The effects of process-induced pectin changes on the viscosity of carrot and tomato sera

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7 2 **tomato sera**
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Abstract

The influence of process-induced pectin changes on the kinematic viscosity of the serum phases of carrot and tomato purées was investigated in this study. Variations in the amount of pectin present in the serum phase and pectin's fine structure were induced by subjecting carrot and tomato to different thermal treatments, i.e. a mild and a strong heat treatment, followed by blending and high-pressure homogenisation at different pressure levels (0, 20 and 100 MPa). Changes in pectin structure were monitored by determination of the degree of methoxylation, analysing the molar mass distribution and immuno-dot blotting using anti-pectin antibodies. Characterisation of serum pectins revealed that a strong thermal treatment caused pectin thermosolubilisation and depolymerisation, especially in carrot sera, and high-pressure homogenisation provoked predominantly mechanical pectin solubilisation in carrot sera and pectin depolymerisation in tomato sera. It was observed that the kinematic viscosity of carrot sera was mainly affected by the amount of solubilised pectin, whereas in tomato sera the kinematic viscosity could mainly be explained in terms of polysaccharide chain length. The degree of methoxylation of pectin, which has a broader distribution in tomato sera than in carrot sera, seemed to have a less pronounced role in determining the kinematic viscosity. In general, it could be concluded that serum viscosity is mainly determined by the amount and size of the solubilised pectin. The influence of serum viscosity on the rheology of the studied purées was limited as the rheology of this type of systems is largely determined by the particle properties of the dispersed phase.

Keywords (max 6, American spelling)

Serum, Pectin, Viscosity, Carrot, Tomato, High-pressure homogenization

34 Introduction

35 Large amounts of fruits and vegetables are nowadays processed into liquid(-like) derived
36 products such as soups, juices and purées. These systems consist of plant-tissue-based particles
37 suspended in a continuous serum phase containing solubilised cell-wall material, in particular
38 solubilised pectin, sugars, salts and organic acids (Caradec & Nelson, 1985; Tanglertpaibul &
39 Rao, 1987; Anthon et al., 2008). The rheological properties of these food suspensions are
40 considered to be determined by the particle properties and the concentration of the dispersed
41 phase as well as by the characteristics of the serum. Particle properties include size, size
42 distribution, shape and deformability (Yoo & Rao, 1994; Den Ouden & Van Vliet, 1997; Servais
43 et al., 2002; Sato & Cunha, 2009; Day et al., 2010; Moelants et al., 2012). In serum, mainly the
44 amount of solubilised pectin and its characteristics, such as chain length, and molar mass
45 distribution, have been suggested to be important for the rheology (Tanglertpaibul & Rao, 1987;
46 Beresovsky et al., 1995; Anthon et al., 2008).

47 Pectin is a plant cell-wall polysaccharide, rich in galacturonic acid (GalA), which can be
48 methoxylated at C-6 and/or acetylated on O-2 and O-3 (Vincken et al., 2003). Pectin structural
49 features can be changed by enzymatic as well as chemical conversion reactions (Van Buren,
50 1979; Sila et al., 2009). Pectin methylesterase (PME), which is present in most fruits and
51 vegetables, and polygalacturonase (PG), which has been detected in large amounts in a.o.
52 tomatoes, are the most important enzymes for the conversion of pectin. The first enzyme
53 catalyses pectin demethoxylation, whereas PG hydrolyses α -1,4-linked D-GalA causing pectin
54 depolymerisation. Both enzymes can be activated or inactivated by specific heat or high pressure
55 treatments (Duvetter et al., 2009). Chemical demethoxylation and depolymerisation, on the other
56 hand, occur without the aid of enzymes under appropriate conditions of temperature and pH
57 (Keijbets & Pilnik, 1974; Van Buren, 1979; Sila et al., 2009).

58 Pectin properties can influence the rheology of plant-tissue-based purées in two ways. Besides
59 influencing particle interactions (Beresovsky et al., 1995), pectin properties can also affect the
60 serum viscosity. Several studies reported an increase in serum viscosity of tomato products with
61 increasing pectin content (Luh et al., 1984; Errington et al., 1998). Tanglertpaibul & Rao (1987)
62 suggested a quantitative, non-linear correlation between the pectin content and the serum
63 viscosity in tomato products. Besides the amount of pectin, also other pectin properties such as

degree of methoxylation (DM), pattern of methoxylation, degree of branching, size, composition and conformation, may affect the serum viscosity because they can change the interactions between pectin chains. In this context, Hurtado et al. (2002) showed that, besides a decrease in the amount of pectin, also pectin depolymerisation causes a drop in the serum viscosity of tomato products. By the inactivation of pectin-degrading enzymes, pectin depolymerisation can be prevented resulting in a higher serum viscosity and higher consistency of tomato purée (Luh & Daoud, 1971; Ural, 2003; Anthon et al., 2008).

Although many studies emphasise the importance of the amount of pectin for the serum viscosity, very few studies actually examined which particular pectin properties play a role. Therefore, the present study investigated which pectin properties are actually important for the kinematic viscosity of the serum phase of plant-tissue-based purées. Carrot and tomato were selected as model systems because they are often consumed as processed products and because of their differences in pectin properties (Houben et al., 2011) and pH. Variations in the amount of pectin present in the serum phase and pectin's fine structure were deliberately induced by subjecting carrot and tomato to different thermal treatments, i.e. a mild and a strong heat treatment, followed by blending and high-pressure homogenisation (HPH) at different pressure levels (0, 20 and 100 MPa). Mild thermal treatments were intended to inactivate PME (and PG in case of tomato), creating reference serum samples in which enzyme activity does not interfere during further analysis. Intense thermal treatments on the other hand were intended to provoke a remarkable degree of pectin solubilisation and degradation. Finally, HPH was used to further mechanically disrupt plant material compared to conventional blending. The effect of this relatively new processing technique in the area of fruit and vegetable processing on pectin structural properties is largely unknown. Changes in pectin structure were monitored by determination of the DM, analysing the molar mass distribution and immuno-dot blotting using anti-pectin antibodies. In a final step, the contribution of the serum viscosity to the consistency of the original carrot and tomato purées was evaluated.

Materials and methods

Plant material

Carrots (*Daucus carota* cv. Nerac) were purchased from a local supplier in Belgium and stored at 4 °C until further use, while tomatoes of a processing variety (*Solanum lycopersicum* cv. Patrona) were obtained from a wholesaler in Spain. Tomatoes were successively washed, cut into slices of 0.5-1 cm and vacuum-packed in plastic bags before high-temperature blanching. Blanching, intended to inactivate the pectinolytic enzymes in tomato, was performed in a temperature-controlled water bath at 95 °C for 8 min. Afterwards, tomato slices were frozen with liquid nitrogen and stored at -40 °C until further processing.

Given the objective of this study, a single variety was selected to prepare the carrot and tomato samples. As such, a single starting point in terms of pectin structures was created for both matrices and focus could be laid on the effect of processing/solubilisation.

Preparation of carrot sera

Fresh carrots were peeled, cut into pieces and vacuum-packed in a plastic bag. The samples were heated in a temperature-controlled water bath at 95 °C for 5 min (further indicated as mildly heat-treated carrots and considered as the reference sample) or at 95 °C for 45 min (further indicated as strongly heat-treated carrots). Both treatments were sufficiently intense to inactivate pectin methylesterase (PME) (results not shown). After heating, samples were immediately cooled on ice. Treated carrot pieces were blended with deionised water in a 1:1 ratio for 1 min using a kitchen blender (Waring blender 7010G, Torrington, CT, USA). Carrot blends were subsequently homogenised with a high-pressure homogeniser (Panda 2K, Gea Niro Soavi, Mechelen, Belgium) at 20 MPa or 100 MPa; one part of the carrot blend was not homogenised ('0 MPa'). For all six carrot purées, the Bostwick consistency and the rheology of the purée were measured immediately. The remaining amount of each purée was centrifuged for 30 min at $12400 \times g$ at 20 °C and the obtained supernatant was vacuum-filtered (MN 615, pore size of 8 µm) to separate the pulp from the serum phase, based on the procedure of Caradec et al. (1985). All carrot sera were prepared in duplicate (same variety of carrots, but different day of purchase), frozen with liquid nitrogen and stored at -40 °C until pectin characterisation and viscosity measurements.

Preparation of tomato sera

Blanched tomatoes were defrozen by the use of two different thermal treatments. Tomatoes were either thawed at 25 °C for 20 min (further indicated as mildly heat-treated tomatoes and considered as the reference sample; the blanching step represents the mild heat treatment) or thawed/treated at 95 °C for 40 min (further indicated as strongly heat-treated tomatoes).

After the strong thermal treatment, samples were immediately cooled on ice. Treated tomatoes were mixed (3×5 s) (Büchi Mixer B-400, Flawil, Switzerland) and sieved (pore size 1.0 mm) in order to obtain a tomato pulp free from skin and seeds. HPH, centrifugation and vacuum-filtration were performed as discussed in the previous section. For all six tomato purées, the Bostwick consistency and the rheology of the purée were measure immediately. All tomato sera samples were prepared in triplicate (same batch of blanched tomatoes), frozen with liquid nitrogen and stored at -40 °C until pectin characterisation and viscosity measurements.

Determination of galacturonic acid content

All chemicals and solvents were of analytical grade and were purchased from Sigma.

To determine their galacturonic acid (GalA) content, sera were subjected to an acid hydrolysis step with concentrated sulphuric acid to depolymerise the pectin polysaccharides according to the method of Ahmed & Labavitch (1978). In short, 2 mL of serum was mixed with 8 mL H₂SO₄ (98%) and 2 mL deionised water was added. Subsequently, the sample was stirred for 1 h to complete the hydrolysis. All serum samples were hydrolysed in duplicate. Subsequently, the GalA content was determined colorimetrically as described by Blumenkrantz & Asboe-Hansen (1973). All hydrolysed serum samples were analysed in triplicate.

Determination of the degree of methoxylation

The degree of methoxylation (DM) of the serum pectins was calculated for each serum sample as the ratio of the molar amount of methoxyl esters (the “corrected” methanol content, see below) to the molar amount of GalA residues.

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4 149 *“Free” methanol content*
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7 150 The amount of methanol (MeOH) present in the sera (here denominated “free” methanol), as a
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9 151 result of hydrolysis of pectin methoxyl esters during the serum preparation steps, was determined
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11 152 using a headspace gas chromatography-mass spectrometry (GC-MS) method with solid-phase
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13 153 microextraction (SPME), based on the method described by Savary & Nunez (2003). To
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15 154 determine the amount of free MeOH, 1 mL serum and 2 μ mol of deuterated methanol (d_3 -
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17 155 MeOH) were diluted to 5 mL in 10-mL vials with ultrapure water (organic free, 18 M Ω •cm
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19 156 resistance), supplied by a Simplicity water purification system (Millipore, Billerica, MA, USA).
20 157 Four vials of each serum sample were prepared, capped and analysed.

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22 158 The GC-MS system consisted of a 6890N Network chromatograph with a 5973 Inert Mass
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24 159 Selective Detector and CTC-PAL autosampler (Agilent Technologies, Diegem, Belgium). Before
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26 160 injection, the sample was equilibrated at 40 °C for 5 min in an aluminium block heater (Peltier
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28 161 Tray Cooler). Subsequently, a Carboxen-PDMS fibre (Supelco, Bellefonte, PA, USA) was
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30 162 exposed to the headspace for 5 min, after which the fibre was retracted and immediately
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32 163 transferred to the GC injection port for sample desorption. Fibers were conditioned prior to use,
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34 164 as recommended by the manufacturer. For the separation of MeOH from other volatile
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36 165 compounds, a HP-INNOWax capillary column (30 mL $\times$ 0.25 mm i.d., film thickness 25 μ m,
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38 166 Agilent Technologies) was used. All injections were conducted in splitless mode with the
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40 167 injection port set at 300 °C and the detector at 250 °C, using helium as carrier gas at 1.5 mL/min.
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42 168 The oven temperature was programmed to increase from 40 °C to 250 °C at 50 °C/min and was
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44 169 subsequently held at the maximum temperature for 5 min. The detector was set at electron
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46 170 impact ionisation mode (200 eV) with data collection using the selected ion recording mode for
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48 171 selected ions at 1.2 scans/s.

49 172 For calibration, samples containing 1 mL carrot or tomato serum and 2 μ mol d_3 -MeOH were
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51 173 spiked with known amounts of MeOH (0.01–20 μ mol MeOH) and subsequently analysed.
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53 174 Blanks (without addition of MeOH) were measured to correct the calibration lines for the free
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55 175 MeOH present in the calibration samples. MeOH concentrations were calculated by plotting the
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57 176 peak area ratios for base ion pairs (m/z 31/35_d) over the indicated range of concentration ratios
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59 177 (normal to deuterated form).
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4 179 *“Corrected” methanol content*

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7 180 To determine the amount of methoxyl esters on the serum pectins, pectin was saponified to
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9 181 pectate and MeOH according to the procedure of Ng & Waldron (1997). In 10-mL vials, 1 mL of
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11 182 serum and 2 μ mol d₃-MeOH were saponified with 0.4 mL of 2 M NaOH for 1 h at 20 °C.
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13 183 Samples were neutralised with 0.4 mL of 2 M HCl and diluted to 5 mL with ultrapure water. All
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15 184 serum samples were saponified in quadruplicate. The amount of MeOH in the samples after
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17 185 saponification was measured using GC-MS as described above (referred to as the “total” MeOH
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19 186 content). This amount was corrected for the amount of “free” MeOH in the serum prior to NaOH
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21 187 saponification to obtain the amount of methoxylesters (referred to as the “corrected” MeOH
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23 188 content).
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25 190 Immuno-dot-assays with anti-pectin antibodies

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27
28 191 Carrot and tomato serum samples were analysed with the monoclonal anti-homogalacturonan
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30 192 antibodies JIM5, JIM7, LM18, LM19, LM20 and PAM1 through immuno-dot-assays. These
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32 193 antibodies have different affinities towards pectins with different degrees and patterns of
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34 194 methoxylation. The procedure used was based on the method described by Christiaens et al.
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36 195 (2011).

37 196 Serum samples and ten-fold dilution series of these samples were dotted (1 μ L) onto pieces of
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39 197 nitrocellulose (Hybond ECL, GE Healthcare, Uppsala, Sweden). After 30 min of air-drying, the
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41 198 nitrocellulose membranes were blocked for 1 h with phosphate-buffered saline (PBS) (140 mM
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43 199 NaCl, 2.7 mM KCl, 8.0 mM Na₂HPO₄, 1.5 mM KH₂PO₄, pH 7.4) containing 5% milk powder
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45 200 (MPBS; Applichem, Darmstadt, Germany). After washing, the nitrocellulose membranes were
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47 201 incubated with primary antibody (diluted in 1% MPBS) for 1.5 h at room temperature.
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49 202 Hybridoma supernatants of JIM5, JIM7, LM18, LM19 and LM20 were diluted 1/20, whereas
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51 203 PAM1 was used at a concentration of 10 μ g/mL. After another washing step, membranes were
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53 204 incubated with secondary antibody for 1 h. A 1/1000 dilution of anti-rat Ig horseradish
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55 205 peroxidase conjugate (Nordic Immunology, Tilburg, The Netherlands) in 1% MPBS was used as
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57 206 secondary antibody for JIM5, JIM7, LM18, LM19 and LM20, whereas for PAM1, an anti-
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59 207 polyhistidine peroxidase antibody (Sigma-Aldrich, St. Louis, United States), diluted 1/2000 in
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61 208 1% MPBS, was used. In a final step, a color reagent (25 ml PBS, 5 ml chloronaphthol, 17 μ l 27%

(v/v) H₂O₂) was added which stained positive blots blue-purple. Because of the high reproducibility of this test, immuno-dot-assays were performed only once.

Analysis of molar mass distribution by high-performance size exclusion chromatography

The molar mass (MM) distribution of the polysaccharides present in the sera was assessed by high-performance size exclusion chromatography (HPSEC). Serum samples were dialysed against deionised water (MWCO 3500 Da) and the concentration of NaNO₃ was adjusted to 0.05 M before analysis. This analysis was conducted on an Äkta Purifier HPLC system (GE Healthcare AB, Uppsala, Sweden), equipped with a mixed-bed column of Bio-Gel TSK (GMPW_{XL}, 300 × 7.8 mm, pore size = 100–1000 Å, particle size = 13 µm, theoretical plates ≥ 7000; Tosoh Bioscience, Stuttgart, Germany) and a TSK guard column (PW_{XL}, 40 × 6 mm). Samples (20 µL) were injected and eluted with 0.05 M NaNO₃ for 25 min at 35 °C and a flow rate of 0.7 mL/min. The eluent was monitored using a Shodex RI-101 refractive index detector (Showa Denko K.K., Kawazaki, Japan). Monogalacturonic acid (0.1% in 0.05 M NaNO₃) was used daily to validate the system. Pullulan standards (180 - 788 000 Da) (Polymer Laboratories Inc., Amherst, MA, USA) were eluted to estimate the MM range of the eluting peaks. The MM distribution of the polysaccharides present in each sera sample was analysed only once.

Kinematic viscosity

Kinematic viscosity of the serum samples (and of several dilutions thereof) was measured using an Ubbelohde capillary viscometer (Ubbelohde, SI Analytics, Mainz, Germany) at 25 °C. Serum was brought into a reservoir and sucked through the capillary into the measuring bulb marked with two calibration lines. Subsequently, the serum was allowed to flow under gravity from the measuring bulb through the capillary to the reservoir. The time required for the serum to pass along two calibrated marks was used to calculate the kinematic viscosity. The Hagenbach correction was used. All sera samples were analysed in triplicate by the use of three capillaries with a different capillary diameter. For all sera, the obtained viscosities were independent of the diameter of the capillary, which confirmed the absence of shear rate dependency and slip at the studied shear rates.

Bostwick consistency

The consistency of the purées was measured using a Bostwick consistometer (CSC Scientific Compagny, VA, USA). The purée was placed into the metal trough of the instrument. One wall of the trough is a metal gate that can be opened almost instantaneously and allows the purée to flow under its own weight into a calibrated trough. The distance travelled by the purée (± 0.5 cm) after 30 s was defined as its Bostwick consistency. Measurements were performed in duplicate.

Rheology of the purées

The rheological properties of the purées were measured with a stress-controlled rheometer (MCR 501, Anton Paar, Graz, Austria) at 25 °C. A six-bladed vane geometry with a diameter of 11 mm and a height of 16 mm was used to avoid wall slip. Approximately 50 mL of sample was loaded into a cup with 28.9 mm diameter. To avoid possible effects of the loading history on the structure, samples were presheared followed by a rest period before all measurements.

The rheology of the purées was studied by the execution of steady-shear tests for the estimation of viscosity and yield stress and by oscillatory tests to study the viscoelastic behaviour. The shear rate ramp, stress ramp, amplitude and frequency sweep were performed as described by Moelants et al. (2012). All measurements were performed in duplicate. For each measurement, a fresh sample was loaded into the cup.

Statistical analysis of data

To evaluate significant differences among the results of different serum samples, all pairwise differences were checked using the Tukey-test (Enterprise Guide 4.2, SAS Inst. Inc., Cary, NC, USA). The level of significance was set at $P < 0.05$.

Results and discussion

Serum pectin characteristics

Pectin content of the sera

The GalA content of the serum represents a measure for the amount of serum pectin. In Table 1 and 2, the GalA content per mL serum is shown for carrot and tomato samples, respectively. For carrot sera, the GalA content was significantly higher for the samples obtained from strongly heat-treated carrot pieces as compared to the ones that received a mild thermal treatment. Continued thermal processing can provoke progressive solubilisation of pectin mainly due to β -elimination of internal cellular pectin whereby the GalA content in the serum increases (Hurtado et al., 2002; Anthon et al., 2008). Moreover, for sera obtained from strongly heat-treated carrots, an increase in pressure level during HPH also caused an increase in GalA content, which turned out to be statistically significant for samples homogenised at 0 MPa compared to 100 MPa. When higher homogenisation pressures are applied, higher velocities and velocity gradients resulting in higher turbulence intensity and more cavitation are expected (Floury et al., 2004). Hence, higher pressure levels result in a higher extent of tissue breakdown, which seems to have affected pectin extractability.

Also for tomato samples, an increase in intensity of the thermal process caused an increase in GalA solubilised in the serum phase (Table 2). Although an increase in pressure during HPH appears to provoke an increase in GalA content of the serum samples prepared from strongly heat-treated tomatoes, this increase was not statistically significant.

Although the thermal treatments applied for carrot and tomato samples were not exactly the same, the results clearly show that intense thermal treatments increased the GalA content, and thus the amount of serum pectin, in carrot sera more (factor ≥ 5 as compared to a mild heat treatment) than in tomato samples (factor ≤ 2). The latter can be linked to the low pH of tomato (ca. 4.5) which limits the extent of thermal depolymerisation and solubilisation (Keijbets & Pilnik, 1974; Sila et al., 2005; Anthon et al., 2008).

Degree of methoxylation of serum pectin

When treating plant material at sufficiently high temperatures, chemical de-esterification of cell-wall pectin can occur, leading to the release of methanol (MeOH) in the serum phase. The amount of MeOH released during the thermal treatment is referred to as “free” MeOH. In Table 1 and 2 respectively, the concentrations of free MeOH in the carrot and tomato sera are presented. As expected, a clear increase in the amount of free MeOH in carrot sera was observed by increasing the thermal treatment time. For tomato samples on the other hand, no significant

change in the amount of free MeOH could be observed by increasing the thermal treatment time. The pH of tomato probably impeded chemical de-esterification during the intense thermal treatment. Furthermore, increasing the homogenisation pressure did not have a significant effect on the amount of free MeOH in both carrot and tomato sera indicating HPH does not cause pectin demethoxylation. Finally, it can be observed that tomato sera display a higher free MeOH content than carrot sera. This observation might be attributed to the presence of a higher level of enzymatic de-esterification in tomatoes prior to the enzyme-inactivating treatments performed in this study. In this context, tomatoes are known to contain a higher amount of intrinsic pectin methylesterase (PME) than for example carrots (Jolie et al., 2010).

The amount of MeOH per mL serum after saponification was corrected by the amount of free MeOH in the serum, released during the heat and pressure treatment of the plant material. The result is referred to as the “corrected” MeOH content and represents the amount of MeOH originally esterified on the serum pectins. The results are presented in Table 1 and 2 for the carrot and tomato samples, respectively. Increasing the intensity of the thermal treatment or the homogenisation pressure resulted in an increase of the corrected MeOH content for both carrot and tomato sera (although the latter was only statistically significant for the sera prepared from the strongly heat-treated carrots homogenised at 100 MPa).

For carrot sera (Table 1), an increase in intensity of the thermal treatment provoked an increase in DM from 40-45% in sera of mildly heat-treated carrots to 65-70% in sera of strongly heat-treated carrots. Enzymatic de-esterification can be neglected because PME was inactivated during both thermal treatments (data not shown). Probably, mainly pectins with low DM are solubilised into the serum during the first minutes of the thermal treatment, while afterwards also pectins with higher DM follow. The β -elimination reaction is promoted for high-methoxylated pectin (Keijbets & Pilnik, 1974; Fraeye et al., 2007). Enrichment of carrot serum with high-methoxylated pectin can dominate chemical de-esterification after a prolonged heat treatment which might explain this increase in DM.

In Table 2, no significant differences between the DM of tomato sera prepared from strongly heat-treated tomatoes as compared to the sera from mildly heat-treated tomatoes were noticed. This can be explained by the inactivation of PME and the low pH of tomato sera whereby enzymatic and chemical de-esterification are impeded.

Since the amount of MeOH on serum pectins in function of homogenisation pressure followed the trend of the amount of GalA as a function of homogenisation pressure, the DM of both carrot or tomato sera appeared to be constant as a function of homogenisation pressure.

Immuno-dot-assays of the sera

In Fig. 1, the results of immuno-dot-assays of the sera samples with antibodies JIM5, JIM7, LM18, LM19, LM20 and PAM1 are shown. Knowledge on the binding specificities of these antibodies is at hand: JIM 5 binds to pectin with a medium DM, whereas JIM7 has a broad affinity towards pectins with very diverse degrees and patterns of methoxylation. Furthermore, LM18 is specific for pectin with a low DM, LM 19 binds both medium- and low-methoxylated pectin and LM20 specifically recognises high-methoxylated pectin. Finally, PAM1 binds to long blocks of approximately 30 non-esterified GalA residues (Willats et al., 1999; Christiaens et al., 2011). Samples of pectic polysaccharides were applied at a single point, which provided the opportunity for differential migration of molecules across the nitrocellulose membrane (Willats & Knox, 1999). In this way, two components, an outer ring and a central dot, were resolved when samples were dotted onto the membrane. For the undiluted carrot sera, the orange colour of the samples themselves should not be confused with a positive reaction in the immuno-dot-assay. The strong binding of JIM7 and LM20 to carrot serum pectin compared to the weak binding of JIM5, LM18, LM19 and PAM1, suggests that carrot sera contain especially high-methoxylated pectin. For sera derived from strongly heat-treated carrots, the outer ring of the dot from LM20 disappeared. According to Willats and Knox (1999), unbranched homogalacturonan-rich components migrate further on the nitrocellulose membrane (outer ring) compared to pectic components with abundant side chains (central dot). Hence, strongly heat-treated carrot sera contain less linear, high-methoxylated homogalacturonan chains than mildly heat-treated carrot sera. Strong depolymerisation of linear, high-methoxylated pectin during the more intense heat treatment may lead to the disappearance of the epitope for LM20 and hence explain this observation. Another possible explanation might be the demethoxylation of linear, high-methoxylated pectin during the more intense heat treatment. Nevertheless, the overall DM of the carrot serum pectin was higher with increasing heat intensity (cf. Table 1). By using a more intense heat treatment, solubilisation of pectin will proceed, whereby dots of PAM1 become more pronounced, due to the solubilisation of low-methoxylated pectin or demethoxylation

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4 359 whereas the global DM turned out to increase due to the extra solubilisation of high-
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6 360 methoxylated pectin. At last, dots of PAM1 became more visible with increasing homogenisation
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8 361 pressure, meaning more blocks of low-methoxylated pectin are occurring. Since the global DM
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10 362 of carrot sera was not affected by the use of HPH, the latter observation is probably caused by
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12 363 the release of more pectin into the serum during HPH rather than demethoxylation of the
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14 364 solubilised pectin. Mechanical release and solubilisation of pectin due to HPH was already
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16 365 demonstrated.

17 366 Whereas pectin in carrot sera turned out to have a small distribution in DM, tomato sera appear
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19 367 to have a large distribution of DM since both LM20 (specific for high-methoxylated pectin) and
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21 368 PAM1 (specific for low-methoxylated pectin) bind strongly to the six different tomato samples.
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23 369 Further analysis of the binding of LM20 and JIM7 to tomato sera, showed that an outer ring
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25 370 around the dot was visible pointing out that high-methoxylated pectins are linear. Besides linear,
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27 371 high-methoxylated pectin, tomato sera also comprise branched pectin (inner dot) with both high
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29 372 and low DM (inner dot was visible for all antibodies). As for carrot sera, the intensity of the dots
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31 373 of PAM1 became stronger with increasing homogenisation pressure, meaning more blocks of
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33 374 low-methoxylated pectin are occurring when a higher homogenisation pressure was used. The
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35 375 effect was, however, less outspoken for tomato sera compared to carrot sera. Moreover, in
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37 376 contrast to the carrot sera, binding of PAM1 for all tomato sera, including the mildly heat-treated
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39 377 tomato samples, was observed.

40 378 In summary, it can be concluded that the distribution of DM of pectin in carrot sera is rather
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42 379 small, whereas this distribution appears to be broader in tomato sera. No clear changes in DM
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44 380 with processing could be found in tomato pectin. For carrot sera on the other hand, an intense
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46 381 heat treatment resulted in the loss of linear, high-methoxylated pectin despite the overall increase
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48 382 in DM.

49 383 50 384 *Molar mass distribution of serum polysaccharides*

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52 385 In addition to the amount and the DM of the serum pectin, the molar mass (MM) of the pectin
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54 386 polysaccharides was analysed. Representative results of the molar mass distribution of the
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56 387 polysaccharides solubilised in the serum phase, as determined by HPSEC analysis in
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58 388 combination with refractive index detection, are presented in Fig. 2.

For carrot sera, MM distributions are shown in Fig. 2A. In these chromatograms, two major unresolved peaks in the polymeric range can be seen. When comparing sera prepared from mildly and intensely heat-treated carrots, an increase in the amount of serum pectin, as well as a shift to polysaccharides with lower MM ($MM \approx 112000$ Da) was noticed. The first observation can be explained by thermosolubilisation whereas the second observation is probably due to the promotion of depolymerisation during more intense heating, as was also reported by De Roeck et al. (2008) for heat-processed carrots. Both thermosolubilisation and depolymerisation are caused by β -elimination of pectin, insoluble pectin in case of the first process and soluble pectin for the latter. The present observations are in line with earlier reports (Nyman et al., 1993; Hurtado et al., 2002; Diaz et al., 2009). Thermosolubilisation of pectin after prolonged heating was confirmed by an increase in the GalA content of serum prepared from more intensely treated carrots.

For carrot sera prepared from both mildly heat-treated and strongly heat-treated carrot pieces, increasing the homogenisation pressure led to an increased release of pectin. Especially at a homogenisation pressure of 100 MPa, pectin solubilisation was unmistakable. As shown in Table 1, in particular a homogenisation pressure of 100 MPa turned out to cause an increase in GalA content. When comparing the MM distribution of serum pectins obtained from carrots that received the same heat treatment, it can be seen that by increasing the pressure level not only the amount of pectin with a lower MM was increased, but solubilisation of pectin over the entire range of MM occurred. Thus, HPH turns out to provoke mechanical pectin solubilisation, but no measurable depolymerisation. Therefore, it can be concluded that the range of MM of serum pectin is mainly determined by the intensity of the thermal treatment and not by the homogenisation pressure. In this context, Corredig & Wicker (2001) observed changes in the MM distribution of certain commercial pectins after HPH at 124 MPa, but no change in the MM distribution was found after HPH at 17 MPa.

The MM distributions of polysaccharides in tomato sera are presented in Fig. 2B. In contrast to the MM distributions in carrot sera (Fig. 2A), only one major broad peak in the polymeric range could be observed for tomato sera. Tomato polysaccharides were, generally spoken, also larger than carrot polysaccharides. Similarly, Houben et al. (2011) showed that tomato pectins have a higher MM than carrot pectins.

As was observed for carrot samples, more intense thermal treatments resulted in a higher detected signal and MM distributions were shifted to lower MM. The former is caused by an increase in the amount of serum pectin (also visible in the GalA content (Table 1)), while the latter is due to pectin depolymerisation. Thermosolubilisation was, however, less pronounced for the tomato samples as compared to the carrot samples, which is probably due to the lower pH of tomato which may hamper beta-eliminative pectin solubilisation (Keijbets & Pilnik, 1974; Anthon et al., 2008).

By increasing the homogenisation pressure, the MM distribution of polysaccharides of tomato sera samples prepared from mildly heat-treated tomatoes was shifted to lower MM. For tomato samples obtained from strongly heat-treated tomatoes, only a homogenisation pressure of 100 MPa was able to cause a similar decrease in MM. Two possible explanations can be given for this decrease in MM with increasing homogenisation pressure: or larger pectins are mechanically disrupted into smaller pectin polymers, or HPH causes conformational changes of pectin. Corredig & Wicker (2001), who also observed changes in the MM distribution of commercial pectin after HPH at sufficiently high pressure levels, did not provide any explanation for these changes. Furthermore, Flourey et al. (2002) and Harrington & Zimm (1965) demonstrated that the strong elongational flow encountered by polymers (like methylcellulose) at the entrance of the high-pressure homogeniser valve can disrupt covalent bonds. In the present study, the use of HPH on the MM distribution of pectin was clearly different for carrot and tomato sera. Whereas HPH provoked mechanical pectin solubilisation in carrot samples, the use of HPH resulted especially in a decrease in MM in tomato samples.

Kinematic viscosity of the sera

The kinematic viscosity of the different sera at 25 °C is shown in Table 3-4 for the carrot and tomato sera, respectively, and will first be commented. Secondly, an attempt will be made to explain differences in kinematic viscosity on the basis of differences in pectin properties, as discussed in previous paragraphs.

For carrot sera, the kinematic viscosity was higher (only significantly higher in case of homogenisation at 100 MPa) for serum samples obtained from strongly heat-treated carrot pieces as compared to those obtained from mildly heat-treated carrots. For the former, an increase in homogenisation pressure to 100 MPa also led to a significant increase in kinematic viscosity.

As compared to carrot sera, the kinematic viscosity of tomato sera was significantly higher. Despite the lower GalA content in tomato sera prepared from strongly heat-treated tomatoes in comparison to the GalA content in similarly prepared carrot sera (see Table 1 and 2), the kinematic viscosity of the tomato sera was higher, irrespective of thermal treatment (cf. below). Furthermore, an increase in homogenisation pressure did not lead to a rise in the kinematic viscosity of tomato sera. Actually, the kinematic viscosity seemed to be independent of the homogenisation pressure in the serum samples prepared from strongly heat-treated tomatoes. For the serum samples prepared from mildly heat-treated tomatoes, the kinematic viscosity of the homogenised samples were, however, significantly lower than the kinematic viscosity of the non-homogenised sample.

In addition, the intrinsic viscosity $[\eta]$ of most sera samples was determined in order to study the effect of different pectin structural properties on the serum viscosity. In contrast to the total serum viscosity, this property is in principle independent of the pectin concentration (Kulicke & Clasen, 2004). $[\eta]$ was obtained as the slope from the plot of the kinematic viscosity of diluted sera *versus* the GalA concentration in these diluted sera. Curves were constrained to reduce to the viscosity of water at zero concentration of GalA and only datapoints with a relative viscosity between 1.2 and 2.5 were taken into account as the following linear relationship is only expected in this dilute region (Kulicke & Clasen, 2004):

$$\eta = \eta_s (1 + [\eta] \varphi)$$

with η = kinematic viscosity of the serum, η_s = solvent viscosity, $[\eta]$ = the intrinsic viscosity and φ = the GalA concentration. An example of this type of curves is given in Fig. 3 for carrot and tomato serum prepared from strongly heat-treated samples homogenised at 0 MPa. The values obtained for the intrinsic viscosities of some representative sera are given in Table 5. For carrot and tomato sera, $[\eta]$ was decreased by a more intense heat treatment. This decrease in $[\eta]$ upon thermal processing can be caused by the solubilisation of smaller pectin chains or by the depolymerisation of pectin after a more intense heat treatment. The use of HPH turned out to affect the chain length of pectin, and hence $[\eta]$, only in mildly heat-treated tomato samples, whereas pectin was not mechanically broken by the use of HPH in the other samples. For the mildly heat-treated tomatoes, the disappearance of large polysaccharides can be an explanation for the decrease in $[\eta]$ with increasing homogenisation pressure. Differences in $[\eta]$ (Table 5) are thus confirmed by observations concerning MM in Fig. 2. Values of $[\eta]$ were larger for tomato

sera as compared to carrot sera, irrespective of thermal treatment. Larger values of $[\eta]$ can be caused by the presence of longer and more linear pectin chains (Houben et al., 2011). In addition, differences in pH and ion concentration might also be responsible for the larger value of $[\eta]$ for tomato in contrast to carrot sera since these parameters can affect the expansion of polyelectrolytes in solution (Kulicke & Clasen, 2004).

Next to structural properties, the concentration regime of polymer solutions partially determines the interaction of polymer chains, e.g. the ability of the chains to overlap and entangle (Macosko, 1994). Entanglement and overlap can cause the viscosity to increase. To examine whether interactions between polymer chains are possible, an overlap parameter $C \cdot [\eta]$ was calculated by multiplying the polymer concentration in solution C (g/L) with the intrinsic viscosity $[\eta]$ (L/g), which forms the basis for the classification of polymer solutions into dilute ($C \cdot [\eta] < 1$), semi dilute ($1 < C \cdot [\eta] < 10$) or concentrated ($C \cdot [\eta] \geq 10$) solutions. C was calculated as the ratio of the GalA concentration to the degree of polymerisation. The latter could be estimated from the average MM, obtained from Fig. 2, divided by the MM of GalA. The coil overlap parameter appeared to be on average between 1 and 10 for all tomato sera, meaning overlap between polymers is possible (semi-dilute regime). For carrot sera on the other hand, this coil overlap parameter was smaller than 1 for sera prepared from mildly heat-treated carrots, whereas it was larger than 1 for sera prepared from strongly heat-treated carrots. In the latter sera, entanglements between polymers can thus occur. The presence of overlap and entanglements in the pectin solutions enables gel formation, if conditions of pH and ion content are suitable. Hence, the serum viscosity will not merely depend on the pectin properties in solution, but also on the gelation behaviour of different pectins.

To evaluate a possible link between the kinematic viscosity and the serum pectin properties, different properties of the different samples were summarised in Table 6. From this table, it can be observed that for carrot sera, changes in GalA content with heating time or homogenisation pressure were similar as those observed for the kinematic viscosity. Therefore, it can be concluded that changes in the kinematic viscosity of carrot purée serum can be mainly explained by changes in the amount of serum pectin: the more serum pectin, the higher the kinematic viscosity. The effect of the GalA concentration on the kinematic viscosity is clearly illustrated in Fig. 4A. Although the serum viscosity turned out to be mainly affected by the GalA content, the

concentration dependence of the kinematic viscosity appeared to be also influenced by the processing conditions (intensity of heat treatment and pressure during HPH), as shown by the variations in $[\eta]$ in Table 5.

When considering tomato sera in Table 6, it turns out that, in contrast to carrot sera, trends in the GalA content did not match with the changes observed for the kinematic viscosity. Therefore, it can be concluded that the amount of pectin in tomato sera is not the main determinant for the kinematic viscosity. Fig. 4B illustrates the effect of the GalA concentration on the kinematic viscosity more clearly. Similarly as for carrot sera, it can be seen that processing conditions affected the concentration dependence of the kinematic viscosity. However, this concentration dependence was clearly stronger for tomato sera than for carrot sera, as shown by the slopes of the curves (or $[\eta]$ in Table 5), irrespective of thermal treatment.

In conclusion, the kinematic viscosity of both carrot and tomato sera from purées prepared of plant material that underwent different thermal and homogenisation treatments, is influenced by a combination of the amount of serum pectin and other pectin properties (like MM distribution, DM). Similarly, Diaz et al. (2009) concluded that serum viscosity in tomato paste was primarily determined by the amount and molecular weight of polymeric material in the serum. Tanglertpaibul & Rao (1987), however, attributed changes in serum viscosity to changes in the pectin content solely and even formulated a quantitative correlation between the pectin content and the serum viscosity for tomato products. In contrast to our study, Tanglertpaibul & Rao (1987) prepared sera with different amounts of pectin by concentrating a non-concentrated serum to various °Brix concentrations. During this concentration process, other pectin properties (like pectin size) may remain unchanged. In our study, however, pectin concentrations were increased by using a strong thermal treatment and/or HPH also provoking changes of pectin properties like MM distribution. High values of serum kinematic viscosity as compared to the viscosity of water (1.5-6x) were found. It is worth noting that, in addition to pectin, also the presence of very small particles ($<10\mu\text{m}$), cell wall polysaccharides other than pectin, sugars and ions may contribute to the serum viscosity.

The higher kinematic viscosity of tomato serum, compared to carrot serum, irrespective of thermal treatment, may be attributed to the occurrence of, in general, higher MM of polysaccharides in tomato serum. However, it should also be noted that the serum conditions (pH, ion concentration) in tomato serum may be more suitable for generating high serum

viscosities than for carrot serum. After all, high-methoxylated pectins (DM > 50%) can form gels in the presence of soluble solids such as sugars and under low pH, whereas low-methoxylated pectins (DM < 50%) can form gels by divalent cations (especially Ca^{2+}) under high pH conditions (Thibault & Ralet, 2003). In addition, other pectin properties (degree of branching, DM and pattern of methoxylation) are also different in carrot and tomato sera (Houben et al., 2011). In the current work, it was revealed that the range of DM was much broader in tomato serum than that in carrot sera. These differences can affect the serum gelling behaviour and is for sure worthwhile studying in the near future.

Based on the results, it can be concluded that, the serum kinematic viscosity can be increased by increasing the amount of serum pectin or by the presence of larger serum pectins which can be obtained by targeted processing of carrot and tomato purée. Whereas the use of thermal and/or HPH treatments however mainly causes an increase of the amount of serum pectin in case of carrot purée, the use of these unit operations causes mainly variations in the MM distributions of tomato serum pectin. Knowledge obtained in this study can thus be used to tailor the serum viscosity in the future.

Rheology of the purées

The Bostwick consistency of the purées was measured and a relation between this rheological property and the serum viscosity was strived for (Fig. 5). However, no unambiguous correlation between the Bostwick consistency and the serum viscosity could be observed. Also other rheological properties (such as the dynamic and static yield stress or the storage modulus at low angular frequency) of the original purées were measured. Again, no unambiguous correlation between these parameters and the serum viscosity could be observed. As an example, the kinematic viscosity of the carrot and tomato sera is plotted versus the static yield stress in Fig. 5B. From these results, it can be concluded that, not unexpectedly, the influence of the serum viscosity on the purée consistency is probably rather limited. In this context, Errington et al. (1998) also observed differences in tomato juice consistency that could not be explained by differences in serum viscosity. They concluded that differences in the amount and nature of solids were responsible for the differences in consistency. In a previous study on the rheology of carrot suspensions (Moelants et al., 2012), it was also reported that rheological properties of

these suspensions were highly dependent on particle concentration and particle properties such as size. In turn, these properties are strongly affected by the intensity of the thermal treatment and HPH and dominate over the effect of the serum viscosity. Also Tanglertpaibul & Rao (1987) and Anthon et al. (2008) concluded that the relative contribution of the pulp content to tomato juice viscosity was larger than that of the serum viscosity. Finally, Sesmero et al. (2009) showed that the serum viscosity was negligible as compared to the overall viscosity of strawberry juice. Hence, rheological properties of plant-tissue-based purées are probably mainly determined by the particle properties and to a lesser extent by the serum viscosity. Although the serum viscosity itself turned out to have little effect on the consistency of the purée, it is possible that components in the serum phase play a role in the consistency of the purée in other ways than by altering the serum viscosity. For example, serum pectin might influence the final rheology by changing interactions between particles rather than by influencing the serum viscosity.

586 Conclusion

587 Serum pectins, originating from plant-tissue-based purées, were characterised and an attempt
588 was made to identify which of the pectin properties were dominating the serum viscosity.
589 Variations in the amount of pectin present in the serum phase and pectin's fine structure were
590 induced by subjecting carrot and tomato to different thermal treatments, i.e. a mild and a strong
591 heat treatment, followed by blending and high-pressure homogenisation at different pressure
592 levels (0, 20 and 100 MPa). More intense heat processes caused pectin thermosolubilisation and
593 depolymerisation, whereas high-pressure homogenisation provoked mechanical solubilisation of
594 polysaccharides. In carrot samples, large as well as small polysaccharides were solubilised into
595 the serum phase by the use of high-pressure homogenisation whereas in tomato sera, the amount
596 of mainly small polysaccharides was increased by high-pressure homogenisation.
597 The kinematic viscosity of both carrot and tomato sera was influenced by a combination of the
598 amount of serum pectin and other pectin properties (like the pectin size and the degree of
599 methoxylation). Beside the galacturonic acid content, a measure for the amount of serum pectin,
600 the pectin size appeared to influence the viscosity of tomato sera. On the other hand, the amount
601 of pectin in carrot sera turned out to be the dominant factor in the serum viscosity.
602 This study proved that the possibility exists to use directed processing (thermal treatment and
603 high-pressure homogenisation) to tailor pectin characteristics and thereby also the serum
604 viscosity. The influence of the serum viscosity on the rheology of the purées appeared limited for
605 the plant-tissue-based systems studied here. Probably, particle properties will dominate the
606 rheological properties of the purée itself. Nevertheless, pectin from the serum phase might play a
607 role in the final rheology, not only by influencing the serum viscosity but by changing
608 interactions between particles. Profound understanding on how to create serum with specific
609 pectin properties, and how this influences the rheology of fruit and vegetable-based food
610 products, is in other words necessary in the context of industrial application in which a viscosity
611 increase (for example to create mouthfeel of fruit- and vegetable-based products) or decrease (for
612 example to make pumpable fruit and vegetable-based products) is desired.

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Fig. 1 Immuno-dot-assays of JIM5, JIM7, LM18, LM19, LM20 and PAM1 binding to serum pectin of carrot and tomato purée prepared from mildly or strongly heat-treated carrots or tomatoes in combination with a high-pressure homogenisation at different pressures levels (0, 20 or 100 MPa) (DM = degree of methoxylation; HG = homogalacturonan). Samples were applied in dilution series at the levels indicated.

Fig. 2 Molar mass distribution of serum polysaccharides in carrot (A) and tomato (B) sera prepared from plant material that received a mild (grey) or strong (black) thermal treatment, homogenised at 0 MPa (····), 20 MPa (— —) or 100 MPa (—). Elution times of pullulan standards are indicated to allow for a rough estimation of the molar masses (in Da).

Fig. 3 Plot of the kinematic viscosity versus the galacturonic acid (GalA) concentration for several dilutions of the carrot (●) and tomato (▲) serum from strongly heat-treated samples homogenised at 0 MPa. The intrinsic viscosity $[\eta]$ was calculated from the slope of the line.

Fig. 4 Effect of the galacturonic acid (GalA) concentration on the kinematic viscosity for carrot (A) and tomato (B) sera prepared from plant material that received a mild thermal treatment in combination with HPH at 0 (◆) or 100 MPa (◇) or strong thermal treatment in combination with HPH at 0 (●) or 100 MPa (○). Data point with largest GalA content of each series represents an undiluted serum.

Fig. 5 Parity plot of the kinematic viscosity of the sera versus the Bostwick consistency (A) or static yield stress (B) of the purées (mild-treated carrot ▲, strongly heat-treated carrot ●, mildly heat-treated tomato ◆ and strongly heat-treated tomato ■).

Table 1 Pectin properties as a function of homogenisation pressure for carrot sera prepared from plant material that received a mild or strong thermal treatment (GalA = galacturonic acid; MeOH = methanol; DM = degree of methoxylation).

Table 2 Pectin properties as a function of homogenisation pressure for tomato sera prepared from plant material that received a mild or strong thermal treatment (GalA = galacturonic acid; MeOH = methanol; DM = degree of methoxylation).

Table 3 Kinematic viscosity ($n=2$, $\pm$ standard error) as a function of homogenisation pressure for carrot sera prepared from plant material that received a mild or strong thermal treatment.

Table 4 Kinematic viscosity ($n=2$, $\pm$ standard error) as a function of homogenisation pressure for tomato sera prepared from plant material that received a mild or strong thermal treatment.

Table 5 The intrinsic viscosity $[\eta]$ and overlap parameter $C \cdot [\eta]$ of carrot (A) and tomato (B) sera.

Table 6 Summary of serum pectin characterisation and relation with the kinematic viscosity of the carrot (A) and tomato (B) sera (GalA = galacturonic acid; MeOH = methanol; DM = degree of methoxylation; MM = molar mass).

Table 1
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Table 1

Intensity of heat treatment	Homogenisation pressure (MPa)	GalA (μmol/ml)			Free MeOH (μmol/ml)			Corrected MeOH (μmol/ml)			DM (%)					
Mild	0	2.74	±	0.30 ^s	c [*]	0.82	±	0.12 ^s	b [*]	1.04	±	0.14 ^s	c [*]	37.93	±	6.59 ^s
	20	2.82	±	0.71	c	0.82	±	0.02	b	1.18	±	0.53	c	41.81	±	21.71
	100	3.66	±	0.61	c	0.86	±	0.00	b	1.81	±	0.54	c	49.30	±	16.93
Strong	0	15.26	±	0.34	b	1.48	±	0.11	a	10.08	±	0.22	b	66.04	±	2.08
	20	16.89	±	1.25	ab	1.56	±	0.09	a	11.10	±	0.65	ab	65.72	±	6.20
	100	20.05	±	1.17	a	1.64	±	0.10	a	13.02	±	0.45	a	64.96	±	4.43

^s sterror
* values with the same letter are not significant different (level of significance was set at $P < 0.05$)

Table 2
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Table 2

Intensity of heat treatment	Homogenisation pressure (MPa)	GalA (μmol/ml)			Free MeOH (μmol/ml)			Corrected MeOH (μmol/ml)			DM (%)		
Mild	0	5.60	± 0.87 ^s	ab [*]	1.98	± 0.06 ^s	ab [*]	3.36	± 0.31 ^s	c [*]	60.04	± 10.80 ^s	
	20	5.20	± 0.57	b	1.74	± 0.04	b	3.02	± 0.25	c	58.09	± 7.96	
	100	6.04	± 0.83	ab	1.80	± 0.05	ab	3.70	± 0.30	bc	61.24	± 9.83	
Strong	0	8.34	± 0.62	ab	1.97	± 0.05	ab	5.32	± 0.33	ab	63.82	± 6.13	
	20	8.67	± 0.55	ab	1.88	± 0.03	ab	5.07	± 0.37	ab	58.48	± 5.63	
	100	9.51	± 1.31	a	1.99	± 0.08	a	6.33	± 0.50	a	66.59	± 10.59	

^s sterror
* values with the same letter are not significant different (level of significance was set at $P < 0.05$)

Table 3
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Table 3

Intensity of heat treatment	Homogenisation pressure (MPa)	Kinematic viscosity (mm ² /s)			
Mild	0	1.46	±	0.05 ^s	b [*]
	20	1.41	±	0.13	b
	100	1.57	±	0.18	b
Strong	0	1.89	±	0.09	b
	20	2.00	±	0.13	b
	100	2.97	±	0.20	a

^s sterror
^{*} values with the same letter are not significant different (level of significance was set at *P* < 0.05)

Table 4

Intensity of heat treatment	Homogenisation pressure (MPa)	Kinematic viscosity (mm ² /s)			
Mild	0	5.52	±	0.34 ^s	a [*]
	20	3.13	±	0.01	cd
	100	2.41	±	0.00	d
Strong	0	4.57	±	0.02	ab
	20	3.98	±	0.07	bc
	100	4.19	±	0.27	b

^s sterror

^{*} values with the same letter are not significant different (level of significance was set at *P* < 0.05)

Table 5

	Intensity of heat treatment	Homogenisation pressure (MPa)	$[\eta]$ (L/g)	$C \cdot [\eta]$
A	Mild	0	1.57	0.75
		100	1.27	0.84
	Strong	0	0.49	1.35
		100	0.55	1.95
B	Mild	0	5.35	5.33
		100	2.47	2.64
	Strong	0	2.15	3.17
		100	1.84	3.11

Table 6
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Table 6

A					
Changes in process conditions	<i>Gala</i>	Pectin properties <i>Corrected MeOH</i>	<i>DM</i>	<i>Average MM</i>	Kinematic Viscosity
Increasing intensity of thermal treatment	↑	↑	↑	↓	↑
Increasing homogenisation pressure	≈ (mild) or ↑ (strong)	≈ (mild) or ↑ (strong)	≈	≈	≈ (mild) or ↑ (strong)

B					
Changes in process conditions	<i>Gala</i>	Pectin properties <i>Corrected MeOH</i>	<i>DM</i>	<i>Average MM</i>	Kinematic Viscosity
Increasing intensity of thermal treatment	↑	↑	≈	↘	no clear effect
Increasing homogenisation pressure	≈ (mild) or ↗ (strong)	≈ (mild) or ↗ (strong)	≈	↓	↓ (mild) or ≈ (strong)

mild or strong = intensity of heat treatment
↗ or ↘ = slight increase/decrease
↑ or ↓ = clear increase/decrease

Figure 1
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Fig. 1

Figure 2
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Fig. 2

Fig. 3

Figure 4
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Fig. 4

Figure 5
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Fig. 5