Deliberate processing of carrot purées entails tailored serum pectin structures

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[1]Abbreviations

[1] HG, homogalacturonan; RG, rhamnogalacturonan; GalA, galacturonic acid; HTT, high temperature treatment; LTT, low temperature treatment; HPH, high pressure homogenization; PME, pectin methyl-esterase; UA, uronic acid; MWCO, molecular weight cut off; DM, degree of methyl-esterification; DAc, degree of acetylation; MM, molar mass

30 **Abstract**

31 A combination of mechanical tissue disintegration techniques (i.e blending and high pressure
32 homogenization) and heat treatments (i.e. high and low temperature) were deliberately applied
33 in processing carrot purées. The chemical structure of serum pectin and its influence on the
34 consistency of the differently prepared purées was evaluated. High temperature treatment of
35 carrot pieces prior to high pressure homogenization (HTT+HPH) resulted in high apparent
36 molar mass (MM) serum pectin, while the reverse order of purée preparation
37 (HPH+HTT) generated a relatively lower MM. The exceptional high apparent
38 MM of HTT+HPH sample is possibly related to proteins bound to pectin. The importance of
39 the order of heat treatment and tissue disruption was also reflected in largely different
40 carrot purée consistencies in which HTT+HPH was more consistent. Low temperature treated
41 (LTT) carrot purées, whereby endogenous pectin methyl-esterase was stimulated, had less
42 consistent purées and low molar mass serum pectins.

43 **Keywords:** carrot purée, consistency, serum pectin, structure, high pressure homogenization

Introduction

The edible portions of fruits and vegetables are commonly processed into dispersed food systems such as soups, purées and juices. This enables the preservation of these highly perishable commodities, facilitates easier distribution and offers a variety of high quality products to consumers that can lead to increased daily intake of fruits and vegetables. The incorporation of particulated/puréed vegetables in a variety of foods could increase vegetable intake (Blatt, Roe, & Rolls, 2011). The production of these particulated/puréed fruit and vegetable products mainly involves tissue disintegration (e.g. blending and high pressure homogenization) and preservation (e.g. thermal processing). These processes result in a complex multi-scale and multi-phase food system structured by particles dispersed in a continuous liquid (serum) phase. The particle phase consists of cell fragments, clusters and insoluble cell wall components, while the serum phase is characterized by soluble cell contents and cell wall polysaccharides such as pectin (Lopez-Sanchez et al., 2011a; Augusto et al., 2012; Moelants et al., 2014). Pectin is present in both the particle and serum phase, and is thus a component that significantly contributes to the textural, rheological and/or nutritional functionalities of fruit and vegetable derived products. Specifically, changes in pectin structure during processing can lead to changes in the flow behavior of particulated fruit and vegetable products (Christiaens et al., 2012; Houben et al., 2013) or pectin can act as an emulsifier in low fat containing systems thereby influencing the bio-accessibility of lipophilic nutrients such as carotenoids (Verrijssen et al. 2014).

Pectin, an important cell wall polysaccharide, is a structurally intricate biopolymer that comprises several polysaccharide domains. The most abundant pectic polysaccharides are homogalacturonan (HG) and rhamogalacturonans (RG) that are believed to be covalently cross-linked to one another (Ridley, Neill, & Mohnen, 2001). HG, the most abundant pectic domain, is a linear chain of 1,4 linked α-D-galacturonic acid (GalA) residues that are

69 methyl-esterified on the C-6 carboxyl groups up to 70% to 80% and O-acetylated at O-3 or
70 O-2 depending on the plant source (Voragen et al., 2009). RG-I consists of a backbone of the
71 repeating disaccharide [-α-D-GalA-1,2-α-L-Rha-1-4-]n and represents 20-35% of pectin. Side
72 chains containing individual, linear, or branched oligosaccharide residues are attached to the
73 rhamnose residues of the RG-I backbone. Linear arabinan and (arabino)galactan are the
74 predominant RG-I side chains (Voragen, Beldman & Schols, 2001; Caffall & Mohnen, 2009).
75 Finally, RG-II is the most structurally complex among the pectic polysaccharides and makes
76 up 10% of pectin. It has a HG rather than a RG backbone which consists of at least 8 GalA
77 residues with side branches of either structurally distinct disaccharide or oligosaccharide.
78 (Mohnen, 2008; Caffall & Mohnen, 2009).

79 Pectin structure in relation to its functionality under various conditions (e.g. pH) has
80 been widely explored and exploited. The gelling and stabilizing abilities of pectin have been
81 ascribed to the degree and pattern of methyl-esterification of HG (Thakur et al. 1997; Fraeye
82 et al., 2010). The emulsifying capacity of pectin which is continuously being investigated has
83 been attributed to a number of structural properties such as a combination of molar mass and
84 degree of methyl-esterification, degree of acetylation, neutral sugar side chains and the
85 proteinaceous moiety of pectin (Akhtar et al., 2002; Leroux et al., 2003; Nakauma et al.,
86 2008). Moreover, process induced changes in the structure of pectin and its influence on the
87 texture of intact tissues has been thoroughly examined (Sila et al., 2009; Christiaens et al.,
88 2011a; Christiaens et al., 2011b). On the other hand, pectin structural modifications in
89 particulated fruit and vegetables and its functionality have also been sought. Christiaens et al.
90 (2012) showed that different (pre)processing operations (e.g. low and high temperature
91 blanching, blending and high pressure homogenization) in the preparation of carrot purées
92 result in pectin structural changes that affect the flow properties of carrot purée. They showed
93 that high pressure homogenization, a more intense mechanical tissue disruption technique,

94 and high temperature blanching significantly reduced the separation of particle and serum
95 phases of the purée due to pectin solubilization. High pressure homogenization compared to
96 conventional blending was also shown to lower the consistency of carrot purée due to the
97 subsequent reduction of carrot tissue particles. In contrast, an increase in carrot purée
98 consistency was observed with low temperature blanching of carrot pieces followed by either
99 blending or high pressure homogenization because of increased resistance to particle
100 disintegration as a result of stronger intercellular adhesion by Ca^{2+} cross-linking of
101 de-methylesterified pectin. Furthermore, Kyomugasho et al. (2015a) also reported that pectins
102 with different characteristics leached into the serum of carrot dispersions depending on the
103 applied treatment (i.e. high or low temperature blanching) during subsequent mechanical
104 disintegration. Moelants et al. (2012) revealed that strong thermal treatment of carrot tissues
105 and intense high pressure homogenization enhances pectin solubilization into the serum of
106 carrot purées. They found that serum viscosity had a limited influence on the rheology of
107 carrot dispersions. However, they inferred that serum pectin may play a role in the final
108 rheology, not only by influencing the serum viscosity but by changing interactions between
109 particles. On the other hand, Diaz, Anthon and Barrett (2009) reported that pectin
110 conformational changes rather than pectin depolymerization might cause the decrease of
111 serum viscosity during industrial tomato paste production. From these aforementioned
112 studies, the relevance and potential functionalities of solubilized pectin in the serum of plant-
113 derived dispersions can be deduced. Nonetheless, information on the detailed structure of
114 pectins in the serum phase of plant-derived dispersions is still very limited.

115 The objective of this current work was to characterize the chemical structure of serum
116 pectin as influenced by different purée preparations and determine its influence on the
117 consistency of the purées. Carrot as a pectin-rich vegetable that is commonly processed into
118 dispersions (e.g. purées, soups) and is of economic relevance was selected. The influence of

the order of processing (thermal treatment before or after mechanical tissue disintegration) and the effect of a low temperature treatment (to stimulate endogenous pectin methyl-esterase activity) on carrot pectin structure were investigated. The quantity and type of pectin that leached into the serum of each differently prepared carrot purée was determined. Finally, the influence of different processing conditions on the composition of the dialyzed serum was also investigated.

2. Materials and methods

A schematic overview of the experimental set-up is shown in Figure 1. The sample codes (in italic) are based on the corresponding processing conditions of the purées.

2.1 Preparation of carrot purées

2.1.1 Plant material

Fresh carrots (*Daucus carota* cv. Nerac) were purchased from a local shop in Belgium and then stored at 4 °C for maximally 3 days. Prior to processing, carrots were both peeled and cut into pieces with an average thickness of 0.5 cm.

2.1.2 Mechanical tissue disruption

Raw (or high temperature treated) carrot pieces were added to demineralized water in a 1:1 (w/w) ratio and mechanically disrupted using a kitchen blender (Waring Commercial, Torrington, Connecticut, USA), operating the first 20 s at low speed and the next 40 s at high speed. To further disrupt the tissue, a high pressure homogenizer (Panda 2K, Gea Niro Soavi, Mechelen, Belgium) was used at 100 MPa via a single pass. The mechanical disruption is associated with pectin solubilization in carrot sera (Moelants et al., 2012).

2.1.3 Heat treatments

Purées (or carrot pieces) were vacuum-packed in polyethylene bags (DaklaPack® Lamigrip Stand-up Pouch Transparent; 220 mm × 300 mm + 65 mm bottom fold). The high

144 temperature treatment (HTT) was done at 95 °C for 30 min in a temperature-controlled water
145 bath. In addition, for some samples, a low temperature treatment (LTT) at 60 °C for 40 min,
146 5 h or 24 h was performed prior to the HTT to allow pectin methyl-esterase (PME) activity.
147 HTT inactivated PME and allowed pectin thermo-solubilization into the serum phase. After
148 HTT, samples were cooled to ambient temperature in an ice-water bath.

149 *2.2 Isolation of carrot sera*

150 The serum and particle fraction of a purée were separated according to the work of Houben et
151 al. (2014). Purées were centrifuged at 12 400 x *g* for 30 min at 20 °C (J2-HS centrifuge,
152 Beckman, CA, USA). Supernatants, the serum phases, were filtered (Machery-Nagel MN 615
153 Ø 90 mm) under vacuum to exclude remaining pulp fragments. The sera were dialyzed
154 (3.5 kDa, MWCO) against demineralized water for 48 h to remove small molecules (e.g. ions
155 and monomeric sugars). Prior to dialysis, pH was adjusted to 6.0 to ionize the carboxylic
156 groups of pectin (Manrique & Lajolo, 2002). Furthermore, to concentrate the sera and obtain
157 the dry matter composition, lyophilization was done using a freeze-dryer (Christ alpha 2-4,
158 Osterode, Germany). Lyophilized sera were stored over P_2O_5 in a desiccator until further
159 analysis.

160 *2.3 Determination of physico-chemical properties of the purées*

161 *2.3.1 pH*

162 The pH of the different purées were measured at room temperature (22 ± 1 ^{o}C) using a pH
163 meter with a glass electrode (Meterlab PHM210, Radiometer Analytical, Lyon, France),
164 calibrated with calibration buffers of pH 4.00 and 7.00 (IUPAC, Radiometer Analytical,
165 Lyon, France). All measurements were done in triplicate.

166 *2.3.2 Bostwick consistency*

167 The empirical Bostwick test was used to evaluate the consistency of the purées. Samples were
168 placed into the Bostwick consistometer (CSC Scientific Company, VA, USA) and were

169 allowed to flow under their own weight along a level surface for 30 s at room temperature
170 (22 ± 1 ^{o}C). The distance (in centimetres) covered by the purée was recorded as the Bostwick
171 consistency index. For each sample, the measurement was performed in triplicate.

172 *2.3.3 Particle size distribution*

173 The particle size distribution of the purées was measured using a laser diffraction instrument
174 (Beckman Coulter Inc., LS 13 320, Miami, Florida). A few drops of the purée were poured
175 into a stirred-tank filled with demineralized water. The diluted sample was then pumped into
176 a measuring cell wherein the laser light (H–Ne laser, wavelength 633 nm) was scattered by
177 the particles. Based on the intensity profile of the scattered light, the volumetric particle size
178 distributions were automatically calculated with the instrument's software by use of the Mie
179 theory (Verrijssen et al., 2014). The measurement was performed in triplicate.

180 *2.3.4 Dry matter and ash content*

181 Purées (2 g) were dried (in triplicate) in a convection oven at 103 °C for 16 h. Subsequently,
182 to determine the ash content, the dried samples were incinerated in a muffle furnace
183 (Nabertherm GmbH, Controller P330, Lilienthal, Germany) operating for 3 h at 350 °C and
184 21 h at 550 °C.

185 *2.4 Determination of sera components*

186 *2.4.1 Protein content*

187 The nitrogen content of the sera was measured using an EA 1110 CHNS-O elemental
188 analyzer (CE-Instruments/Thermo Fisher Scientific). About 2 mg of lyophilized serum was
189 placed in crimped tin capsules (8 mm x 5 mm) prior combustion in the elemental analyzer. A
190 conversion factor of 6.25 was used to calculate the amount of proteins in the sample
191 (Immerzeel et al., 2006). The analysis was done in duplicate.

192 *2.4.2 Pectin content*

193 *2.4.2.1 Uronic acid analysis*

194 The uronic acid (UA) content of the sera was determined following the method of
195 Ahmed & Labavitch (1977). Hydrolysis of 10 mg of lyophilized serum in 8 ml concentrated
196 sulphuric acid was performed in duplicate. Afterwards, a spectrophotometric measurement for
197 each hydrolysate (in triplicate) was performed at 520 nm at 25 °C according to the method of
198 Blumenkrantz & Asboe-Hansen (1973).

199 *2.4.2.2 Neutral sugar analysis*

200 The neutral sugar profile of the sera was determined based on the method of Houben et al.
201 (2011). First, acid hydrolysis of the polysaccharides to monosaccharides was done. Briefly,
202 5 mg of the lyophilized serum was hydrolysed in 4 M trifluoroacetic acid (TFA) at 110 °C for
203 1.5 h. Then, the samples were cooled, dried under N_2 at 45 °C, washed with 1 M NH_4OH, and
204 dried again under N_2 at 45 °C to remove and neutralize TFA. Afterwards, the samples were
205 dissolved in demineralized water (organic free, 18 MΩ cm resistance) and diluted to a final
206 concentration of 0.1% (w/v). Before chromatographic analysis, the samples were filtered
207 through a 0.45 μm syringe filter (Chromafil A-45/25, Macherey-Nagel, Duren, Germany).
208 The monosaccharides were analyzed using high performance anion exchange chromatography
209 (HPAEC) combined with pulsed amperometric detection (PAD). A Dionex HPLC system
210 (DX600), equipped with a GS50 gradient pump, a CarboPac™ PA20 column (150 × 3 mm,
211 pH range = 0–14), a CarboPac™ PA20 guard column (30 × 3 mm), and an ED50
212 electrochemical detector (Dionex, Sunnyvale, USA) were used. The detector was equipped
213 with a reference pH electrode (Ag/AgCl) and a gold electrode and was used in the PAD
214 mode, performing a quadruple potential waveform. The applied gradients were based on the
215 method described by Arnous and Meyer (2008). Diluted hydrolysate (10 μl) was injected and
216 eluted at 30 °C with a flow rate of 0.5 ml/min after equilibration (−10 → −5 min: 100 mM

217 NaOH and −5 → 0 min: elution gradient). Two elution gradients (0.5 mM NaOH and 15 mM
218 NaOH at 0→20 min) were respectively applied for a complete chromatographic separation of
219 the analyzed monosaccharides (Jamsazzadeh Kermani et al., 2014). The column was
220 regenerated using 500 mM NaOH (20 → 30 min). Mixtures of sugar standards (L-Fuc,
221 L-Rha, L-Ara, D-Gal, D-Glc, D-Xyl and D-Man) at varying concentrations (1–10 ppm) were
222 used as standards for identification and quantification. Acid hydrolysis of these standards was
223 also performed to correct the degradation of the monosaccharides during the hydrolysis step.
224 Peak areas of unhydrolysed and hydrolysed sugar standards were compared and the recovery
225 values were considered in the quantification of the monosaccharides. The hydrolysis and
226 chromatographic measurement were done in duplicate.

227 *2.4.3 Ash content*

228 The ash content of the lyophilized serum (0.1 g) was determined as in section 2.3.4

229 *2.5 Characterization of serum pectin*

230 *2.5.1 Degree of methyl-esterification*

231 The degree of methyl-esterification (DM) of pectin in the sera was measured using Fourier
232 transform infra-red (FT-IR) spectroscopy as explained and described in the work of
233 Shpigelman et al. (2014) and Kyomugasho et al. (2015b). Briefly, the lyophilized serum was
234 firmly pressed to remove entrapped air and ensure smooth surfaces. The compacted sample
235 was placed on the sample holder of the attenuated total reflectance Fourier transform infrared
236 spectrometer (ATR-FTIR, Shimadzu FTIR-8400S, Japan) and 100 scans were taken. The
237 transmittance was recorded at wavenumbers from 4000 cm^{-1} to 400 cm^{-1} at resolution 4 cm^{-1}.
238 The spectra were converted into absorbance mode before baseline correction and reading of
239 the absorption at the maxima of peaks at 1740 cm^{-1} (due to ester carbonyl group (C=O)
240 stretching) and at 1600-1630 (due to carboxylate group (COO^{-})). Since peak intensities at
241 1530 and 1650 cm^{-1} were detected due to the presence of proteins, peak deconvolution was

242 performed. The obtained ratio (*R*) between the peak intensity at 1740 cm^{-1} to the sum of the
243 peak intensities at 1740 cm^{-1} and 1600 cm^{-1} was used to predict the DM of the samples based
244 on the calibration line: DM (%) = 123.45 x R + 6.59 (Kyomugasho et al., 2015b).

245 *2.5.2 Degree of acetylation*

246 The degree of acetylation (DAc) of pectin was measured using an enzyme kit (Megazyme,
247 K-ACETRM, Ireland). Prior to acetic acid measurement, lyophilized serum was hydrolysed at
248 25 °C for 1 h with 2 M sodium hydroxide and neutralized with 2 M hydrochloric acid.
249 Hydrolysis and colorimetric measurement at 340 nm were done in triplicate. The DAc was
250 determined as the ratio of the molar amount of the released acetic acid to the molar amount of
251 uronic acid multiplied by 100.

252 *2.5.3 Molar mass distribution*

253 The molar mass distribution of pectin in the sera was analyzed based on the work of
254 Shpigelman et al. (2014; 2015). Size exclusion chromatography (SEC) coupled to multi-angle
255 light scattering (MALS) (PN3621, Postnova analytics, Germany), refractive index (RI)
256 (Shodex RI-101, Showa Denko K.K., Kawazaki, Japan) and a diode array detector (G1316A,
257 Agilent technologies, Diegem, Belgium) at 280 nm to detect the presence of UV absorbing
258 compounds, was used. Lyophilized serum (0.5% w/v) dissolved in 0.1 M acetate buffer was
259 stirred overnight and then filtered through 0.45 µm filter (Millex-HV). 100 µl of sample
260 solution was injected to a series of three Waters columns (Waters, Milford, MA), namely,
261 Ultrahydrogel 250, 1000 and 2000 with exclusion limits of 8×10^4, 4×10^6, and 1×10^7
262 g/mol, respectively. The columns were kept at 35 °C and the flow rate of the eluent (0.1 M
263 acetic acid buffer with 0.1 M $NaNO_3$) was 0.5 ml/min. A dn/dc value of 0.146 ml/g was used.
264 The molar masses were calculated using the Debye fitting method (second order) by the
265 software provided by the manufacturer of the MALS detector (NovaMals, version 1.0.0.18,
266 Postnova analytics, Germany). Samples were analyzed in duplicate.

267 **3. Results and discussion**

268 *3.1 Influence of processing conditions on the physico-chemical properties of carrot purées*

269 *3.1.1 pH*

270 The pH values of the differently prepared purées are presented in Table 1. The pH of the sera
271 was similar to the purées. These pH values are in close agreement with the results of Talcott
272 and Howard (1999) on pre-treated and thermally processed (121 °C for 30 min) carrot purées,
273 but lower than the pH of 6.27 of untreated (no heat treatment and no high pressure
274 homogenization) carrot purée reported by Houben et al. (2013). Moreover, in this work, a
275 lower pH for the low temperature treated carrot purées is noticeable. The pH of the 24 h low
276 temperature treated (24 h LTT) purée had a 1.1 unit decrease compared to the carrot purées
277 that were not subjected to low temperature treatment (HTT+HPH and HPH+HTT). This
278 decrease in pH can be due to the protons (H_3O^+) released by the de-methoxylation of pectin
279 and the solubilization of organic acids (Anthon & Barrett, 2012).

280 *3.1.2 Dry matter and ash content*

281 The dry matter of the purées is composed of the organic and inorganic compounds in both
282 serum and particle fractions. The latter contributes more to the bulk of the determined dry
283 matter. There was no discernible change in dry matter content in all differently prepared
284 carrot purées as displayed in Table 1. Also, Lopez-Sanchez et al. (2011b) and Houben et al.
285 (2014) did not find significant changes in the dry matter of carrot purées prepared through
286 different processing conditions. On the other hand, the ash content is composed of the
287 inorganic residues which represent the total amount of minerals in the purées. Based on Table
288 1, there was no difference in the ash content of the differently prepared purées. This suggests
289 that the processing conditions applied had no influence on the quantities of dry matter and
290 inorganic matter in carrot purées.

291 *3.1.3 Particle size distribution*

292 Compared to conventional blending, high pressure homogenization has been demonstrated to
293 reduce the particle size and obtain a more homogenous particle size distribution of the
294 dispersed plant-based food systems (Lopez-Sanchez et al., 2011a; 2011b). As shown in
295 Figure 2, a unimodal particle size distribution was observed for all differently prepared
296 purées. This was expected since the intensity of mechanical tissue disruption was similar for
297 all purées. A slight shift to the right of the particle diameter of the HTT+HPH sample can be
298 observed compared to the other purées. This shift only indicates a slightly larger particle size
299 diameter in the HTT+HPH purée whereby the median (D_{v50}) was 84.7 μm compared to an
300 average of 75.1 μm for the other carrot purées. Only the sample in which tissue disintegration
301 was performed after high temperature treatment had a slightly larger particle size distribution.

302 *3.1.4 Bostwick consistency*

303 The empirical Bostwick consistency test was used to determine the flow behavior of the
304 differently prepared purées. The Bostwick consistency indices of the purées are shown in
305 Figure 3. Syneresis, the separation of the pulp and serum fractions, was not observed in any of
306 the carrot purées. This observation confirms the result of Christiaens et al. (2012) wherein
307 high pressure homogenized carrot purées clearly displayed less syneresis compared to purées
308 that were only blended. Among the differently prepared purées, the HTT+HPH sample had
309 the lowest Bostwick consistency index which indicates that it was more resistant to flow (i.e.
310 high consistency). On the contrary, the other carrot purées had similar high consistency
311 indices that indicate less resistance to flow (i.e. low consistency). LTT seems to have no
312 influence on Bostwick consistency while reversing the order of HTT and HPH had a great
313 influence. Differences in the particle shape and particle packing probably influenced the
314 consistencies of the purées as the particle size distribution had no large difference. Besides
315 particle characteristics, the solubilized polymers (e.g. pectin) in the serum may also influence

316 purée consistency (Moelants et al., 2012). Differences in serum polymer characteristics,
317 discussed in the next sections, may support in explaining these observations.

318 *3.2 Influence of processing conditions on carrot serum composition*

319 In this research work, serum components are the compounds that leached into the serum of
320 the purée during processing and remained after isolation, dialysis (MWCO, 3.5 kDa) and
321 freeze drying of the serum. The sum of the amounts of all compounds in the lyophilized
322 serum from differently prepared carrot purées is presented in Table 2. Differences in the
323 quantities of the analyzed compounds in the different sera were observed. The carrot sera
324 predominantly contained polysaccharides consisting largely of uronic acid and to a smaller
325 extent of neutral sugars. The amount of uronic acid, which is mainly galacturonic acid in most
326 vegetables such as carrot, is commonly used to represent and express the amount of pectin
327 (Koubala et al., 2008; Christiaens et al., 2012; Houben et al., 2014). It can be noticed that
328 HTT+HPH and HPH+HTT sera had a relatively high uronic acid content which indicates
329 leaching of the galacturonic acid rich domain of pectin into the sera. In literature, intense
330 thermal treatment of carrots has been shown to result in high uronic acid content in the serum
331 fraction (Moelants et al., 2012) which is attributed to a large extent of temperature-induced
332 pectin solubilization into the serum by β-eliminative degradation of pectin present in the
333 middle lamellae and primary cell walls (Diaz et al., 2009; Sila et al., 2009). With LTT prior to
334 the intense thermal treatment of the purées, a lower amount of uronic acid in the sera was
335 noticeable. During low temperature treatment of the purées, PME activity is stimulated
336 thereby de-methoxylating pectin. In this context, Christiaens et al. (2012) observed a shift in
337 pectin solubility (decrease in water soluble pectin and an increase in both chelator soluble and
338 sodium carbonate soluble pectin) in carrot purée prepared from low temperature blanched
339 (60 °C for 40 min) carrot pieces. Recently, Kyomugasho et al. (2015a) also reported a low
340 galacturonic acid content in the serum of carrot purée prepared from low temperature

341 blanched carrot pieces. The decreased pectin solubility, due to the increased amount of free
342 carboxyl groups that can cross-link with Ca^{2+} or other ions, explains the lower uronic acid
343 (Thakur et al., 1997). Next to (galact)uronic acid, the presence of different neutral sugar side
344 chains in pectin is well recognized (Kravtchenko et al., 1992; Houben et al., 2011). As shown
345 in Table 2, carrot sera evidently contained high amounts of pectic neutral sugars (fucose,
346 rhamnose, arabinose, galactose and xylose). Among these, galactose and arabinose were
347 higher compared to rhamnose, fucose and xylose which may also originate from free arabinan
348 or arabinogalactan. A decrease in the neutral sugar content can also be noticed specifically
349 with LTT that can be linked to reduced pectin solubility as compared to non-LTT treated
350 samples. Besides pectic neutral sugars, glucose and mannose were found in appreciable
351 quantities in all carrot sera. Since the samples were dialyzed and the sugar analysis was done
352 in hydrolysed samples, this could indicate that the glucose present in the sera comes from a
353 polymeric material (e.g. starch). Mannose can originate from fragments of hemicellulosic
354 polysaccharides that leached into the serum.
355 Furthermore, the different carrot sera also contained proteins (ranging from 81.0 to 309.5 μg/g
356 purée) which might be free proteins or fragments from glycoproteins (e.g. arabinogalactan
357 protein) possibly associated with pectin. HTT+HPH, when thermal treatment preceded tissue
358 disintegration, had the highest level of proteins. The ash content of the sera was also
359 determined. This ash content represents the amount of total minerals that leached into the
360 serum during purée preparation. Leaching of minerals is common during cooking and/or
361 processing of vegetables (Rees & Bettison, 1991). The HTT+HPH sera had a relatively higher
362 ash content which suggests the greater extent of leaching of the minerals from the softened
363 carrot tissues prior to high pressure homogenization.

364 From these results, it is clear that the serum phase of the differently prepared carrot purées
365 primarily contains pectic polysaccharides. Hence, the structure of pectin in the serum was
366 further characterized as will be discussed in the succeeding section.

367 *3.3 Influence of processing conditions on the structure of carrot serum pectin*

368 *3.3.1 Degree of methyl-esterification*

369 Pectin chemical structure is commonly characterized based on the degree of
370 methyl-esterification (DM) which is the number of moles of methyl esters per 100 moles of
371 galacturonic acid residues (Schols & Voragen., 2002). The DM has been identified as the
372 most important property of pectin especially for its influence on gelling ability as well as for
373 its influence on other functional properties (e.g. stabilizing, emulsifying). As shown in Table
374 3, HTT+HPH and HPH+HTT resulted in a similar serum pectin DM of 66.3% and 66.1%,
375 respectively. This indicates that there was no PME activity despite enzyme and substrate de-
376 compartmentalization during the blending and high pressure homogenization of raw carrot
377 pieces prior to the heat treatment of HPH+HTT samples. These values are comparable with
378 the previously reported serum pectin DM (64.96%) in carrot purée prepared by strong heat
379 treatment of carrot pieces (95 ^{o}C for 45 min) followed with blending and high pressure
380 homogenization (100 MPa) (Moelants et al., 2012). Furthermore, a decreasing DM of the
381 serum pectins in (40 min, 5 h and 24 h) low temperature treated carrot purées (60 °C) was
382 apparent. This was expected because at 60 °C the PME activity in carrots is stimulated
383 thereby lowering the DM of pectin (Ni et al., 2005; Sila et al., 2005; Kyomugasho et al.,
384 2015a).

385 *3.3.2 Degree of acetylation (DAc)*

386 The acetylation of galacturonic acid residues has been pointed to be a factor causing the
387 emulsifying property of pectin and it is recognized to negatively influence pectin’s gelling
388 ability (Thakur et al., 1997; Voragen, Beldman and Schols, 2001; Leroux et al., 2003). The

389 DAc of serum pectin in the differently prepared carrot purées is presented in Table 3. These
390 values are in close agreement with the reported DAc (13%) of carrot pectin (Endress et al.,
391 2006). It can also be observed that low temperature treatment of carrot purées for a long time
392 (5 h and 24 h) resulted to leaching of more acetylated pectic polysaccharides. It was reported
393 that RG-I from carrot is acetylated at mainly O-3 of the galacturonic acid moieties
394 (Komalavilas & Mort, 1989).

395 *3.3.3 Linearity/ degree of branching*

396 To gain an insight into the linearity/ branching of serum pectin, molar ratios of the pectin
397 associated sugars were determined (Houben et al., 2011). A linear pectin structure is
398 presumed with the backbones of RG-I and RG-II being continuous with the linear HG
399 structure (Christiaens et al., 2015). The linearity of pectin is estimated from the molar ratio of
400 the pectic (galact)uronic acid to neutral sugars(Fuc, Rha, Ara, Gal and Xyl). Conversely, the
401 extent of branching of RG-I is estimated based on the molar ratio of RG-I sugar side chains
402 (Ara and Gal) to Rha. As displayed in Table 3, serum pectins with varying linearity and
403 degree of branching were generated. It is apparent that HTT+HPH and HPH+HTT samples
404 contained more linear and less branched serum pectin. On the contrary, serum pectins of low
405 temperature treated samples were less linear and more branched. Pectin in low temperature
406 treated purées was probably more strongly bound in the cell walls through Ca^{2+} cross-links
407 due to the previously observed lower DM (Kyomugasho et al., 2015a). Ca^{2+} cross-linking of
408 the linear GalA-rich de-methoxylated pectic domain in LTT samples makes this type of pectin
409 less likely to leach out (Thakur et al., 1997), and thus, a more branched fraction of pectin
410 leached into the serum. This observation is in accordance with the lower GalA content in the
411 low temperature treated samples. In samples with higher DM, Ca^{2+} cross-linking is less and
412 thus linear GalA-rich pectin was more easily leached out.

413 *3.3.4 Molar mass distribution*

414 To determine the molar mass distribution of serum polymers and qualitatively detect the
415 presence of UV absorbing compounds (e.g. proteins, polyphenols) at 280 nm, size exclusion
416 chromatography coupled to MALS, RI and DAD detectors was used. The serum polymers
417 were analyzed based on their hydrodynamic volume in which large molecules elute at a
418 shorter time than small molecules. Figure 4a presents the molar mass distribution profile and
419 the concentration chromatograms, while Figure 4b shows the corresponding light scattering
420 profile superimposed with the UV absorbance chromatograms. From the concentration
421 chromatograms, two peaks can be distinguished that are characterized by high molar mass
422 (1st peak) and lower molar mass (2nd peak) polymers. For the HTT+HPH sample, the weight
423 average molar mass (MM) was 6 220 kDa for the 1st peak and 527 kDa for the 2nd peak which
424 suggests thermo-solubilization of large pectic polymers (De Roeck et al., 2008). Moreover, a
425 clear peak in the UV chromatogram at around 40 min is noticeable that can be related to the
426 very large LS peak (Figure 4b) and high MM of the HTT+HPH sample at the same elution
427 time. From this observation, it can be inferred that these pectic polymers were compact,
428 aggregated polymers which are possibly associated to proteins. In this context, Christiaens et
429 al. (2015) also observed proteins attached to the high molar mass pectic polymers in water
430 soluble pectin fractions from carrot-derived waste streams. Perhaps this can be ascribed to
431 arabinogalactan proteins that are hypothesized to be linked with pectin in carrots (Vincken et
432 al., 2003; Immerzeel et al., 2006). Conversely, a higher concentration of lower molar mass
433 polymers can be observed in HPH+HTT and low temperature treated sera. In these samples,
434 there were no discernible peaks in the UV chromatograms at the elution time of 35-58 min but
435 an apparent higher UV peak intensity at around 62 min was observed. This is probably
436 indicative of the presence of low molar mass UV absorbing molecules. The HPH+HTT
437 sample had a MM of 1 210 kDa for the 1st concentration peak at around 43 min and 85 kDa

438 for the 2nd peak at 50 min elution time. Similarly, the low temperature treated samples had a
439 MM of 823 kDa (40 min LTT); 712 kDa (5 h LTT) and 552 kDa (24 h LTT) for the 1st peaks
440 while the 2nd peaks had a MM of 102 kDa, 114 kDa and 121 kDa, respectively. This suggests
441 that more small molecules and less large molecules leached into the serum especially for the
442 low temperature treated samples. Furthermore, a remarkable difference in terms of MM can
443 be noticed between HTT+HPH and HPH+HTT whereby the latter had smaller pectic
444 molecules. For the HTT+HPH sample, this shows that predominantly large pectic polymers
445 leached out into the serum from the thermally softened carrot tissue. High pressure
446 homogenization of softened carrot tissue facilitated the leaching of large polymers into the
447 serum. While for the HPH+HTT sample, there was no softening prior to tissue disintegration
448 thus only lower MM ploymers leached and less high MM polymers. In contrast, LTT resulted
449 in generally lower MM serum pectins probably due to the retention of high molar mass pectic
450 polymers in the tissues/particles owing to a low DM that possibly promoted cross-linking
451 through Ca^{2+} bridges (Kyomugasho et al., 2015a). This consequently led to the leaching of
452 only lower molar mass pectic polymers into the serum.
453

454 **Conclusion**

455 Tailored serum pectin structures can be obtained by deliberate processing of carrot purées.
456 The order of high temperature treatment and high pressure homogenization was shown to be
457 very important because it results in totally different carrot purée consistencies. A high purée
458 consistency was observed when thermal treatment preceded intense tissue disintegration
459 (HTT+HPH). On the other hand, less consistent purées were observed when the reverse order
460 of processing (high pressure homogenization prior to thermal treatment, HPH+HTT) and low
461 temperature treatment were applied. In terms of serum pectin structure, HTT+HPH and
462 HPH+HTT had comparable DM, DAc, linearity and degree of branching but different molar

mass distributions. HTT+HPH had relatively high apparent molar mass pectic polymers which is probably bound to proteins, while HPH+HTT had lower molar mass. The difference in purée consistencies can possibly be related to serum pectin and its association with proteins besides the influence of the particles. Low temperature treatment of carrot purées also generated different serum pectin structures, however this did not result in different Bostwick consistencies. Yet, it may be important for other functionalities (e.g. emulsifying property). Further research on the functional properties of serum pectin, for instance on its stabilizing and emulsifying properties in dispersed plant-based food systems, will enable the production of more natural plant-based food products by tailored processing.

Acknowledgements

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626 **Figures**
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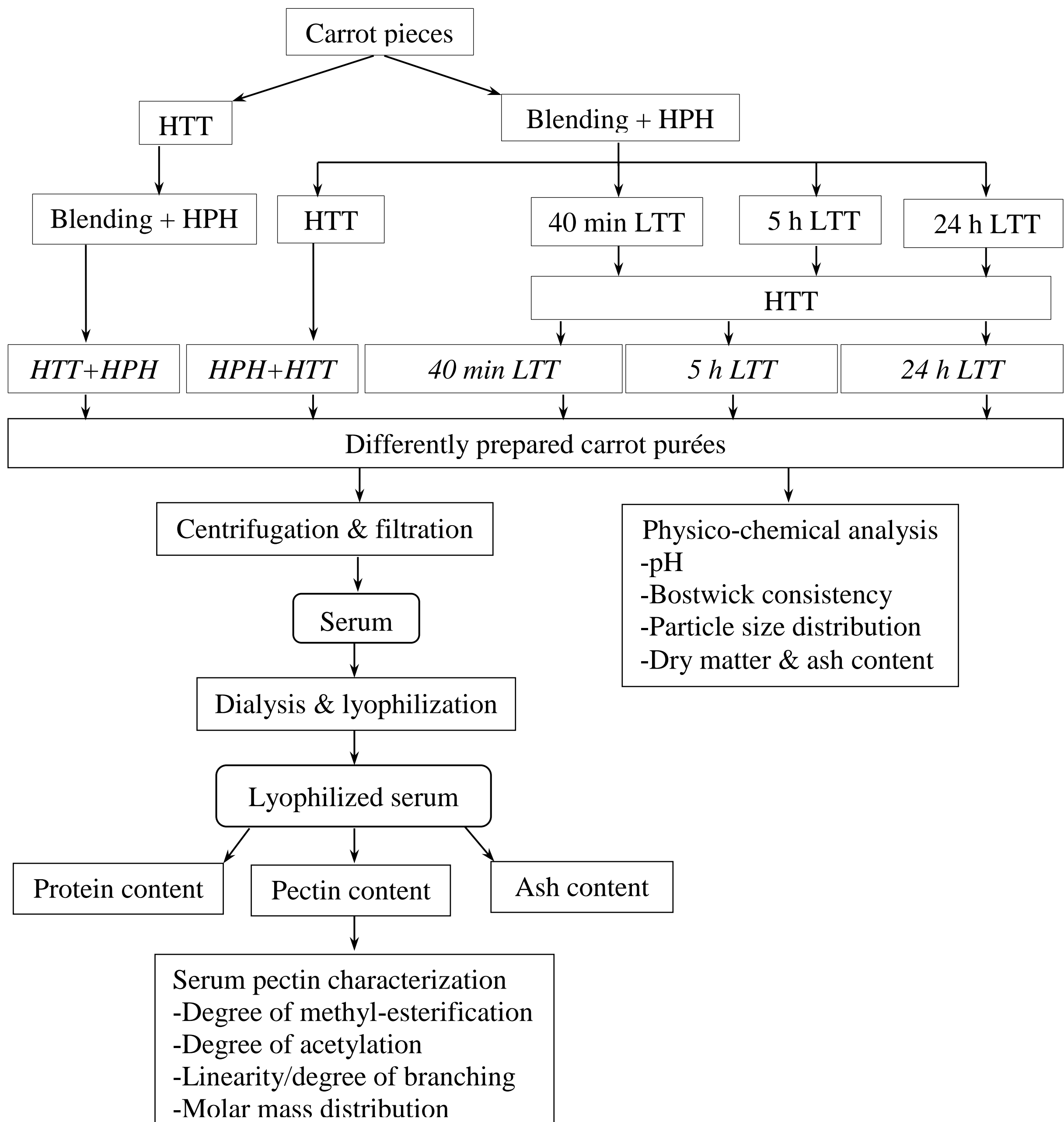

Figure 1. Schematic overview of the experimental set-up (HTT= high temperature treatment, HPH= high pressure homogenization, LTT= low temperature treatment).

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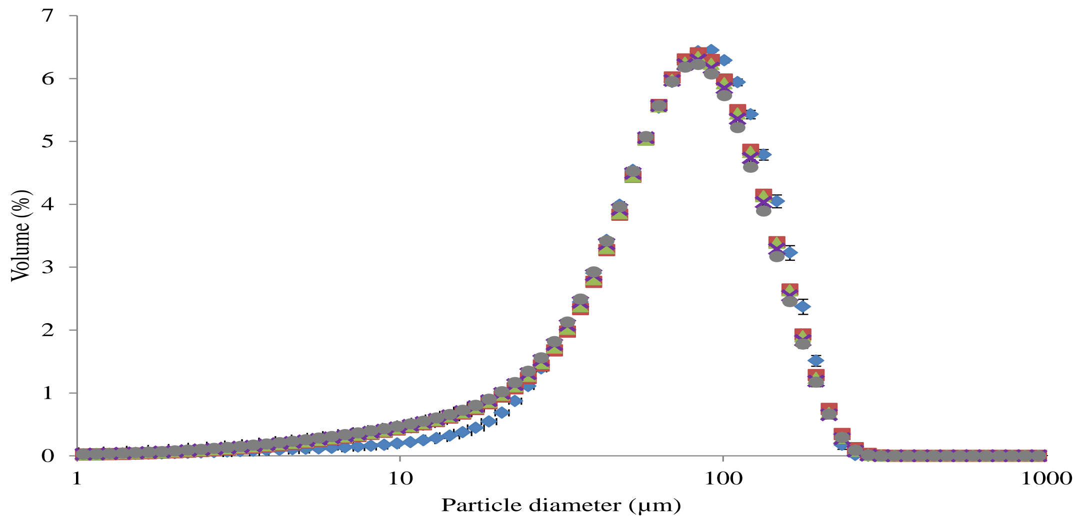

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630 Figure 2. Particle size distribution of the differently prepared carrot purées (HTT+HPH (♦);
631 HPH+HTT (■); 40 min LTT (▲); 5 h LTT (X); 24 h LTT (●)) (For interpretation, the reader
632 is referred to the colored version of this figure legend in the article.)

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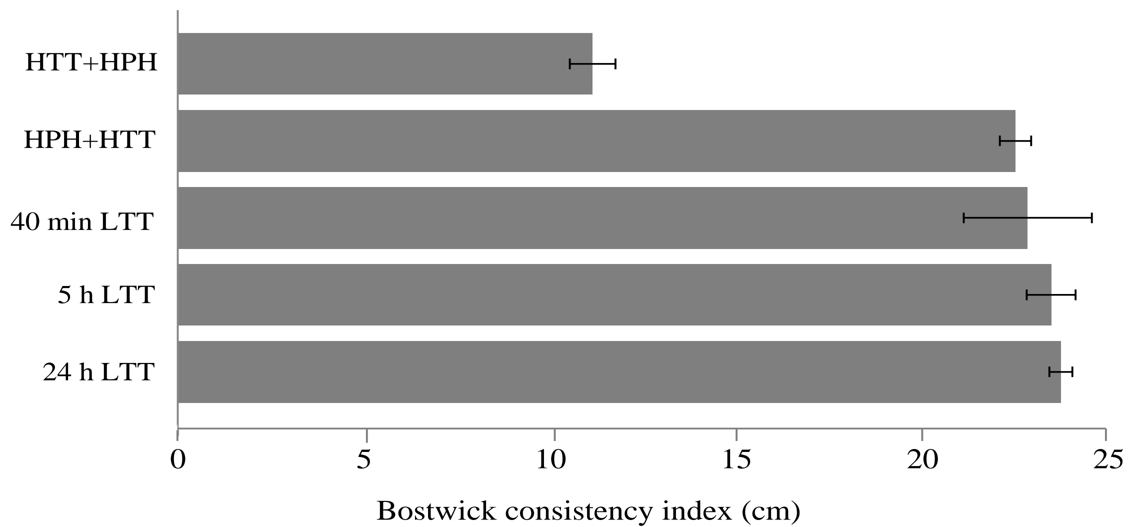

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638 Figure 3. Bostwick consistency of the differently prepared carrot purées

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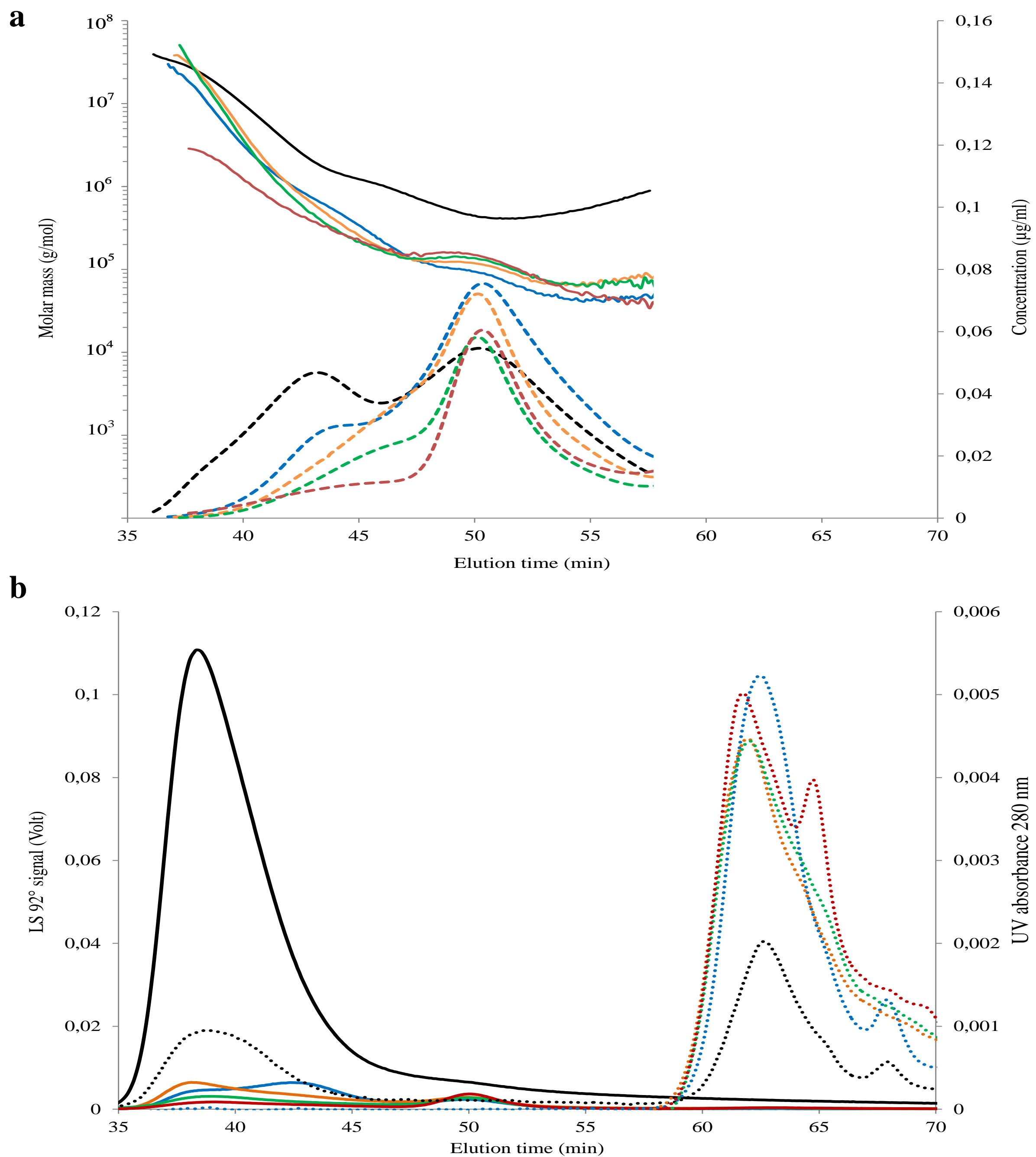

645 Figure 4. Size exclusion elution profile of serum polymers from differently prepared carrot
646 purées (a) log molar mass (thick solid line) superimposed on concentration chromatogram
647 (square dot curve) (b) Light scattering signal at 92° angle (solid curve) superimposed on UV
648 absorbance chromatogram at 280 nm (round dot curve). HTT+HPH – black; HPH+HTT –
649 blue; 40 min LTT – orange; 5 h LTT – green; 24 h LTT – red . (For interpretation, the reader
650 is referred to the colored version of this figure legend in the article.)

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Tables

Table 1. Average pH values, dry matter and ash contents (± standard deviation) of the differently prepared carrot purées

Sample	pH	Dry matter (%)	Ash (%)
HTT+HPH	5.77 ± 0.04	4.60 ± 0.01	0.27 ± 0.02
HPH+HTT	5.72 ± 0.03	4.61 ± 0.02	0.23 ± 0.01
40 min LTT	5.47 ± 0.04	4.52 ± 0.17	0.24 ± 0.02
5h LTT	5.18 ± 0.05	4.47 ± 0.03	0.22 ± 0.02
24h LTT	4.67 ± 0.05	4.46 ± 0.01	0.24 ± 0.01

655

656 Table 2. Total amount of compounds and their corresponding quantity (± standard deviation) for each dialyzed serum sample expressed in
657 µg/g purée

Sample	Monosaccharides								Crude protein	Ash content	Total amount of compounds
	Fuc	Rha	Ara	Gal	Glc	Xyl	Man	UA			
HTT+HPH	3.8 ± 0.1	79.9 ± 1.7	228.7 ± 21.2	272.1 ± 32.0	103.4 ± 6.0	3.5 ± 0.5	12.9 ± 0.5	1 536.6 ± 55.9	309.5 ± 1.1	130.3 ± 5.5	2 680.4 ± 68.3
HPH+HTT	2.5 ± 0.2	41.7 ± 6.9	143.2 ± 18.9	193.4 ± 10.6	104.8 ± 7.5	1.6 ± 0.2	13.8 ± 0.6	1 119.8 ± 19.2	155.6 ± 3.8	80.5 ± 3.3	1 856.9 ± 31.1
40 min LTT	1.6 ± 0.2	15.3 ± 0.3	103.7 ± 10.5	168.9 ± 2.5	99.3 ± 1.5	1.1 ± 0.1	15.7 ± 0.1	520.3 ± 3.6	187.4 ± 2.0	73.7 ± 3.7	1 187.1 ± 12.2
5 h LTT	1.3 ± 0.0	11.6 ± 0.2	93.1 ± 3.0	158.7 ± 4.5	120.4 ± 6.1	0.9 ± 0.0	15.4 ± 0.7	272.2 ± 2.8	151.6 ± 0.2	84.1 ± 1.5	909.4 ± 8.8
24 h LTT	0.5 ± 0.0	8.8 ± 0.0	78.4 ± 0.4	123.0 ± 0.2	109.0 ± 0.2	0.6 ± 0.0	11.0 ± 0.2	112.1 ± 2.4	81.0 ± 0.1	67.5 ± 1.9	591.8 ± 3.1

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Table 3. Average DM, DAc and linearity/branching (± standard deviation) of serum pectin from differently prepared carrot purées

Sample	% DM	% DAc	Linearity of pectin (UA:Rha+Ara+Gal)	Branching of RG-I (Ara+Gal/Rha)
HTT+HPH	66.3 ± 0.55	11.2 ± 0.38	2.22 ± 0.11	6.23 ± 0.52
HPH+HTT	66.1 ± 1.20	8.4 ± 0.57	2.40 ± 0.05	7.10 ± 0.71
40 min LTT	53.1 ± 1.80	9.7 ± 0.14	1.54 ± 0.04	17.48 ± 0.95
5h LTT	37.9 ± 2.67	16.3 ± 0.54	0.88 ± 0.03	21.26 ± 0.30
24h LTT	18.4 ± 1.02	19.6 ± 0.76	0.46 ± 0.01	22.53 ± 0.04

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