2	1.2	1.5
234 rha	*t*-rha	0.9	2.2	2.0
34 rha	1 → 2-rha	0.4	2.1	0.3
3 rha	1 → 2,4-rha	1.0	3.2	0.4
		2.3	7.5	2.7
2346 glc	*t*-glc	6.2	0.1	0.8
236 glc	1 → 4-glc	28.6	4.1	2.7
23 glc	1 → 4,6-glc	3.9	1.2	1.1
		38.7	5.4	4.6
236 man	1 → 4-man	3.7	0.5	0.8
		3.7	0.5	0.8
2346 gal	*t*-gal	3.7	9.0	5.7
246 gal	1 → 3-gal	4.6	7.8	9.0
236 gal	1 → 4-gal	2.6	12.1	8.8
234 gal	1 → 6-gal	5.7	9.1	10.8
23 gal	1 → 4,6-gal	0.5	2.7	2.5
26 gal	1 → 3,4-gal	3.0	2.6	1.8
24 gal	1 → 3,6-gal	10.2	17.7	25.7
		30.4	60.9	64.4

investigate the nature of the high molecular weight population, containing both pectic polysaccharides and protein. Galactose was found to be mainly (1 → 3)-, (1 → 6)-, and (1 → 3,6)-linked, indicating the presence of type II arabinogalactans, although some (1 → 4)-linked galactose was present as well. Arabinose was found to be predominantly (1 → 5)-and terminally linked. The ratio of branched molecules to terminally linked molecules for arabinose and galactose indicates that arabinose is mainly present as side-chain of (arabino)galactan molecules. The glucose content of pool I as determined with the sugar linkage analysis was very high in comparison with the results found for the sugar analysis (Table 4). No explanation for this observation was found. The presence of (1 → 3)- and (1 → 3,6)-linked galactose in pool I combined with a high amount of protein linked to it is a strong indication for the presence of AGPs (Fincher & Stone, 1983). The amino acid composition of this pool was determined to substantiate this.

3.4. Amino acid composition

In order to further investigate the possible presence of AGPs in the hop pectin extract, the amino acid compositions of the preparative SEC pools were determined (Table 2). Pools I and II had a similar amino acid composition, which was significantly different from the initial extract. When compared to the initial extract these pools were enriched in cystein, threonin, serinin, alanin, and hydroxyprolin. Serinin, alanin, tyrosin, and hydroxyprolin together accounted for approximately 55% of the amino acids in pools I and II. The amino acid composition of these pools resembles that of AGPs from rose cell walls, tabacco, and lolium multiflorum (Akiyama & Kato, 1981; Anderson, Clarke, Jermyn, Knox, & Stone, 1977; Serpe & Nothnagel,1995). Pool III was enriched in glutamin, iso-leusin, leusin, lysin, histidin, arginin, and prolin as compared with pool I and II and resembled the amino acid composition of the spent hops and the hop pectin it originated from.

3.5. Enzymatic degradation of hop pectin

In order to gain information about the location of the arabinogalactan-protein in the pectin, the pectin extract was incubated with several enzyme combinations (Fig. 1). The combinations used were: arabinofuranosidase B plus *endo*-arabinanase (EA + AF), *endo*polygalacturonase combined with pectin methyl esterase (PG + PE), and rhamnogalacturonase in combination with rhamnogalacturonan acetyl esterase (RG + RGAE). The molecular weights and intrinsic viscosities of the pectin extract before and after enzyme treatment are shown in Table 3.

Treatment of the hop pectin with the enzymes EA + AF did not result in a decrease in M_w or $[\eta]_w$, probably as a result of the low arabinose content of the hop pectin. However, two small populations eluting at 25 and 37 min (based on UV_{280}) disappeared after enzyme treatment. These populations also disappeared after treatment with RG + RGAE. This is an indication that some of the UV-absorbing material may be linked to the rhamnogalacturonan regions of the pectin. RG + RGAE also caused a decrease in the molecular weight, but not in the intrinsic viscosities. Although it is generally assumed that rhamnogalacturonan stretches are present throughout the pectin (Schols & Voragen, 1996), the small decrease in viscosity indicates that the degraded rhamnogalacturonan backbone was predominantly present at the extremities of the pectin molecules as was also seen for acid extracted sugar beet pectins (Oosterveld, Beldman, & Voragen, 2000c). Apparently, the backbone of the rhamnogalacturonan regions was partly hydrolized during the extraction as a result of the acidic extraction conditions. Guillon and Thibault (1990) showed that both rhamnose and galacturonic acid were released from sugar beet pectins during mild acid treatment, indicating hydrolysis in the rhamnogalacturonan backbone, although these sugars were released at a lower rate than

arabinose and galactose. The release of arabinose and galactose due to the acidic extraction conditions probably resulted in a further decrease of the molecular weight of the rhamnogalacturonan regions. As a result of these processes apparently the rhamnogalacturonan regions remained after the extraction as relatively small stretches at the extremities of the pectin molecules. This may explain that treatment of the hop pectin with RG + AE only resulted in a small decrease in molecular weight and viscosity. Treatment of the hop pectin with PG + PE resulted in a large decrease of the M_w and a huge decrease of the $[\eta]_w$. Also the population containing the arabinogalactan-protein, eluting at 22.5 min, shifted towards higher retention times, suggesting that the arabinogalactan-protein itself has a fairly low molecular weight. These results show that the arabinogalactan-protein was somehow linked to the pectin population, but was not released after degradation of the rhamnogalacturonan backbone or the arabinan side-chains. Possibly, the presence of highly branched arabinogalactan side-chains prevented the release of the arabinogalan-proteins with the enzyme combinations RG + RGAE and EA + AF.

4. Concluding remarks

Spent hops are an important by-product from the hop processing industry. In the current study we showed that it is possible to extract high M_w pectins from spent hops, which have an intrinsic viscosity comparable to that of commercially available apple and citrus pectins. However, the low DM of these hop pectins, as well as the rather high DA and neutral sugar content limit the use of hop pectins as gelling agents (Oosterveld et al., 2000a; Pippen, McCready, & Owens, 1950). Future research will be directed towards the removal of part of the acetyl groups and neutral sugars, for instance by enzymatic modification, which is expected to improve the properties of the pectins obtained from spent hops.

This investigation has shown that a high molecular weight fraction is present in hop pectin which contains AGPs. The arabinogalactan-protein in this population consisted predominantly of (1 → 3)- and (1 → 3,6)-linked galactans highly branched with arabinose and galactose, while the protein part was found to be rich in cystein, threonin, serinin, alanin, and hydroxyprolin. The arabinogalactan-protein present in hop pectin is suggested to be linked to the pectin, thus explaining its high molecular weight. The molecular weight distribution of the hop pectin after degradation with PG + PE suggests that the arabinogalactan-protein itself has a fairly low molecular weight. To our knowledge this is the first report of the presence of AGPs in hop pectin.

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References

Akiyama, Y., & Kato, K. (1981). An extracellular arabinogalactan-protein from Nicotiana Tabacum. *Phytochemistry*, *20*, 2507–2510.

Anderson, R. L., Clarke, A. E., Jermyn, M. A., Knox, R. B., & Stone, B. A. (1977). A carbohydrate-binding arabinogalactan-protein from liquid suspension cultures of endosperm from lolium multiflorum. *Australian Journal of Plant Physiology*, *4*, 143–158.

Baron, A., Rombouts, F. M., Drilleau, J. F., & Pilnik, W. (1980). Purification et propriétés de la pectinestérase produite par Aspergillus niger. *Lebensmittel Wissenschaft und Technologie*, *13*, 330–333.

Brillouet, J. -M., Williams, P., Will, F., Muller, G., & Pellerin, P. (1996). Structural characterization of an apple juice arabinogalactan-protein which aggregates following enzymic dearabinosylation. *Carbohydrate Polymers*, *29*, 271–275.

Carpita, N. C., & Gibeaut, D. M. (1993). Structural models of primary cell walls in flowering plants: Consistency of molecular structure with the physical properties of the walls during growth. *The Plant Journal*, *3*, 1–30.

Clarke, A. E., Gleeson, P. A., Jermyn, M. A., & Knox, R. B. (1978). Characterization and localization of β-lectins in lower and higher plants. *Australian Journal of Plant Physiology*, *5*, 707.

Clarke, A. E., Anderson, R. L., & Stone, B. A. (1979). Form and function of arabinogalactans and arabinogalactan-proteins. *Phytochemistry*, *18*, 521–540.

De Clerck, J. (1957). *A textbook of brewing* (pp. 58–63). London: Chapman & Hall.

Fincher, G. B., & Stone, B. A. (1983). Arabinogalactan-proteins: Structure, biosynthesis, and function. *Annual Reviews of Plant Physiology*, *34*, 47–70.

Guillon, F., & Thibault, J. -F. (1990). Oxidative cross-linking of chemically and enzymatically modified sugar-beet pectin. *Carbohydrate Polymers*, *12*, 353–374.

Hwang, J., Pyun, Y. R., & Kokini, J. L. (1993). Sidechains of pectins: Some thoughts on their role in plant cell walls. *Food Hydrocolloids*, *7*, 39–53.

Jermyn, M. A., & Yeow, Y. M. (1975). A class of lectins present in tissues of seed plants. *Australian Journal of Plant Physiol.*, *2*, 501.

Kravtchenko, T. P., Voragen, A. G. J., & Pilnik, W. (1992). Analytical comparison of three industrial pectin preparations. *Carbohydrate Polymers*, *18*, 17–25.

Nothnagel, E. A. (1997). Proteoglycans and related components in plant cells. *International Review of Cytology*, *174*, 195–291.

Oosterveld, A., Beldman, G., Schols, H. A., & Voragen, A. G. J. (1996). Arabinose and ferulic acid rich pectic polysaccharides extracted from sugar beet pulp. *Carbohydrate Research*, *288*, 143–153.

Oosterveld, A., Beldman, G., Searle-van Leeuwen, M. J. F., & Voragen, A. G. J. (2000a). Effect of deacetylation on gelation of sugar beet pectin in the presence of calcium. *Carbohydrate Polymers*, *43*, 249–256.

Oosterveld, A., Beldman, G., Schols, H. A., & Voragen, A. G. J. (2000b). Characterization of arabinose and ferulic acid rich polysaccharides and hemicelluloses from sugar beet pulp. *Carbohydrate Research*, *328*, 185–197.

Oosterveld, A., Beldman, G., & Voragen, A. G. J. (2000c). Enzymatic modification of pectic polysaccharides obtained from sugar beet pulp. *Carbohydrate Polymers*, In Press.

Pasculli, R., Gereads, C. C. J. M., Voragen, A. G. J., & Pilnik, W. (1991). Characterization of polygalacturonases from yeast and fungi. *Lebensmittel Wissenschaft und Technologie*, *24*, 63–70.

Pippen, E. L., McCready, R. M., & Owens, H. S. (1950). Gelation properties of partially acetylated pectins. *Journal of the American Chemical Society*, *72*, 813–816.

Renard, C. M. G. C., Voragen, A. G. J., Thibault, J. -F., & Pilnik, W. (1990). Studies on apple protopectin: I. Extraction of insoluble pectin by chemical means. *Carbohydrate Polymers*, *12*, 9–25.

Rolin, C., & De Vries, J. (1991). Pectin. In P. Harris, *Food gels* (pp. 401–434). Amsterdam: Elsevier.

Rombouts, F. M., Voragen, A. G. J., Searle-van Leeuwen, M. J. F., Gereads, C. C. J. M., Schols, H. A., & Pilnik, W. (1988). The arabinases of Apergillus niger-purification and characterisation of two α-L-arabinofuranosidases and an *endo*-1,5-α-L-arabinanase. *Carbohydrate Polymers*, *8*, 25–47.

Rudemo, M., Bech-Andersen, S., & Mason, V. C. (1980). Hydrolysate preparation for amino acid determinations in feed constituents. 5. The influence of hydrolysis time on amino acid recovery. *Zeitschrift fuer Tierphysiologie, Tierernaehrung und Futtermittelkunde*, *43*, 27–34.

Scalbert, A., Monties, B., & Janin, G. (1989). Tannins in wood: Comparison of different estimation methods. *Journal of Agriculture and Food Chemistry*, *37*, 1324–1329.

Schols, H. A., Gereads, C. C. J. M., Searle-van Leeuwen, M. J. F., Kormelink, F. J. M., & Voragen, A. G. J. (1990). Rhamnogalacturonase: A novel enzyme that degrades the hairy regions of pectins. *Carbohydrate Research*, *206*, 105–115.

Schols, H. A., & Voragen, A. G. J. (1996). Complex pectins: Structure elucidation using enzymes. In J. Visser & A. G. J. Voragen, *Pectins and Pectinases* (pp. 3–19). Amsterdam: Elsevier.

Schols, H. A., Bakx, E. J., Schipper, D., & Voragen, A. G. J. (1995). A xylogalacturonan subunit present in the modified hairy regions of apple pectin. *Carbohydrate Research*, *279*, 265–279.

Searle-van Leeuwen, M. J. F., van den Broek, L. A. M., Schols, H. A., Beldman, G., & Voragen, A. G. J. (1992). Rhamnogalacturonan acetylesterase: A novel enzyme from Aspergillus aculeatus, specific for the deacetylation of hairy (ramified) regions of pectins. *Applied Microbiology and Biotechnology*, *38*, 347–349.

Sedmak, J. J., & Grossberg, S. E. (1977). A rapid, sensitive, and versatile assay for protein using Coomassie Brilliant Blue G250. *Analytical Biochemistry*, *79*, 544–552.

Serpe, M. D., & Nothnagel, E. A. (1995). Fractionation and structural characterization of arabinogalactan-proteins from the cell wall of rose cells. *Plant Physiology*, *109*, 1007–1016.

Thibault, J. -F. (1979). Automatisation du Dosage des substances pectiques par la méthode au métahydroxydiphenyl. *Lebensmittel Wissenschaft und Technologie*, *12*, 247–251.

Thibault, J. -F., Guillon, F., & Rombouts, F. M. (1991). Gelation of sugar beet pectins by oxidative coupling. In R. H. Walter, *The chemistry and technology of pectin* (pp. 119–133). San Diego, California: Academic Press.

Thibault, J. -F., Renard, C. M. G. C., Axelos, M. A. V., Roger, P., & Crépeau, M. -J. (1993). Studies of the length of homogalacturonic regions in pectins by acid hydrolysis. *Carbohydrate Research*, *238*, 271–286.

Tollier, M., & Robin, J. (1979). Adaptation de la méthode à l'orcinol-sulfurique au dosage automatique des glucides neutres totaux: Conditions d'application aux extraits d'origine végétale. *Annales de Technologie Agricole*, *28*, 1–15.

Turmucin, F., Ungan, S., & Yilder, F. (1983). Pectin production from sun flower. *Journal of Pure Applied Science*, *16*, 263.

Turquois, T., Rinaudo, M., Taravel, F. R., & Heyraud, A. (1999). Extraction of highly gelling pectic substances from sugar beet pulp and potato pulp: Influence of extrinsic parameters on their gelling properties. *Food Hydrocolloids*, *13*, 255–262.

Voragen, A. G. J., Schols, H. A., & Pilnik, W. (1986). Determination of the degree of methylation and acetylation of pectins by hplc. *Food Hydrocolloids*, *1*, 65–70.

Voragen, A. G. J., Pilnik, W., Thibault, J. -F., Axelos, M. A. V., & Renard, C. M. G. C. (1995). Pectins. In A. M. Stephen, *Food polysaccharides and their applications* (pp. 287–339). New York: Marcel Dekker.