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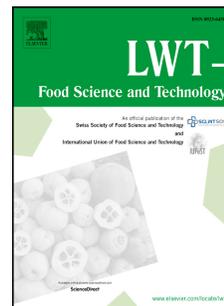
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Pectin characterisation in vegetable waste streams: a starting point for waste valorisation in the food industry

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1 **Abstract**

2 Vegetable waste streams, which are a major disposal issue in food industry, present a promising

3 source of compounds which may be valorised because of their favourable functional features. In

4 this context, the potential of five vegetable waste streams (rejected carrots, carrot steam peels,

5 green beans cutting waste, leek cutting waste and celeriac steam peels) as a source for the

6 extraction of pectin with interesting structural, and hence functional, properties was evaluated.

7 Specifically, cell-wall components were extracted from the waste streams as alcohol-insoluble

8 residue and subsequently fractionated into different (pectin) fractions based on their solubility.

9 The pectic polysaccharides were characterised in terms of GalA content, neutral sugar content,

10 linearity/branching, degree of methyl-esterification (DM), molar-mass distribution and the

11 presence of bound protein. Pectin characterisation revealed considerable differences between the

12 pectic polymers present in the investigated waste streams. For example, pectin in carrot steam

13 peels showed a low DM, whereas the cutting waste of leek contained pectin with a very high

14 DM. Furthermore, the level of protein bound to pectin was generally highest in carrot-derived

15 waste streams. Depending on the intended pectin functionality, a deliberate choice for one of

16 these vegetable waste streams as a source for pectin extraction can be made.

17 **Keywords:** carrot, leek, green beans, celeriac, protein

18 **1. Introduction**

19 Waste streams originating from vegetable processing present a major disposal issue for the food

20 industry concerned. Nowadays, waste of plant materials is generally used for animal feed, as

21 fertilizer or disposed as such. In addition, concepts such as composting and biogas production

22　have been exploited for the conversion of waste (Laufenberg, Kunz, & Nystroem, 2003).

23　Vegetable waste streams however present a promising source of compounds which may be

24　valorised because of their favourable functional (e.g. rheological, nutritional, …) properties

25　(Schieber, Stintzing, & Carle, 2001). Among the different compounds comprised in plant

26　material waste, pectin represents an interesting polysaccharide, which can be used as functional

27　food ingredient in many applications.

28　Pectin is a complex polysaccharide, rich in galacturonic acid (GalA), present in the cell wall of

29　all higher plants. Structurally, three main pectin domains can be distinguished:

30　homogalacturonan (HG), the linear region of pectin, and rhamnogalacturonan-I (RG-I) and

31　rhamnogalacturonan-II (RG-II), branched domains of pectin (Voragen, Coenen, Verhoef, &

32　Schols, 2009). Specifically, HG is a homopolymer consisting of α-D-GalA residues in which

33　some of the C-6 carboxyl groups are methyl-esterified. RG-I on the other hand is a family of

34　highly branched pectic polymers that contain a backbone of the repeating disaccharide [→4)-α-

35　D-GalA-(1→2)-α-L-Rha-(1→] of which the Rha residues can be substituted with side chains

36　mainly consisting of galactosyl and/or arabinosyl residues. Finally, RG-II consists of a backbone

37　of around nine GalA residues which is substituted by four hetero-oligomeric side chains with

38　known and consistent composition and length (O'Neill et al., 1996). The nanostructural

39　properties of pectin depend on the plant source as well as on the tissue part from which pectin

40　was obtained. Specifically, pectic polymers can differ in their composition, solubility, degree of

41　methyl-esterification (DM), degree of acetylation, distribution of ester groups, degree of

42　branching and degree of polymerisation (molar mass) (Sila et al., 2009). Moreover, during food

43　processing, several conversion reactions can take place modifying pectin's fine structure.

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44 Extracted pectin (EU code E440) is widely used as a functional food ingredient in innumerable

45 food products, whereby in all its applications as a food ingredient, the nanostructure of pectin

46 profoundly affects its functionality. Pectin is primarily known as a gelling agent and is

47 extensively applied in the production of jams and jellies, fruit juice, confectionary products and

48 bakery fillings (Willats, Knox, & Mikkelsen, 2006). Depending on the DM of pectin, two

49 different mechanisms of pectin gelation can be distinguished. High-methoxylated pectins (DM >

50 50%) on the one hand can form gels in the presence of co-solutes (typically sucrose at a

51 concentration > 55%) and under acidic conditions (pH < 3.5) (Endress, Mattes, & Norz, 2006).

52 Low-methoxylated pectins (DM < 50%) on the other hand form gels in the presence of divalent

53 cations, particularly Ca^{2+}. Gelation is due to the formation of junction zones between HG regions

54 of different pectin chains through calcium bridges between dissociated carboxyl groups (Fraeye,

55 Duvetter, Doungla, Van Loey, & Hendrickx, 2010). Besides its use as a gelling agent, pectin is

56 also often employed for the stabilisation of acidified milk drinks and yoghurts. Specifically,

57 pectin adsorbs onto the casein micelles as a result of electrostatic interaction thereby preventing

58 the flocculation of these milk proteins at acidic pH (Tromp, de Kruif, van Eijk, & Rolin, 2004).

59 Finally, pectin can be applied as an emulsifying agent. This functionality of pectin has not been

60 so widely exploited as its gelling and stabilizing features. The emulsion-stabilising potential of

61 pectin has been related to the hydrophobic character of acetyl groups, ferulic acid groups and

62 residual protein moieties present within the pectin as well as the molar mass of the pectic

63 polymers (Leroux, Langendorff, Schick, Vaishnav, & Mazoyer, 2003; Williams, Sayers, Viebke,

64 Senan, Mazoyer, & Boulenguer, 2005).

65 Nowadays, commercial pectin production is limited to a few sources, i.e. citrus peel and apple

66 pomace (Endress et al., 2006). In this study, the potential of five vegetable waste streams

3

67 (rejected carrots, carrot steam peels, green beans cutting waste, leek cutting waste and celeriac

68 steam peels) as a source for the extraction of pectin with interesting structural, and hence

69 functional, properties was evaluated. Specifically, cell-wall components were extracted from the

70 waste streams as alcohol-insoluble residue and subsequently fractionated into different (pectin)

71 fractions based on their solubility. By applying this type of procedure, insight into the native

72 state of pectin in the plant cell wall can be obtained, hence revealing the full endogenous

73 potential of pectin present in the different vegetable waste streams. Conversely, a direct pectin

74 extraction would not have allowed for this information as, depending on the extraction

75 conditions used, the structure of pectin is modified in terms of molar mass, DM, etc. Pectic

76 polysaccharides were characterised in terms of GalA content, neutral sugar content,

77 linearity/branching, DM and molar-mass distribution. Finally, by combining state-of-the-art

78 techniques (size exclusion chromatography coupled with refractive index, multi angle laser light

79 scattering and diode array detection), the level of protein bound to pectic polymers with a certain

80 molar mass could be examined qualitatively.

81 **2. Materials and methods**

82 *2.1. Vegetable waste streams*

83 Five different vegetable waste streams were acquired from food companies in Belgium. Rejected

84 carrots, carrot steam peels, celeriac steam peels and the cutting waste of green beans were

85 obtained from a canning company, while the cutting waste of leek was provided by a leek

86 producer. The rejected carrots and the cutting waste of leek were first frozen under liquid

87 nitrogen and then packed in plastic bags. The other three waste streams were first vacuum-

88   packed in plastic bags before freezing with liquid nitrogen. All samples were stored at -40 °C

89   until further use.

90   *2.2. Isolation of cell-wall components as alcohol insoluble residue (AIR)*

91   The cell wall material of the different waste streams was isolated as alcohol-insoluble residue

92   (AIR) using ethanol and aceton as described by Christiaens, Van Buggenhout, Houben, Fraeye,

93   Van Loey, & Hendrickx (2011). The GalA content of AIR was determined as described in

94   section 2.4, while an estimation of the total pectin content of AIR was made based on the

95   amounts of GalA and neutral sugars present in the pectin fractions (see sections 2.4 and 2.5)

96   divided by the fractionation yield (i.e. the amount of GalA retrieved in the different fractions

97   compared to the level of GalA in the AIR).

98   *2.3. Fractionation of AIR based on solubility*

99   Cell-wall material, extracted as AIR, was fractionated into various polysaccharide fractions

100   according to Houben, Jolie, Fraeye, Van Loey, & Hendrickx (2011). Specifically, water-soluble

101   pectin (WSP), chelator-soluble pectin (CSP), sodium-carbonate-soluble pectin (NSP) and a

102   hemicellulose fraction (HF) were obtained after subsequent extraction of the AIR with boiling

103   water, 0.05 mol/l cyclohexane-trans-1,2-diamine tetra-acetic acid (CDTA) in 0.1 mol/l potassium

104   acetate, 0.05 mol/l Na_2CO_3 containing 0.02 mol/l $NaBH_4$ and 4 mol/l KOH containing 0.02

105   mmol/l $NaBH_4$ and 35 g/l borate respectively. It is assumed that WSP consists of pectic polymers

106   that are loosely bound to the cell wall through non-covalent and non-ionic bonds, while CSP

107   mainly contains ionically cross-linked pectin and NSP is predominantly linked to cell wall

108   polysaccharides through covalent ester bonds. HF on the other hand still contains some pectic

5

109 polymers that are very strongly bound to cellulose or hemicelluloses. All polysaccharide

110 fractions as well as the remaining residue were frozen with liquid nitrogen and stored at -40 °C.

111 For certain analyses (determination of neutral sugar content and analysis of pectin fractions using

112 high-performance size exclusion chromatography), WSP, CSP, NSP and HF were lyophilised

113 using a freeze-dryer (Christ alpha 2-4 LSC, ice condenser temperature = -85 °C).

114 *2.4. Determination of GalA content in AIR and all polysaccharide fractions*

115 The GalA content in AIR as well as in the different polysaccharide fractions was determined

116 spectrophotometrically as described by Christiaens et al. (2011). Specifically, samples were

117 hydrolysed with concentrated sulfuric acid in a first step after which the concentration of GalA

118 was determined with the m-hydroxydiphenyl method.

119 *2.5. Determination of neutral sugar content in all polysaccharide fractions*

120 Quantification of the neutral sugars (fucose, rhamnose, arabinose, galactose, glucose, xylose and

121 mannose) in all polysaccharide fractions was performed via high-performance anion exchange

122 chromatography according to Houben et al. (2011) after hydrolysis of the samples with

123 trifluoroacetic acid. A Dionex Bio-LC system (DX600) equipped with a GS50 gradient pump, a

124 CarboPac™ PA20 column (150 x 3 mm, pH range = 0–14), a CarboPac™ PA20 guard column

125 (30 x 3 mm) and an ED50 electrochemical detector (Dionex, Sunnyvale, USA) was used. The

126 detector was equipped with a reference pH electrode (Ag/AgCl) and a gold electrode and was

127 used in the PAD mode, performing a quadruple potential waveform. Elution of the samples was

128 performed with 4 mmol/l NaOH at 30 °C.

129 *2.6. Determination of the degree of methyl-esterification of AIR, WSP and CSP*

130   The DM of pectin in AIR, WSP and CSP was determined as the ratio of the molar amount of

131   methyl-esters to the molar amount of GalA residues in the AIR. The amount of methyl-esters

132   was analysed according to Christiaens et al. (2011) while the GalA content was measured as

133   described in section 2.4. To quantify the amount of methyl-esters, the ester bonds in pectin were

134   saponified with NaOH after which the amount of methanol released was measured with the

135   pentanedione method.

136   *2.7. Analysis of pectin fractions via high-performance size exclusion chromatography*

137   To analyse the pectin fractions via high-performance size exclusion chromatography (HPSEC),

138   lyophilised WSP, CSP and NSP were first dialysed. Lyophilised WSP and NSP samples were

139   dissolved in demineralised water (WSP: 2.5 mg/ml, NSP: 15 mg/ml) and extensively dialysed

140   against demineralised water during 48 h, using Visking dialysis membranes (cellulose-based,

141   molecular weight cut-off = 12 – 14 kDa, Medicell International Ltd). CSP samples were also

142   dissolved in demineralised water (75 mg/ml), but dialysed against 0.1 mol/l NaCl during 24 h,

143   followed by dialysis against demineralised water for another 24 h. The dialysed fractions were

144   adjusted to a concentration of 0.05 mol/l $NaNO_3$ and filtered using a Millex HV-filter before

145   analysis.

146   HPSEC was carried out with an Agilent 1200 Series (Agilent) equipped with a series of three

147   mixed-bed columns of TSK-GEL ($GMPW_{XL}$, 300 mm x 7.8 mm, pore size = 100-1000 Å,

148   particle size = 13 µm, theoretical plates/column $\geq$ 7000, pH range = 2-12, maximum pressure =

149   300 psi; Tosoh Bioscience). Dialysed and filtered fractions were injected (50 µl) and eluted with

150   0.05 mol/l $NaNO_3$ at a flow rate of 0.5 ml/min at 35 °C. The eluent was examined with a

151   refractive index (RI) detector (Shodex RI-101, Showa Denko), a multi angle laser light scattering

152 (MALLS) detector (Postnova) and a diode array (DAD) detector (Agilent). The RI detector was

153 used as a concentration detector while the MALLS detector allows for the absolute determination

154 of the molar mass of the samples. In addition, through the DAD detector, the absorbance of the

155 fractions at 280 nm was registered which indicates the presence of proteins in the samples

156 (Whitaker & Granum, 1980).

157 **3. <u>Results and discussion</u>**

158 *3.1. Pectin content in vegetable waste streams*

159 The amount of GalA is for most plant matrices a measure of the total pectin content of the

160 sample. The GalA content of the five different vegetable waste streams, expressed per gram AIR,

161 per gram fresh material and per gram dry matter is in this context displayed in **Table 1**. Celeriac

162 steam peels are the vegetable waste stream richest in GalA, whereas the cutting waste of leek

163 shows the second highest level of GalA. Rejected carrots and carrot steam peels contain lower,

164 similar amounts of GalA while the cutting waste of green beans is the vegetable waste stream

165 with the lowest GalA content. An estimation of the total pectin content could be made based on

166 the amounts of GalA and neutral sugars present in the pectin fractions (see section 3.2) and the

167 fractionation yield (results not shown). The total pectin content of the different vegetable waste

168 streams is also presented in Table 1. It appears that the GalA content is indeed a good predictor

169 of the total pectin content. Similar conclusions as based on the GalA content could be drawn for

170 the different waste streams. The level of pectin found in the vegetable waste streams ranges from

171 7.3 mg/g fresh material (for the cutting waste of green beans) to 15.4 mg/g fresh material (for

172 celeriac steam peels). In literature, similar values, on fresh weight basis, were found for cherries

173   (4 mg/g), apples (10-15 mg/g), apricot (10 mg/g), carrots (14 mg/g) and oranges (5-35 mg/g)

174   (Niture & Refai, 2013).

175   *3.2. GalA and neutral sugar content in polysaccharide fractions*

176   **Table 2** presents the amount of GalA and neutral sugars (Fuc, Rha, Ara, Gal, Glu, Man and Xyl)

177   in WSP, CSP, NSP and HF of the different vegetable waste streams. The determined GalA

178   values differ between the different vegetable waste streams as well as between the different

179   polysaccharide fractions within one waste stream. In general, WSP and CSP extracted from the

180   different waste streams display the highest levels of GalA. Remarkably, HF of all vegetable

181   waste streams, and especially of the cutting waste of green beans, also contains a certain amount

182   of GalA. This observation indicates that a (considerable) part of the pectic polymers are strongly

183   bound to the cell wall in all waste streams (1-2% of total GalA content in polysaccharide

184   fractions), and especially in green beans cutting waste (12% of total GalA content in

185   polysaccharide fractions). The strong binding of pectic polysaccharides to other cell wall

186   polymers in green beans has also been observed by Stolle-Smits, Beekhuizen, Recourt, Voragen

187   and van Dijk (1997). Besides GalA, pectin also contains different neutral sugars in its branched

188   domains (i.e. Fuc, Rha, Ara, Gal and Xyl). Similarly as for GalA, the determined neutral sugar

189   values differ between the different vegetable waste streams as well as between the different

190   polysaccharide fractions within one waste stream. Arabinose and galactose appear to be the

191   dominant pectin neutral sugars in the different pectin fractions of all vegetable waste streams.

192   The high amount of glucose in WSP of the different waste streams on the other hand may be a

193   remnant of soluble sugars that were not completely removed during AIR isolation or may

194   originate from co-extracted starch (Houben et al., 2011; Stolle-Smits et al., 1997). Based on the

9

195 amount of GalA and (pectin-related) neutral sugars in the different samples, the distribution of

196 the polysaccharide fractions (see section 3.3) and the linearity/branching of pectin (see section

197 3.4) in the five vegetable waste streams was determined.

198 *3.3. Distribution of polysaccharide fractions in vegetable waste streams*

199 The relative amounts of WSP, CSP, NSP and HF in the different vegetable waste streams are

200 shown in **Figure 1**. Rejected carrots and the cutting waste of leek display a similar distribution of

201 their polysaccharide fractions: ± 35% WSP, ± 30% CSP, ± 20% NSP and a small level of HF.

202 Carrot steam peels and celeriac steam peels also possess a comparable solubility of their pectin

203 and hemicellulose polymers. Steam peels waste streams contain a very large amount of weakly

204 bound WSP (± 60%) and only a little amount of strongly bound pectin (CSP and NSP) and

205 hemicellulose. The high WSP content in carrot steam peels and celeriac steam peels may be

206 attributed to the thermal treatment (i.e. the steam peeling process) these samples were subjected

207 to. Processing at high temperatures and near-neutral pH has been shown to cause solubilisation

208 and/or β-eliminative depolymerisation of pectin (De Roeck, Sila, Duvetter, Van Loey, &

209 Hendrickx, 2008). The cutting waste of green beans finally contains predominantly strongly

210 bound pectin (CSP and NSP) and hemicellulose and only a small amount of WSP.

211 *3.4. Linearity/branching of pectin*

212 To obtain a better insight in the linearity/branching of pectin, different ratios between the

213 monosaccharides present in pectin were determined (Houben et al., 2011). In this context, a

214 linear pectin structure is assumed in which the backbones of RG-I and RG-II are continuous with

215 the linear HG structure.

10

216 The ratio of the molar amount of GalA over the molar amount of Fuc, Rha, Ara, Gal and Xyl

217 gives an idea on the linearity of pectin. A high ratio between GalA and Fuc, Rha, Ara, Gal and

218 Xyl points at pectin molecules that are more linear while a low ratio indicates less linear or more

219 branched pectin. In **Figure 2A**, this ratio is shown for the different pectin fractions of the

220 different vegetable waste streams. A large difference in linearity can be noticed between the

221 different pectin fractions for all waste streams. While WSP and CSP are more linear, and hence

222 consist predominantly of HG, NSP contains more branched pectin. Between the different

223 vegetable waste streams on the other hand, the largest differences in linearity can be observed for

224 WSP. Compared to rejected carrots, carrot steam peels and celeriac steam peels, the cutting

225 waste of green beans and leek possess WSP with a very high level of linearity.

226 The ratio of the molar amount of Rha over the molar amount of GalA is indicative for the

227 contribution of RG to the entire pectin population. As RG consists per definition of branched

228 pectin polymers, a high value for the contribution of RG to the pectin population is related to a

229 low linearity and vice versa. **Figure 2B** presents this ratio for the different pectin fractions of the

230 different vegetable waste streams. For all waste streams, the contribution of RG to the entire

231 pectin population is much higher for NSP than for WSP and CSP. Correspondingly to the very

232 high linearity of WSP of the cutting waste of green beans and leek, these vegetable waste

233 streams display a very low contribution of RG to the entire WSP population.

234 Finally, the ratio of the molar amount of Ara and Gal over the molar amount of Rha is reflective

235 of the extent of branching of RG-I. For the different pectin fractions of the different vegetable

236 waste streams, this ratio is displayed in **Figure 2C**. The degree of branching of RG-I is highest in

237 the pectin fractions originating from the cutting waste of green beans. Pectin in the cutting waste

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238 of green beans thus contains RG-I with longer and/or more arabinan, galactan and/or

239 arabinogalactan side chains. Conversely, pectin in the cutting waste of leek shows the lowest

240 degree of branching of RG-I. Differences in the extent of branching of RG-I between the

241 different pectin fractions in a certain waste stream are generally quite small.

242 The linearity of pectin, the contribution of RG to the pectin population and the degree of

243 branching of RG-I in the different pectin fractions extracted from rejected carrots are in line with

244 the results observed by Houben et al. (2011) for carrot root. Commercially extracted citrus pectin

245 on the other hand contains much less neutral sugars, hence displaying a very high linearity, than

246 all pectic polysaccharides investigated in this study (Kravtchenko, Voragen, & Pilnik, 1992).

247 However, comparison of the pectin linearity/branching of the different vegetable waste streams

248 with the linearity/branching of commercially extracted pectins, such as citrus pectin, is difficult

249 as the extraction conditions used may influence these pectin properties.

250 *3.5. Degree of methyl-esterification of pectin*

251 The DM of AIR, WSP and CSP of the different vegetable waste streams is presented in **Table 3**.

252 The DM of AIR represents the general DM of pectin present in the vegetable waste streams. AIR

253 extracted from the cutting waste of leek displays a very high DM (82%). In this context, a

254 similar, but somewhat lower DM (75%) was determined for the alcohol-insoluble substances

255 extracted from fresh leek by Kratchanova, Nikolova, Pavlova, Yanakieva & Kussovski (2010).

256 Furthermore, a DM between 60 and 70%, which is common for many (fresh) vegetable matrices

257 (Christiaens et al., 2011; Femenia, Garosi, Roberts, Waldron, Selvendran, & Robertson, 1998;

258 Sila, Smout, Vu, Van Loey, & Hendrickx, 2005), was observed for the AIR of rejected carrots

259 and the cutting waste of green beans. Finally, AIR extracted from carrot steam peels and celeriac

260   steam peels shows a rather low DM. The lower DM of pectin in the steam peels waste streams

261   probably results from the chemical demethoxylation of pectin that occurred during thermal

262   treatment of these streams. Similar differences in DM between the different vegetable waste

263   streams, as observed for AIR, can also be noticed for WSP and CSP. However, for each waste

264   stream, the DM of WSP was higher than the DM of AIR, while the DM of CSP was lower than

265   the DM of AIR which was also observed by Christiaens et al. (2011). WSP is generally high

266   methyl-esterified whereas CSP is, as a result of the extraction procedure used, usually low

267   methyl-esterified. Specifically, CSP is rich in Ca^{2+}-crosslinked pectin which implies a low DM

268   of the pectin polymers (Selvendran & O'Neill, 1987).

269   *3.6. Analysis of pectin fractions with HPSEC-RI-MALLS-DAD*

270   Pectin fractions of the different vegetable waste streams were analysed with an integrated

271   HPSEC-RI-MALLS-DAD system. **Figure 3** presents the RI, LS 92° and UV 280 nm profiles of

272   WSP and CSP of the different vegetable waste streams. The RI signal is proportional to the

273   concentration of the pectin polymers while LS signals were recorded at 21 different angles (of

274   which only one angle is shown in Figure 3) to allow for an absolute determination of the molar

275   mass (weight average molar mass, M_w). The UV signal at 280 nm finally is a qualitative measure

276   for the presence of protein in the sample.

277   For carrot steam peels and celeriac steam peels, the majority of WSP polymers has a very high

278   M_w, i.e. in the order of magnitude of 10^6 Da (1 460 000 Da for carrot steam peels and 821 000

279   Da for celeriac steam peels). For rejected carrots, the cutting waste of green beans and the cutting

280   waste of leek on the other hand, the largest part of the WSP polymers shows a lower M_w, i.e. in

281   the order of magnitude of 10^5 Da (114 000 Da for rejected carrots, 97 200 for the cutting

13

282 waste of green beans and 99 200 Da for the cutting waste of leek). WSP of rejected carrots

283 contains, besides the high amount of pectic polymers with lower M_w, a smaller portion of pectin

284 with high molar mass (M_w = 1 380 000 Da), which corresponds, in M_w, to the majority of the

285 WSP polymers of carrot steam peels. In addition, also in the WSP of the cutting waste of leek, a

286 small amount of pectic polymers with high molar mass (M_w = 1 340 000 Da) is present. WSP of

287 carrot steam peels finally contains also very little material with a very high M_w (10 800 000 Da),

288 which is thought to consist of aggregated pectic polymers, and a considerable level of pectic

289 polymers with a lower M_w (179 000 Da), in the order of magnitude of the M_w of the majority of

290 the WSP polymers of rejected carrots. Comparing the RI profile of WSP of carrot steam peels

291 with the RI profile of WSP of rejected carrots, a clear shift towards a higher concentration of

292 pectic polymers with higher M_w can be observed upon thermal treatment of carrots. This shift

293 can be attributed to the thermosolubilisation of, originally, strongly bound pectin with high M_w

294 (see further) to the WSP fraction in carrot steam peels. The thermosolubilisation of pectin after

295 thermal processing of carrots is in agreement with previous research (De Roeck et al., 2008).

296 Considering the molar-mass distribution of CSP of the different vegetable waste streams, it can

297 be noticed that the molar mass of CSP is generally larger than the molar mass of WSP for

298 rejected carrots, the cutting waste of leek and the cutting waste of green beans, but generally

299 lower for carrot and celeriac steam peels. Nevertheless, the majority of the pectic polymers in the

300 CSP fractions of all waste streams have a M_w in the order of magnitude of 10^6 Da (1 390 000 Da

301 for rejected carrots, 1 190 000 Da for carrot steam peels, 730 000 Da for the cutting waste of

302 green beans, 1 050 000 Da for the cutting waste of leek and 796 000 Da for celeriac steam peels).

303 It should also be noted that some of the CSP samples (CSP carrot steam peels and CSP green

304 beans cutting waste) showed weak concentration signals due to the low filterability of these

14

305 samples. Moreover, the high viscosity, and hence low filterability, of the NSP samples hampered

306 their analysis completely (results not shown).

307 The level of protein co-eluting with pectin in the WSP and CSP fractions of the different

308 vegetable waste streams was evaluated through the UV absorbance profiles at 280 nm. The

309 strongest UV signal at 280 nm is observed for pectic polymers of WSP of rejected carrots and

310 carrot steam peels with a M_w around $1.4 * 10^6$ Da. This observation indicates that a certain

311 amount of protein is attached to the high molar mass pectic polymers in WSP of the carrot-

312 derived waste streams. For the other vegetable waste streams, no detectable amount of protein

313 co-elutes with their WSP polymers. Considering the UV absorbance profiles at 280 nm of the

314 CSP fractions of the different vegetable waste streams, only a clear peak can be seen for the CSP

315 of rejected carrots, while a very small peak is visible for the CSP of leek cutting waste. Hence, a

316 (small) amount of protein is attached to the pectic polymers (M_w in the order of magnitude of

317 10^6) present in the CSP fraction of rejected carrots and leek cutting waste. Carrot steam peels, the

318 cutting waste of green beans and celeriac steam peels possess no detectable amount of protein

319 that co-elutes with their CSP polymers.

320 **4. <u>Conclusion</u>**

321 In this study, the potential of five vegetable waste streams (rejected carrots, carrot steam peels,

322 green beans cutting waste, leek cutting waste and celeriac steam peels) as a source for the

323 extraction of pectin with interesting structural, and hence functional, properties was evaluated.

324 An overview of the main structural characteristics of pectin in the different waste streams is

325 presented in **Table 4**. The highest levels of pectin were enclosed in celeriac steam peels and leek

326 cutting waste, while the solubility of pectin, which is an interesting feature for future extraction

15

327 of pectin, was largest in carrot steam peels and celeriac steam peels. The low amount of pectin

328 present in the cutting waste of green beans as well as the strong binding of the pectic

329 polysaccharides to other cell wall polymers in this matrix imply that this waste stream is less

330 interesting for future valorization in terms of pectin extraction. Furthermore, the DM of pectin, a

331 very important structural property of this polysaccharide, varied largely among the different

332 vegetable waste streams. Specifically, pectin in carrot steam peels showed the lowest DM,

333 whereas the cutting waste of leek contained pectin with a very high DM. In food industry, high-

334 methoxylated as well as low-methoxylated pectin can be applied as gelling agent. Furthermore,

335 the level of protein bound to pectin was generally highest in the carrot-derived waste streams. A

336 large amount of bound protein is considered to be a favourable pectin property in the context of

337 emulsifying applications. Finally, carrot steam peels and celeriac steam peels contained the

338 highest level of (water-soluble) pectic polymers with high molar mass, whereas the (water-

339 soluble) pectic polymers retrieved from the cutting waste of green beans and the cutting waste of

340 leek were generally lower in molar mass and displayed the highest linearity. In future pectin

341 extraction from these vegetable waste streams, extraction conditions should be chosen

342 deliberately to obtain certain desired pectin characteristics out of the endogenous potential of the

343 pectin present.

344 **<u>Acknowledgements</u>**

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407

408   **Figure captions**

409   **Figure 1**: Relative amounts of WSP, CSP, NSP and HF in the different vegetable waste streams.

410   (Relative amounts of the different polysaccharide fractions were determined as the sum of GalA

411   and (pectin-related) neutral sugars in the fractions relative to the sum of GalA and (pectin-

412   related) neutral sugars in all fractions.)

413   **Figure 2**: A) Ratio of the molar amount of GalA over the molar amount of Fuc + Rha + Ara +

414   Gal + Xyl (± standard deviation) in WSP, CSP and NSP of the different vegetable waste streams.

415   B) Ratio of the molar amount of Rha over the molar amount of GalA (± standard deviation) in

19

416   WSP, CSP and NSP of the different vegetable waste streams. C) Ratio of the molar amount of

417   Ara + Gal over the molar amount of Rha (± standard deviation) in WSP, CSP and NSP of the

418   different vegetable waste streams (■ WSP, ■ CSP, ■ NSP).

419   **Figure 3**: Analysis of WSP and CSP of the different vegetable waste streams with HPSEC-RI-

420   MALLS-DAD (▬▬▬ RI, ▬ ▬LS 92°, ▬ ▬ ▬ ▬ UV 280 nm).

Table 1: GalA content and pectin content (± standard deviation) of the different vegetable waste streams expressed per g AIR, per g fresh material and per g dry matter (n = 2).

	mg / g AIR		mg / g fresh material		mg / g dry matter	
	GalA	pectin	GalA	pectin	GalA	pectin
Rejected carrots	199 ± 4	286 ± 5	6.2 ± 0.1	8.9 ± 0.2	62 ± 1	89 ± 2
Carrot steam peels	181 ± 4	238 ± 3	6.5 ± 0.1	8.5 ± 0.1	69 ± 1	90 ± 1
Green beans cutting waste	136 ± 6	172 ± 3	5.8 ± 0.2	7.3 ± 0.1	65 ± 3	82 ± 1
Leek cutting waste	208 ± 6	261 ± 4	9.5 ± 0.3	11.9 ± 0.2	86 ± 2	108 ± 2
Celeriac steam peels	243 ± 5	338 ± 10	11.1 ± 0.2	15.4 ± 0.5	114 ± 2	159 ± 5

Table 2: Amount of GalA and neutral sugars (± standard deviation) in WSP, CSP, NSP and HF of the different vegetable waste streams expressed in mg/g AIR (n = 2). 'Pectin sugars' represents the sum of GalA and the pectin-related neutral sugars (Fuc, Rha, Ara, Gal and Xyl) in WSP, CSP and NSP. *'Pectin sugars' only reflects the GalA content in HF as only GalA can exclusively be related to pectin in this fraction. < d.l. = below detection limit.

|		GalA	Fuc	Rha	Ara	Gal	Glu	Man	Xyl	Pectin sugars
Rejected carrots	WSP	81 ± 2	0.12 ± 0.05	1.7 ± 0.6	8 ± 1	13 ± 2	50.6 ± 0.9	1.6 ± 0.5	0.3 ± 0.3	104 ± 4
	CSP	73 ± 2	0.09 ± 0.04	1.8 ± 0.4	7 ± 2	9 ± 1	8 ± 1	0.08 ± 0.01	0.18 ± 0.01	91 ± 2
	NSP	25.8 ± 0.7	0.13 ± 0.03	2.8 ± 0.5	12.9 ± 0.6	23 ± 1	14 ± 1	< d.l.	0.5 ± 0.4	65 ± 2
	HF	2.6 ± 0.6	0.19 ± 0.01	0.63 ± 0.03	2.08 ± 0.02	3.66 ± 0.08	9.58 ± 0.04	2.15 ± 0.05	3.5 ± 0.2	2.6 ± 0.6*
Carrot steam peels	WSP	118 ± 3	0.18 ± 0.02	3.2 ± 0.2	11.9 ± 0.2	24.4 ± 0.2	40.9 ± 0.5	1.9 ± 0.2	0.3 ± 0.2	158 ± 3
	CSP	53 ± 2	0.04 ± 0.01	0.9 ± 0.2	5.0 ± 0.5	4.7 ± 0.3	1.4 ± 0.2	0.13 ± 0.02	0.13 ± 0.02	64 ± 2
	NSP	3.2 ± 0.2	0.02 ± 0.01	0.34 ± 0.08	1.42 ± 0.09	2.5 ± 0.2	0.40 ± 0.02	< d.l.	0.19 ± 0.05	7.7 ± 0.3
	HF	4.4 ± 0.5	0.31 ± 0.06	0.52 ± 0.08	2.7 ± 0.4	5.5 ± 0.7	4 ± 1	1.3 ± 0.1	4.6 ± 0.6	4.4 ± 0.5*
Green beans cutting waste	WSP	38 ± 1	0.04 ± 0.01	0.21 ± 0.01	1.18 ± 0.03	2.16 ± 0.09	25 ± 3	1 ± 1	0.68 ± 0.02	43 ± 1
	CSP	51 ± 2	0.11 ± 0.01	0.50 ± 0.01	2.94 ± 0.09	4.7 ± 0.6	8 ± 3	0.4 ± 0.4	0.5 ± 0.4	60 ± 2
	NSP	22 ± 1	0.10 ± 0.02	1.4 ± 0.5	6.1 ± 0.7	14.1 ± 0.2	14.7 ± 0.4	0.05 ± 0.02	0.31 ± 0.01	44 ± 1
	HF	15 ± 1	0.58 ± 0.04	< d.l.	2.6 ± 0.3	3.8 ± 0.2	26 ± 2	3.42 ± 0.01	7 ± 1	15 ± 1*
Leek cutting waste	WSP	76 ± 2	0.11 ± 0.10	0.7 ± 0.1	1.9 ± 0.2	5 ± 1	13 ± 2	0.53 ± 0.08	0.7 ± 0.1	85 ± 2
	CSP	51 ± 2	0.05 ± 0.01	1.2 ± 0.1	2.5 ± 0.1	6.2 ± 0.5	0.42 ± 0.01	0.1 ± 0.1	1.0 ± 0.1	62 ± 2
	NSP	20 ± 1	0.06 ± 0.01	1.9 ± 0.1	4.2 ± 0.2	13.0 ± 0.7	0.09 ± 0.01	< d.l.	0.41 ± 0.01	39 ± 1
	HF	2.4 ± 0.8	0.81 ± 0.01	0.36 ± 0.05	1.64 ± 0.04	4.5 ± 0.2	6.8 ± 0.2	3.16 ± 0.01	7.7 ± 0.6	2.4 ± 0.8*
Celeriac steam peels	WSP	111 ± 2	0.27 ± 0.03	3.4 ± 0.2	17 ± 1	15 ± 2	23 ± 2	3 ± 1	5 ± 6	151 ± 7
	CSP	43 ± 1	0.08 ± 0.01	1.51 ± 0.03	9.84 ± 0.06	4.5 ± 0.4	1.1 ± 0.3	0.17 ± 0.01	0.46 ± 0.09	59 ± 1
	NSP	5 ± 1	0.02 ± 0.01	0.54 ± 0.05	3.7 ± 0.3	2.3 ± 0.2	0.32 ± 0.01	< d.l.	0.4 ± 0.1	12 ± 1
	HF	3.4 ± 0.8	0.19 ± 0.08	0.7 ± 0.1	4 ± 1	3 ± 1	3 ± 1	1.4 ± 0.6	5 ± 2	3.4 ± 0.8*

Table 3: DM (± standard deviation) of AIR, WSP and CSP originating from the different vegetable waste streams (n = 2).

	DM (%)		
	AIR	WSP	CSP
Rejected carrots	61 ±3	77 ±3	40 ±1
Carrot steam peels	47 ±2	53 ±2	24 ±1
Green beans cutting waste	66 ±3	95 ±3	52 ±2
Leek cutting waste	82 ±3	98 ±2	43 ±2
Celeriac steam peels	55 ±2	71 ±3	27 ±1

Table 4: Overview of main structural characteristics of pectin in different vegetable waste streams (+++++ = highest, + = lowest).

		Rejected carrots	Carrot steam peels	Green beans cutting waste	Leek cutting waste	Celeriac steam peels
Pectin content		+	+	+	++	++(+)
Pectin solubility		++	+++	+	++	+++
Linearity	WSP	+++	++	++++	++++	++
	CSP	+++	+++	+++	+++	++
	NSP	+	+	+	+	+
Degree of branching RG-I		++	++	+++	+(+)	++
DM	AIR	+++	++	+++	++++	++
	WSP	++++	++	+++++	+++++	+++
	CSP	++	+	++	++	+
Molar mass	WSP	+/++	++/+++	+	+	++
	CSP	++	++	+(+)	+/++	++
Protein content	WSP	++	+++	+	+	+
	CSP	++	+	+	++	+

Legend:
- ■ % WSP
- ◢ % CSP
- ▦ % NSP
- ∵ % HF

Categories (top to bottom):
- rejected carrots
- carrot steam peels
- green beans cutting waste
- leek cutting waste
- celeriac steam peels

X-axis: 0, 20, 40, 60, 80, 100
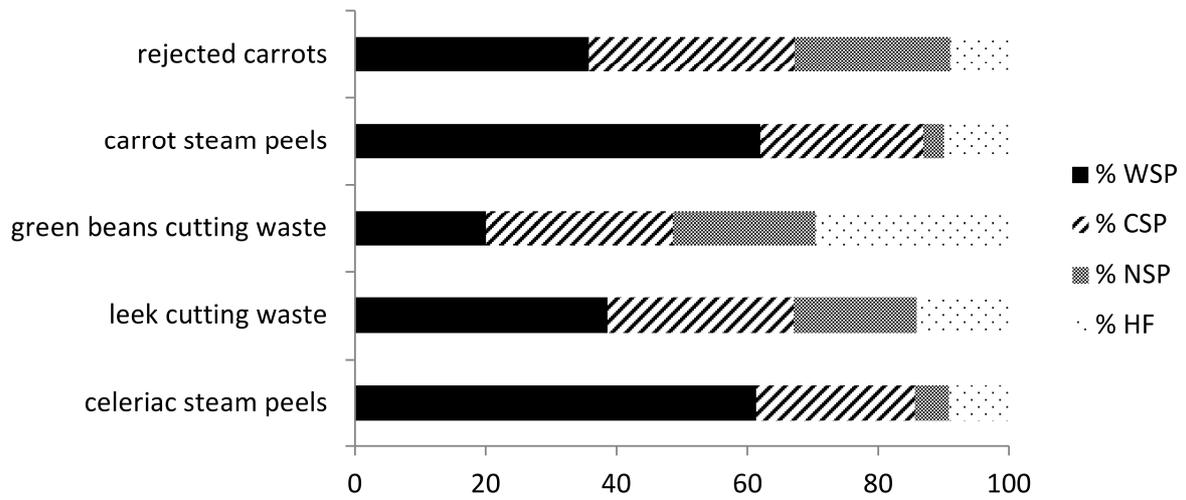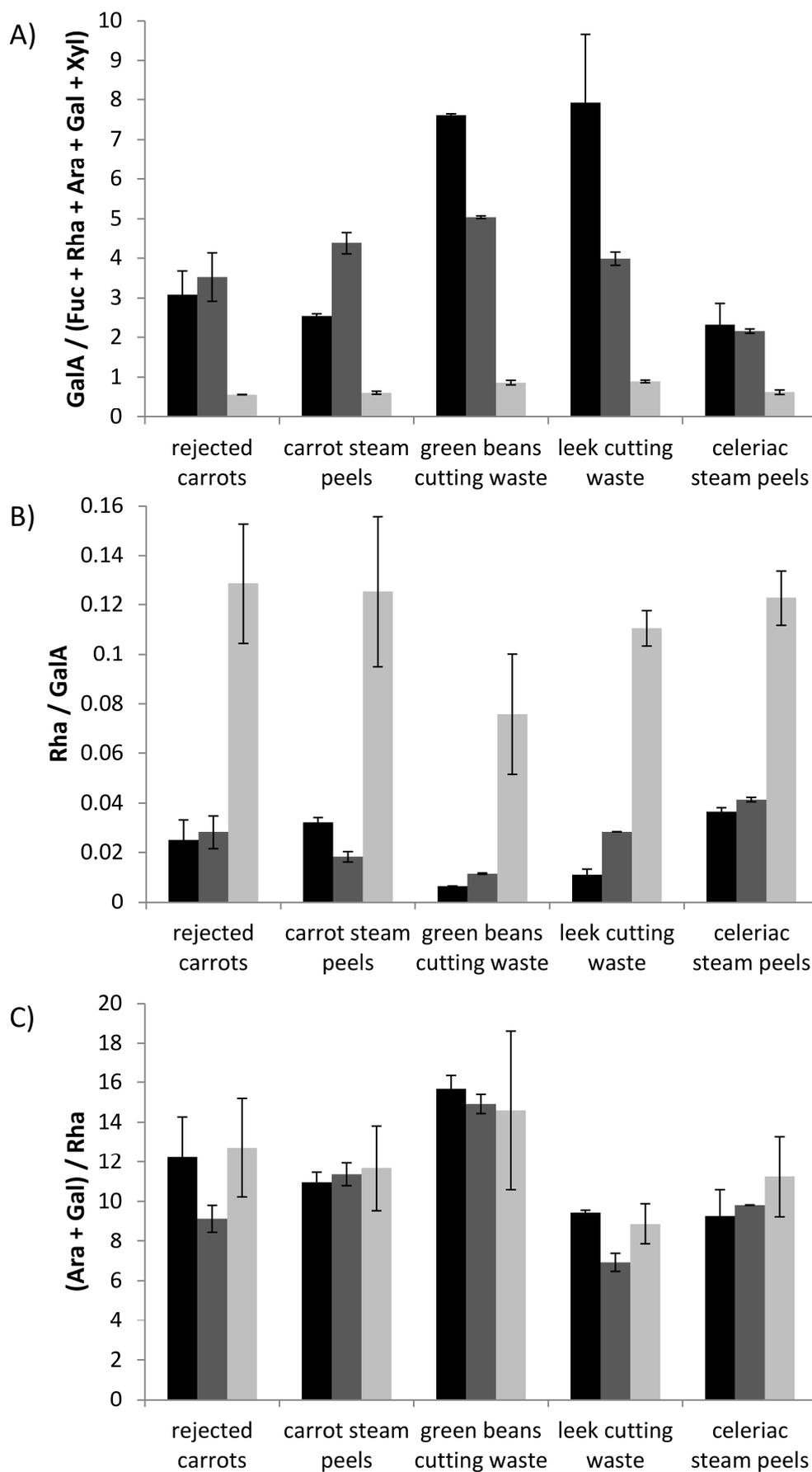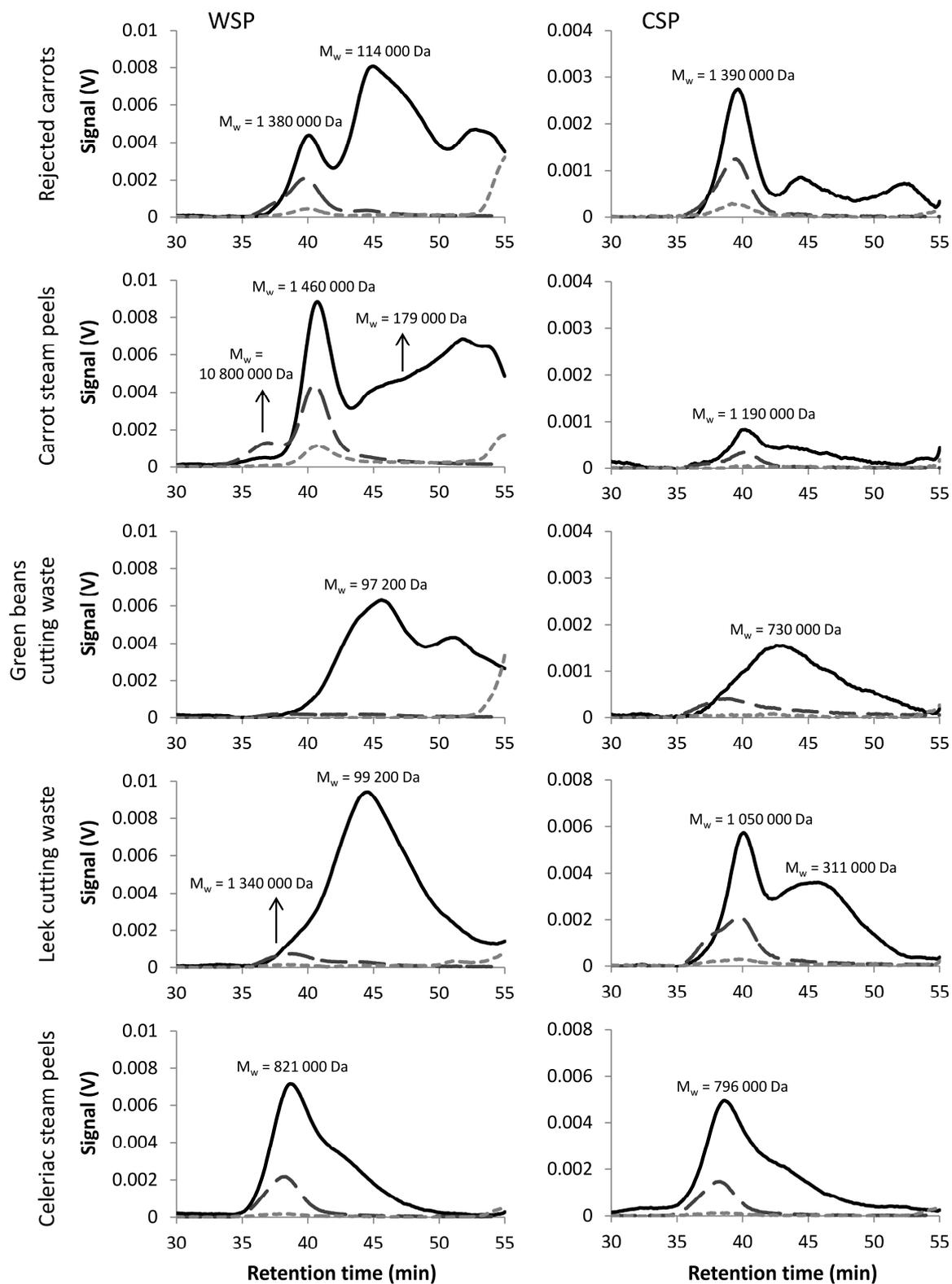
- Vegetable waste streams can be a source for the extraction of pectin
- There are considerable differences in pectin stucture between vegetable waste streams
- Leek cutting waste contains a large amount of very high-methoxylated pectin
- The level of protein bound to pectin is highest in carrot-derived waste streams