Low-temperature blanching as a tool to modulate the structure of pectin in blueberry purees

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Abstract

Blueberry composition was characterized for six cultivars. It contains a good amount of dietary fiber (10-20%) and pectin (4-7%) whose degree of methylation (DM) is sensitive to food processing. Low-temperature blanching (LTB: 60°C/1 h) was applied on blueberry purees to decrease pectin DM, in order to modulate puree properties and functionalities (i.e. viscosity and stability), and to enhance pectin affinity towards other components within food matrices. Fiber content, viscosity, pectin solubility, DM, and monosaccharide composition were determined for both pasteurized and LTB+pasteurized blueberry purees. The results showed that neither the amount of fiber, nor the viscosity were affected by LTB, indicating that this treatment did not result in any significant pectin depolymerization and degradation. LTB caused a decrease both in pectin DM from 58-67% to 45-47% and in the amount of water-soluble pectin fraction, the latter remaining the major fraction of total pectin at 52 to 57%. LTB is a simple and mild process to produce blueberry purees with mostly soluble and low-methylated pectin in order to extend functionality and opportunities for interactions with other food ingredients.

Keywords

Blueberry; Fiber; Pectin; Low-temperature blanching; Degree of methylation.

Practical Application

Naturally structured and functional food products may be designed using a thermal process on raw plant material. In this study, the application of a low-temperature blanching modified *in situ* pectin structure in blueberry purees. These modifications may facilitate puree incorporation among other ingredients, in order to develop new functional foods.

1. Introduction

Native from North America, highbush blueberry (*Vaccinium corymbosum*) is a very popular fruit in Canada, which is the world's second largest producer of blueberry, with 163,000 metric tonnes produced in 2014 (FCC 2016). Blueberry is very rich in phenolic compounds and presents a high antioxidant activity (Moyer and others 2002), making it very appealing to health-conscious consumers. Moreover, blueberry contains a substantial amount of dietary fiber (DF >2%) (USDA 2016). Blueberry purees are thus natural and healthy food ingredients which can be incorporated into high DF functional foods.

Fruit DF are polysaccharides from plant cell walls that consists of cellulose, hemicellulose and pectin. Pectin is a heterogeneous polysaccharide, mainly composed of galacturonic acid (GalA). Its structure and properties are affected by food processing, especially heat treatments such as pasteurization and blanching that cause partial pectin depolymerization through β-elimination and acid hydrolysis (Sila and others 2009; Van Buggenhout and others 2009). Pectin depolymerization is pH and temperature-dependent, and typically results in the softening of plant tissues and a loss in viscosity. Heat treatments also affect the pectin degree of methylation (DM) which corresponds to the percentage of GalA that is methyl-esterified at C-6. DM is an indicator of the global charge density of pectin, and it governs its functional properties, especially its gelling mode. Charge density is a significant characteristic for interactions with other charged molecules like proteins or divalent ions, in particular calcium. Pectin DM varies depending on its source (plant type and species), as well as during ripening, post-harvest storage and processing (Sila and others 2009). Upon plant growth, the DM decreases as a result of pectin methyl-esterase (PME) activity, an endogenous enzyme naturally found in plant tissues, that hydrolyzes methyl ester groups on GalA. PME activity is temperature-dependent: a rise in temperature increases PME activity, resulting in lower DM.

PME activity is optimal between 50-70°C and is inhibited at higher temperatures (Duvetter and others 2009). Several works performed on vegetables and reviewed by Christiaens and others (2014), have shown that a low-temperature blanching (LTB), typically 10-60 min at 50-70°C, is an efficient treatment to reduce tissue softening during subsequent high thermal processing. Indeed, such a blanching enhances endogenous PME activity, resulting in pectin de-methylation. The free carboxylic groups on GalA thus produced, indeed promote pectin ability to interact with divalent ions like calcium, resulting in lower pectin solubility and increased intercellular adhesion. Pectin seems to behave differently between fruits and vegetables, as well as the fruit type. Bengtsson and others (2011) applied a LTB on apple suspensions and observed a decrease of the soluble pectin content. However, neither the pectin chemical structure nor the DM were characterized. After an exogenous PME addition to strawberry samples, Fraeye and others (2009) reported a drop in DM without any loss of pectin solubility. Besides, Fraeye and others (2007b) obtained a very different DM after a prolonged enzymatic de-methylation depending on fruit type: DM of apple pectin was reduced from a value of 79% to 7%, whereas that of strawberry pectin reached 32%, from an initial value of 94%.

Heat processes are a relatively simple method to optimize pectin structure and properties, especially its solubility. To our best knowledge, the effect of heat treatments on blueberry fiber composition and pectin structure has never been reported. The objective of this work was to use heat treatments currently applied in the food industry to modulate blueberry pectin structure. We hypothesized that a LTB could activate PME activity and thus decrease the pectin DM. For this purpose, composition of different blueberry cultivars was characterized, and the impact of both heat treatments on fiber content and viscosity of blueberry purees, as well as on pectin solubility and structure, was investigated.

91 **2. Materials and methods**

92 *2.1. Chemicals*

93 MeOH (certified ACS, Fisher Chemical, Fair Lawn, NJ, USA), 3-phenylphenol (Sigma-Aldrich
94 Inc., Milwaukee, WI, USA), Na_2CO_3 (Fisher Science Education, Rochester, NY, USA), $NaBH_4$
95 (Fluka, St-Louis, MO, USA), CDTA (*trans*-1,2-diaminocyclohexane-*N,N,N',N'*-tetraacetic acid
96 monohydrate, Alfa Aesar, Ward Hill, MA, USA), potassium acetate (EMD Chemicals Inc.,
97 Gibbstown, NJ, USA), and deuterated MeOH (d_3-MeOH, CDN Isotopes, Pointe-Claire, QC,
98 Canada) were acquired.

99 *2.2. Plant material*

100 Highbush blueberries (*Vaccinium corymbosum*) were obtained from Agridor (Beaumont, QC,
101 Canada). Six cultivars ("Blueray", "Duke", "Northland", "Patriot", "Polaris", and "Reka") were
102 harvested at their physiological maturity (July/August 2014), frozen at -18°C, vacuum-packed,
103 and stored at -30°C until analysis (April 2015). After the characterization of these six cultivars,
104 two were selected, Patriot (Pa) and Polaris (Po), to study the effects of heat treatments. They
105 were harvested from the same location (July/August 2015), frozen at -30°C, vacuum-packed,
106 and stored at -30°C until being pureed and heat treated (September/October 2015).

107 *2.3. Characterization of purees from six blueberry cultivars*

108 *2.3.1. Composition and physicochemical characterization*

109 Blueberries (200 g) from each cultivar were thawed at 4°C overnight then mixed for 1 min
110 using a kitchen blender (Magic Bullet, Homeland Housewares, Los Angeles, CA, USA) to
111 obtain homogeneous purees. The pH (Symphony SB20 pH-meter, VWR, Radnor, PA, USA)
112 and the moisture content (72°C for 24 h) were determined (Silva and others 2005). Titratable
113 acidity (method 942.15), soluble solids (932.12), ash (525°C overnight, 940.26), protein
114 (Kjeldahl, conversion factor of 6.25), lipid (Soxhlet extraction for 8 h, 945.16), and dietary

115 fiber (991.43) analyses were made according to AOAC official methods (AOAC 2012). The
116 ratio SS/TA was calculated as an indicator of overall sweetness (Retamales and Hancock
117 2012). Dietary fiber content was analyzed using a commercial assay kit (K-TDFR kit,
118 Megazyme International, Bray, Co. Wicklow, Ireland). Briefly, samples were enzymatically
119 digested to remove starch and proteins. Then, insoluble dietary fiber (IDF) and soluble fiber
120 (SDF) were obtained after filtration, and after precipitation with EtOH, respectively. Proteins
121 (Kjeldahl) and ashes (500°C for 5 h) were subtracted from each residue to obtain the final IDF
122 or SDF quantities. Total dietary fiber (TDF) was the sum of IDF and SDF. All analyses were
123 conducted at least in duplicate.

124 *2.3.2. Extraction of alcohol-insoluble residue (AIR)*

125 Cell wall material was isolated as AIR as described by Deng and others (2014), with slight
126 modifications. Blueberry puree (25 g) was stirred in 95% EtOH (150 mL) to extract low
127 molecular weight solutes, and boiled under reflux with continuous stirring for 30 min to
128 inactivate endogenous enzymes. The suspension was cooled to 23°C then filtered (GF/D,
129 Whatman, Buckinghamshire, UK). The residue was sequentially washed with 85% EtOH (3 x
130 150 mL), chloroform/MeOH (1:1 v/v; 3 x 250 mL), and acetone (3 x 250 mL), yielding the
131 crude cell wall extract (AIR). AIR was dried overnight at 37°C in an air oven, weighed and
132 expressed as g per 100 g of dry weight of fruit. AIR was ground in liquid nitrogen using a
133 mortar and pestle and stored at -18°C until analysis.

134 *2.3.3. Pectin content*

135 The pectin content in AIR was estimated as galacturonic acid content (GalA), the main
136 constituent of pectin, according to Melton and Smith (2001). AIR was hydrolyzed in duplicate
137 in concentrated sulfuric acid, followed by a colorimetric assay with 3-phenylphenol reagent, in

138 order to reduce interferences from neutral sugars. Absorbance was measured at 525 nm against
139 a standard curve of galacturonic acid (75-180 µg.mL^{-1}).

140 *2.3.4. Degree of methylation*

141 The degree of methylation (DM) of pectin was calculated for each AIR sample as the molar
142 ratio of methanol (MeOH) to GalA. MeOH was determined after saponification using
143 headspace gas chromatography-mass spectrometry (GC-MS) based on the method described by
144 Kosmala and others (2010). Samples (10 mg, in triplicate) were suspended in deionized water
145 (0.5 mL) and saponified with NaOH (0.5 M; 1 mL) for at least 1 h at 23°C. d_3-MeOH (10
146 µmol.mL^{-1}; 1 mL) was used as internal standard. A calibration curve was prepared with 0.5 mL
147 of deionized water, 1 mL of MeOH (0.5-10 µmol.mL^{-1}) and 1 mL of d_3-MeOH (10 µmol.mL^{-1}).
148 Analysis was performed by GC-MS (Hewlett-Packard model 6890 Series II gas chromatograph
149 attached to an Agilent model 5973N selective quadrupole mass detector; Palo Alto, CA) under
150 an ionization voltage of 70 eV at 230˚C, and equipped with an Agilent ChemStation software.
151 Samples contained in sealed vials were heat treated (70°C for 15 min) in order to vaporize the
152 MeOH released after saponification treatment of pectin. The headspace vapors (1 mL) were
153 then injected, in split mode (2:1), using a headspace system (Agilent Technologies, model
154 G1888 network headspace sampler), at 250˚C. GLC separation was performed on a DB-wax
155 capillary column (Agilent Technologies, 60 m × 0.25 mm i.d., 0.25 µm film thickness) with a
156 specific temperature program (40˚C for 8 min then to 250˚C at 30˚C.min^{-1}). He was used as the
157 carrier gas under constant flow (1 mL.min^{-1}). Data were collected using selected ions (m/z 29;
158 30; 31; 33; 35). The plot of the peak area ratio (normal to deuterated forms) for ion pairs (m/z
159 $31/35_d$) over a range of concentration ratios (MeOH/d_3-MeOH) allowed determination of
160 MeOH concentrations in the samples. The ion at m/z 35_d is the molecular ion for d_3-MeOH.

161 The ion at m/z 31 was selected for MeOH instead of the molecular ion at m/z 32 in order to
162 distinguish it from the molecular ion of ambient dioxygen.

163 *2.4. Heat treatments and analysis*

164 A schematic overview of the experimental setup is given in Figure. 1.

165 *2.4.1. Heat treatments*

166 Patriot and Polaris blueberry cultivars were thawed (4°C overnight) then homogeneous purees
167 were obtained as described previously (section *2.3.1*), except that they were mixed for 2 min.
168 For each of the following two treatments, puree samples (320 g) were heated in semi-sealed
169 erlenmeyer flasks (500 mL) in which the temperature was monitored by inserting a
170 thermocouple (VWR, Radnor, PA, USA). The first treatment (P) corresponded to a
171 pasteurization to inactivate endogenous enzymes (90-95°C for 5 min). Pasteurized samples
172 were used as reference. The second treatment corresponded to a low-temperature blanching
173 followed by a pasteurization (LTB+P): samples were maintained at 60°C for 1 h in order to
174 favor PME activity and then pasteurized to stop it (90-95°C for 5 min). All puree samples were
175 stirred during treatment, then cooled to 20°C in ice bath. The analyses described in sections
176 *2.4.2* to *2.4.7* were performed.

177 *2.4.2. Fiber characterization*

178 Fibers were characterized as total, insoluble, and soluble fiber, as described in section *2.3.1*,
179 except that the time-consuming filtration, performed after enzymatic digestion, was replaced by
180 a centrifugation (5,000 g x 15 min at 23°C). Moreover, ashes content was not measured
181 because values previously determined were negligible or zero. AIR was extracted from each
182 puree as described in section *2.3.2* with slight modifications. An aliquot of puree (100 g) was
183 stirred in 95% EtOH (500 mL) but the suspension was not boiled because enzymes had already
184 been inactivated by previous pasteurization treatments. After filtration, the residue was

185 sequentially washed with 85% EtOH (4 x 250 mL) and acetone (4 x 200 mL). The step with
186 chloroform/MeOH was not performed in order to ensure the absence of exogenous methanol in
187 AIR, which could otherwise affect the measured DM values. Pectin content was estimated as
188 GalA measured from AIR, as described in section *2.3.3.* All the results were expressed as g/100
189 g of puree, on a dry weight basis. The moisture content was determined by drying 3 g of puree
190 (102°C for 18 h).

191 *2.4.3. Viscosity*

192 Puree viscosity was measured in duplicate at 20°C using a rheometer (ARES G2, TA
193 Instruments, New Castle, DE, USA), equipped with a four-blade vane geometry (diameter
194 27.637 mm, height 41.507 mm, gap 5.849 mm) under steady state measurements. A pre-shear
195 of 50 s^{-1} was applied for 1 min then the shear rate was increased from 1 to 200 s^{-1}. The
196 Herschel-Bulkley model was used to describe the flow behavior (R^2>0.999).

197 *2.4.4. Particle size*

198 The mean particle size of the purees was determined using a laser diffraction analyzer (Master
199 Sizer 3000, Malvern Instruments Ltd., Worcestershire, UK) from diluted samples in deionized
200 water, stirred at 2,500 rpm.

201 *2.4.5. Extraction of pectin-rich fractions*

202 In order to extract pectin-rich fractions and minimize pectin degradation, AIR was fractionated
203 according to a protocol adapted from Vicente and others (2007). An aliquot of AIR (1.5 g) was
204 suspended in deionized water (130 mL) and stirred for 16 h at 23°C. The suspension was
205 centrifuged (10,000 g x 10 min) at 23°C and the pellet was washed twice with water (2 x 10
206 mL). The supernatant and its water washings were pooled, filtered (Whatman GF/C), and were
207 designated as the water-soluble pectin (WSP) fraction. The residue was mixed and stirred with
208 130 mL of 50 mM CDTA in 50 mM potassium acetate buffer, at pH 6.5, for 8 h at 23°C. The

209 mixture was then centrifuged and washed as described above and the combined filtrate was
210 designated as the chelator-soluble pectin (CSP) fraction. The residue was suspended and stirred
211 in 130 mL of 50 mM Na_2CO_3 in 20 mM $NaBH_4$ solution for 16 h at 4°C then for 3 h at 23°C.
212 The suspension was centrifuged and washed as above. The combined filtrate was designated as
213 the sodium carbonate-soluble pectin (NSP) fraction. The pH of the NSP fraction was adjusted
214 to pH 6-6.5 with glacial acetic acid. The CSP fraction was dialyzed against 0.1 M NaCl for 24
215 h at 4°C. The three fractions were then dialyzed against deionized water for 3 days at 4°C to
216 remove salt residues (M_w 15 kDa cut-off, Spectra/Por® 6, Spectrum Laboratory Inc., Rancho
217 Dominguez, CA, USA). One serial extraction was performed by AIR sample. GalA content
218 was estimated from each fraction as described in section *2.3.3*. Degree of methylation was
219 determined for the AIR, WSP and CSP fractions, as described in section *2.3.4*.

220 *2.4.6. Monosaccharide composition*

221 Neutral monosaccharides and galacturonic acid were identified as trimethylsilyl derivatives
222 after acidic methanolysis of the polysaccharides from each pectin-rich fractions and subsequent
223 GC analysis (Montreuil and others 1986; Kamerling and others 1975). Methanolysis was
224 performed in 3 M MeOH-HCl for 4 h at 100°C and the methyl glycosides were then converted
225 to the corresponding per-*O*-trimethylsilylated derivatives. Separation and quantification were
226 achieved using a GC 2010 Plus System (Shimadzu Corporation, Mandel Scientific Co. Inc.
227 Guelph, ON, Canada) equipped with an FID detector and a CP-Sil-5CB fused silica column
228 (Chrompack, Varian, Mississauga, ON, Canada; 60 m × 0.32 mm i.d., 0.25 µm film thickness)
229 in a split/splitless mode using He at a flow of 1.5 $mL.min^{-1}$ as the carrier gas. Monosaccharides
230 were identified according to their retention times and quantified using an internal standard
231 method involving *myo*-inositol. All analyses were performed in triplicates.

232 *2.4.7. Total phenolic content*

233 Total phenolic content was determined by the Folin-Ciocalteu method (Singleton and Rossi
234 1965) adapted for microplate measurement, after extractions with 80% MeOH (2 x 20 mL, 20
235 min at 37°C) on lyophilized puree samples (0.2 g). The results are expressed as g of gallic acid
236 equivalents/100 g of dried blueberry puree.

237 *2.5. Statistical analysis*

238 Statistical analysis was conducted with SAS® 9.3 (SAS Institute Inc., Cary, NC, USA.).
239 Analysis of variance (ANOVA) using the GLM procedure was executed. For the
240 characterization of cultivars, all dependent variables were analyzed using a one-way ANOVA
241 and significance of the differences between the cultivars were determined using Tukey's
242 multiple comparisons test (α=0.05). For heat-treatments, twelve purees were produced (2
243 cultivars x 2 heat treatments x 3 repetitions) with a complete randomized design. Two-way
244 ANOVAs were used according to a factorial experiment 2x2, considering the effects "cultivar"
245 (Cult) and "heat treatment" (HT). For significant interaction (P≤0.05), the means of the
246 treatments and cultivars were individually compared with the Tukey's multiple comparisons
247 test (α=0.05). For significant individual effect(s), the two means per cultivars or per heat
248 treatments were compared with the same test and represented by bars with letters.

249 **3. Results and discussion**

250 *3.1. Characterization of purees from six blueberry cultivars*

251 The physicochemical characteristics and the composition of the six blueberry cultivars of 2014
252 are given in Table 1. pH values ranged from 2.8 (Patriot) to 3.3 (Blueray). The SS/TA ratio
253 (sugar/acid ratio) varied between 13.3 (Duke) and 19.6 (Blueray) meaning that Duke was the
254 most acidic cultivar and Blueray the sweetest. Polaris tended to have the highest TDF content
255 (19.0%), whereas Patriot had the lowest (13.6%). These values are in agreement with other

256 studies on highbush blueberry: the USDA value is 15.2% (USDA 2016), and by summing
257 lignin, cellulose, hemicellulose and pectin, Silva and others (2005) measured 13.0% of dietary
258 fiber. TDF content may also be estimated by measuring the AIR content. AIR is mainly
259 composed of cell wall polysaccharides (fiber) with low amounts of co-precipitated soluble
260 proteins (about 3-5%), lignin, salts of organic acids, and phenolic compounds. The AIR
261 contents, similar on fresh weight to that of Vicente and others (2007), confirmed the highest
262 fiber content of Polaris. For the six cultivars, dietary fiber consisted of IDF and SDF in a ratio
263 of approximately of 4:1, except Blueray (2.5:1). IDF is mainly composed of cellulose,
264 hemicellulose, and a minor part of insoluble pectin, whereas SDF consists predominantly of
265 pectin. Galacturonic acid (GalA) content was used to estimate pectin content since GalA is the
266 main monosaccharide of pectin. Results showed the highest GalA content for Polaris (7.4%),
267 while Reka had the lowest (4.7%). Values for the other four cultivars were not significantly
268 different, with values ranging from 5.1 to 5.9%. Values for GalA contents are in the range of
269 the reported value of 3.4% in highbush blueberry (Silva and others 2005). Pectin DM values
270 ranged from 22%, for Polaris, to 41%, for Blueray: all cultivars had therefore a low-methylated
271 pectin (DM<50%). Based on these results, two cultivars were selected for the subsequent work
272 with heat treatments. Polaris was selected for its higher dietary fiber and pectin (GalA)
273 contents, while Patriot was chosen since it is the most popular and the most produced highbush
274 blueberry cultivar in the province of Quebec (AAC 2012), and is representative of the other
275 cultivars for the fiber and pectin contents.
276 In 2015, the selected two cultivars, Polaris and Patriot, were harvested and their
277 physicochemical characteristics and composition were determined (Supplementary Table 1).
278 Globally, both cultivars were significantly less acidic and sweeter compared to 2014, with
279 higher pH values of 3.1 and 3.6 for Patriot and Polaris, respectively. Polaris still had higher

280 contents in dietary fiber, in AIR, and in pectin (GalA) than Patriot and these values were not
281 significantly different than those from the previous year. However, the TDF and IDF contents
282 of Patriot in 2015 significantly differed from that of 2014; IDF content was lower in 2015, at
283 9.0%, compared to a value of 10.9% in 2014, resulting in a lower TDF content (11.8% in 2015
284 vs. 13.6% in 2014). GalA content were also significantly lower in 2015 (3.7% in 2015 vs. 5.9%
285 in 2014). Various factors such as growing conditions, climate and storage time, may cause
286 variations in the physicochemical characteristics from season to season (Retamales and
287 Hancock 2012). It is likely that such parameters also have affected fiber composition from one
288 year to the next. DM values of both cultivars were similar and significantly higher than values
289 in 2014 (44 and 41% in 2015 vs. 30 and 22% in 2014 for Patriot and Polaris respectively).
290 Cultivars harvested in 2015 were frozen at -30°C and analyzed a few weeks after their freezing,
291 whereas cultivars harvested in 2014 were frozen and stored at -18°C for 4 months before being
292 transferred at -30°C for 2 months then analyzed. It is likely that pectin was partially
293 de-methylated during the slower freezing process and over storage time, since PME was likely
294 not completely inactivated by the freezing (Van Buggenhout and others 2009). Reports on DM
295 of blueberry pectin are scarce but Lin and others (2016) also measured DM values lower than
296 50%.

297 *3.2. Influence of heat treatment on the purees*

298 The present results showed that only the cultivar had a significant effect on the fiber amount
299 during the heat treatment (Table 2). The Polaris purees had higher AIR, fiber and pectin (GalA)
300 contents than the Patriot purees, as observed in the previous characterization realized on the
301 raw purees (Table 1). There was no significant effect of LTB+P on the fiber and GalA amounts,
302 compared to P. Yet, according to the literature, pectin is the fiber that is the most affected by
303 heat treatments. The pH of blueberry purees and the moderated temperature (60°C) of the

304 blanching appear to have prevented any fiber degradation. Indeed, the pH of blueberry purees
305 was 3.1 and 3.6 for Patriot and Polaris cultivars, respectively (Supplementary Table 1). These
306 values are in the pH range where pectin is generally very stable because this is near its pKa
307 value (2.9-3.3). Furthermore, these pH values were too acidic to induce substantial β-
308 elimination and not acidic enough to allow fast acid hydrolysis, the two main pectin
309 degradation mechanisms leading to pectin depolymerization and solubilization (Fraeye and
310 others 2009; Diaz and others 2007) and an increase of SDF and TDF (Colin-Henrion and others
311 2009). Indeed, β-elimination occurs mainly at neutral or weakly acidic pH (pH>4.5) and for
312 temperature superior to 85°C, whereas acid hydrolysis is promoted only at pH<3 (Sila and
313 others 2009; Fraeye and others 2007a). On the other hand, the presence of polygalacturonase
314 has been found in blueberry (Deng and others 2014). This enzyme, which hydrolyzes
315 glycosidic links between GalA molecules of pectin, is inactivated by pasteurization, but
316 similarly to PME, it is more active at temperatures around 50-60°C (Duvetter and others 2009).
317 Its activity lowered the pectin amount in tomato paste samples during the concentration process
318 performed at around 65°C (Anthon and others 2008). In blueberry purees, the
319 polygalacturonase activity was likely minimal or inhibited at 60°C, since no decrease in pectin
320 content was perceived with LTB+P treatment when compared to P treatment. Furthermore, the
321 other endogenous enzymes affecting pectin structure, such as β-galactosidase and α-
322 arabinofuranosidase, are thermo-labile and were inactivated at 60°C in tomato (Houben and
323 others 2013).
324 The viscosity of the heat-treated purees confirmed the limited effect of heat-treatment on fiber
325 content and the significant effect of cultivar (Supplementary Table 2). For both Polaris and
326 Patriot cultivars, the viscosity was not affected by LTB+P, reflecting the absence of significant
327 pectin depolymerization. Moreover, regardless of the heat treatment, the Polaris purees had a

328 higher viscosity (3.2 ± 0.2 Pa.s) than the Patriot purees (1.1 ± 0.1 Pa.s) that can be attributed to
329 a higher fiber content (Table 2). Indeed, fiber amount and molecular weight are known for their
330 capacity to increase the viscosity but the latter was not tested in this study. The viscosity was
331 not affected by the mean particle size of purees since it was similar after both heat treatments
332 (Supplementary Table 2).

333 Because of beneficial health effects of phenolic compounds, especially for their antioxidant
334 capacity, the Folin assay was performed to get a global measure of total phenolic content
335 (Table 2). Values for total phenolic contents were similar after both heat treatments and were in
336 the range of reported values for unheated blueberry (Moyer and others 2002), suggesting that
337 the heat treatments performed in the present study did not degrade blueberry phenolic
338 compounds. Nonetheless, beyond the phenolic amounts, heat treatments may affect the
339 interactions between phenolic compounds and proteins or polysaccharides, which can modify
340 their antioxidant activity (Le Bourvellec and Renard 2012). These interactions will be assessed
341 in an upcoming study.

342 *3.3. Influence of heat treatment on the pectin structure and solubility*

343 *3.3.1. Degree of methylation of pectin in AIR*

344 DM of pectin found in AIR is presented in Figure 2. LTB+P treatment had a significant effect
345 on DM of pectin in AIR. For both cultivars, DM decreased with LTB+P treatment in
346 comparison to P treatment: the high-methylated pectin (DM>50%) became low-methylated
347 pectin (DM<50%). This decrease likely reflected the enzymatic de-methylation. Indeed, PME
348 activity is enhanced at temperatures ranging between 50 and 65°C (Duvetter et al. 2009). With
349 high temperature, pectin can also be de-methylated by chemical hydrolysis. However, the
350 extent of the reaction is significant only under alkaline or near neutral conditions (Sila and
351 others 2009). Therefore, in the present study, chemical de-methylation of the blueberry purees

352 was unlikely or limited because of their low pH values of 3.1-3.6 (Supplementary Table 1)
353 (Diaz and others 2007).

354 For the raw purees, DM of both Patriot and Polaris cultivars were higher (>50%) after P
355 treatment than DM determined for the cultivar characterization (<50%). Yet, a short and high
356 heat treatment like P is too fast to significantly change the DM (Sila and others 2005), or it may
357 result in slightly decreased DM because of a slight PME activity occurring before reaching
358 90°C (Christiaens and others 2012a; Sila and others 2006). In the present study, the AIR
359 extraction procedure was not exactly the same for the cultivar characterization and for the heat-
360 treated puree analyzes. In the first case, blueberry purees were mixed with boiling EtOH in
361 order to inactivate the enzymes, which could result in a decrease of DM before the inactivation
362 temperature was reached throughout the purees.

363 *3.3.2. Pectin solubility and distribution of the pectin-rich fractions*

364 To investigate the pectin modifications that may have been induced by the heat treatments,
365 pectin found in AIR was successively extracted into three pectin-rich fractions, depending on
366 the pectin solubility. WSP fraction contains pectin that is weakly bound to cell walls, CSP
367 fraction contains pectin with ionic cross-links, and NSP fraction contains pectin that is strongly
368 bound to cell walls by covalent ester linkages. The relative amounts of each pectin-rich
369 fractions in the different purees are presented in Figure 3. For both cultivars, most of the pectin
370 was water-soluble, the WSP fraction accounting for over 50% of total pectin, in agreement with
371 reported results for highbush blueberry (Vicente and others 2007). Polaris purees contained a
372 higher and lower proportions of pectin from NSP and CSP fractions, respectively, compared to
373 Patriot purees. LTB+P treatment had a significant effect on the WSP amount compared to P
374 treatment. Indeed, WSP amount decreased with LTB+P for both cultivars, whereas CSP
375 amount tended to increase. No significant effect on NSP amount was observed due to LTB+P

376 treatment. A reduction in content of water-soluble pectin (WSP) and a rise in insoluble pectin
377 (CSP and NSP) were also reported after a similar heat treatment (60°C/40 min) for carrot (Sila
378 and others 2006) and broccoli (Christiaens and others 2011; Christiaens and others 2012a). The
379 conversion from WSP to CSP was attributed to a de-methylation of water-soluble pectin that
380 increased pectin overall charge and its ability to form ionic bounds, especially with endogenous
381 calcium. Nevertheless, for both cultivars, the WSP fraction remained the major fraction (>50%)
382 even after the low-temperature blanching (LTB+P), unlike the reported data for vegetables for
383 which the WSP fraction became lower than that of the combined insoluble fractions
384 (Christiaens and others 2012a; Christiaens and others 2012b; Christiaens and others 2011; Sila
385 and others 2006).
386 In contrast to vegetables, the changes in the proportion of pectin-rich fractions after a treatment
387 enhancing PME activity were not very pronounced. Fraeye and others (2009) also reported no
388 significant pectin modifications in strawberry samples subjected to the activity of an exogenous
389 PME. According to these authors, these differences compared to vegetables might be explained
390 by a lower ratio of endogenous calcium to pectin level that could limit the ionic cross-linkage
391 formation, despite a lowered DM. Indeed, they observed a noticeable decrease of WSP amount
392 and an increase of CSP amount after calcium addition during the activation of an exogenous
393 PME, whereas no clear changes in relative pectin-rich fractions were noticed without calcium
394 addition (Fraeye and others 2009). Like strawberry, blueberry also has a very low content in
395 calcium: 0.06 mg/g (USDA 2016), while fresh carrot contains 0.64 mg/g (Sila and others 2005)
396 and raw broccoli 0.32 mg/g (Christiaens and others 2011). In addition to endogenous calcium
397 content, the cross-linkage of pectin with divalent ions, particularly calcium, also depends on
398 calcium location and pectin structure (Christiaens and others 2014), which are highly source-
399 dependent (Houben and others 2011). For instance, a too low linearity in pectin structure might

400 hinder the ionic cross-linkage (Houben and others 2011). Furthermore, beyond the calcium
401 amount, PME activity also plays a role in pectin cross-linkage related to firmness of fruits and
402 vegetables (Javeri and others 1991; Degraeve and others 2003). For example, fruits usually
403 have a lower pH than vegetables, which may reduce PME activity (Duvetter and others 2009),
404 but this was not measured in this study. All this confirms the importance and relevance of
405 studying many types of fruits and vegetables, even several cultivars.

406 *3.3.3. Monosaccharide composition of the pectin-rich fractions*

407 Monosaccharide composition of each pectin-rich fraction was determined to better understand
408 pectin behavior in relation to its solubility and DM. Polysaccharide methanolysis followed by
409 trimethylsilyl derivatization and GC analysis is a fast and convenient method for pectin
410 characterization, because it degrades monosaccharides to a lower extent than acid hydrolysis,
411 and allows for the quantification of both neutral monosaccharides and GalA (Sundberg, 1996).
412 Pectin is mainly composed of linear chains of GalA (55-90% in this study, Supplementary
413 Table 3), known as homogalacturonan (HG). Rhamnose (Rha), accounting for about around
414 1%, is occasionally inserted into the chain forming "hairy" regions called rhamnogalacturonan
415 (RG). The latter is branched with neutral monosaccharide side chains composed mainly of
416 arabinose (Ara) (3-16%) and galactose (Gal) (4-23%). Similar proportions of arabinose and
417 galactose were reported for apple and strawberry pectins (Fraeye and others 2007b).

418 To better visualize the structure of each pectin-rich fraction, the ratio of GalA to neutral
419 monosaccharides was calculated as a measure of the pectin linearity (Fig. 4). A high ratio
420 implies large amounts of HG and consequently low amounts of branched RG regions. For both
421 Patriot and Polaris cultivars, WSP fraction and NSP fraction showed the lowest ratios of GalA
422 to neutral monosaccharides, whereas CSP fraction presented the highest ratio, indicating that
423 pectin in the CSP fraction was more linear (higher in HG). Patriot purees contained more

424 arabinose and galactose than Polaris in WSP and CSP fractions, resulting in lower ratios.
425 Patriot pectin was therefore globally more branched than Polaris pectin.
426 Compared with the P treatment, the LTB+P treatment had no significant effect on the ratio of
427 GalA to neutral monosaccharides for both cultivars. Yet, Christiaens and others (2011) reported
428 a decrease of WSP ratio and an increase of CSP ratio from broccoli samples treated by a low-
429 temperature blanching compared to raw broccoli samples. This observation indicated that it
430 was mainly the HG-rich pectin that forms new ionic cross-links (CSP) following de-
431 methylation. However, as discussed in the previous section, blueberry contains less endogenous
432 calcium than broccoli. In addition, for all cultivars and heat treatments, our values of CSP ratio
433 were higher than for broccoli (5-10 vs. 2 for broccoli), meaning higher pectin linearity and
434 GalA proportion in blueberry. Therefore, most of endogenous calcium was likely already cross-
435 linked with the GalA molecules, leaving no or limited calcium to form new ionic cross-links
436 following the de-methylation. This would confirm that the available calcium amount has a role
437 on converting the water-soluble pectin into cross-linked pectin, as described in *3.3.2*. This
438 suggestion is also supported by the results of Christiaens and others (2011) who observed a
439 larger CSP ratio increase in the presence of added calcium. Therefore, the high pectin linearity
440 and the low endogenous calcium content of blueberry would allow for a reduction of pectin
441 DM without substantial loss the pectin solubility.

442 *3.3.4. Degree of methylation in the pectin-rich fractions*

443 Values of DM for pectin in WSP and CSP fractions is given in Figure 2. For both cultivars and
444 treatments, pectin found in WSP fraction was high-methylated with a DM ranging from 53 to
445 63%, whereas DM of pectin in CSP fraction was low-methylated, at 31 to 43%. DM of pectin
446 found in AIR, which consists of the three pectin-rich fractions, globally had intermediate
447 values. These observations are consistent with the literature data for other fruits and vegetables

448 (Christiaens and others 2012a; Fraeye and others 2009; Houben and others 2011; Sila and
449 others 2006). DM in NSP fraction could not be measured since the extraction of this fraction
450 occurred in alkaline conditions which alter ester linkages.
451 Globally, DM values of the pectin-rich fractions did not significantly differ between the heat
452 treatments. Christiaens and others (2012b) and Sila and others (2006) also noticed no changes
453 of DM in pectin-rich fractions from broccoli and carrot purees, respectively, after a low-
454 temperature blanching (equivalent to LTB+P) compared with a high-temperature blanching
455 (equivalent to P), despite a diminution of DM in AIR. As LTB+P modified pectin-rich fraction
456 proportion, the pectin in AIR globally consisted in less high-methylated pectin (WSP) and more
457 low-methylated pectin (CSP): this might explain the decrease of DM in AIR, although there
458 was no significant changes of DM in fractions.
459 As no studies had been reported on blueberry fiber, the combination time/temperature of LTB
460 was chosen according to the literature and preliminary tests. A temperature of 60°C is the most
461 used to blanch vegetables and to activate endogenous PME (Christiaens and others 2014).
462 Since PME activity also depends on the pH, it would be relevant to test other temperatures to
463 find optimal conditions for PME activation in blueberry purees. Indeed, as seen previously, the
464 pH of blueberry is lower than that of most vegetables. Moreover, reported LTB for vegetables
465 typically lasts between 10 and 60 min (Christiaens and others 2014). Pectin de-methylation
466 seemed to continue beyond 60 min, since our preliminary tests showed that the charge density
467 (Zeta-potential) of AIR samples decreased (60 min: -15.7 mV; 120 min: -17.3 mV; unpublished
468 data). However, a long time heat process is not convenient for the food industries and could
469 lead to the degradation of some bioactive molecules such as phenolic compounds. In addition, a
470 too low DM could result in a higher reduction in pectin solubility through the conversion into
471 CSP, as studied in the present work, and also through the depolymerization by the

polygalacturonase. Indeed, this enzyme shows increasing activity with low-methylated pectin (Duvetter and others 2009). Yet, in order to incorporate the purees into food products, soluble pectin is preferred because it contains more available free carboxylic groups enhancing its anionic nature and promoting interactions with other macromolecules wearing positive charges such as proteins. Interactions between pectin and proteins may provide useful functionalities for the development of food products, including rheological and textural properties (Turgeon and Laneuville 2009). For example, pectin interactions with proteins may protect them against thermal aggregation (Ibanoglu 2005) or may be used as a fat-replacer ingredient, in yoghurt for instance (Krzeminski and others 2014). Finally, with a LTB lasting 60 min, the pectin found in the AIR of both Polaris and Patriot cultivars reached a DM<50% that was sufficient to consider the pectin as low-methylated, while keeping most of the pectin soluble (high WSP). Compared to high-methylated pectin, low-methylated pectin carries more charges, through its free carboxylic groups, promoting even more the interactions with other ingredients, as explained previously, which could also help the development of food products.

4. Conclusion

The potential of a low-temperature blanching as a tool to modulate the pectin structure was investigated in blueberry. The present study showed that fiber content and composition of blueberry were cultivar dependent and were not affected by neither a pasteurization nor a low-temperature blanching followed by a pasteurization. Rheological properties depended also on the cultivar and remained constant between both heat treatments, as well as the monosaccharide composition of pectin. However, pectin DM and solubility were modulated when using a low-temperature blanching treatment before pasteurization. Such a treatment decreased DM values and the amount of the water-soluble pectin, possibly attributed to the enhanced PME activity. A LTB treatment is a relevant and simple process to generate a blueberry puree with a low-

496 methylated pectin, while keeping the pectin mostly soluble and preserving the fiber level and
497 the bioactive compounds (phenolic content). An upcoming study will investigate the impact of
498 LTB+P compared to unheated blueberry purees studying pectin modifications and interactions
499 with proteins. Indeed, these modifications may enhance the affinity of pectin towards other
500 ingredients in order to facilitate the formulation of new functional foods. Further investigations
501 of blueberry puree functionality will be conducted to provide more information in the food
502 engineering field and the development of new applications.

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507 **Author contributions**

508 SLT jointly conceived the study with LMC and LER. LMC performed the experiments,
509 interpreted the results, and drafted the manuscript. LER supervised the statistical analysis
510 design and data interpretation. LER, SLT, and PA revised the manuscript. All authors discussed
511 the results on the manuscript at all stages.

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Tables

Table 1. Physicochemical characteristics and composition (g/100 g dry weight) of highbush blueberry cultivars in 2014

	Blueray	**Duke**	**Northland**	**Patriot**	**Polaris**	**Reka**
Physicochemical characteristics						
pH	3.3 ± 0.0^{a}	3.1 ± 0.1^{b}	3.0 ± 0.0^{b}	2.8 ± 0.0^{c}	3.3 ± 0.1^{a}	3.1 ± 0.0^{b}
SS/TA	19.6 ± 0.1	13.3 ± 0.1	16.3 ± 0.1	13.4 ± 0.1	17.7 ± 0.1	15.6 ± 0.11
Composition						
Moisture	84.5 ± 0.0^{c}	85.8 ± 0.1^{b}	85.9 ± 0.1^{b}	87.3 ± 0.0^{a}	86.4 ± 0.2^{ab}	86.0 ± 0.8^{b}
Protein	2.2 ± 0.3^{b}	3.6 ± 0.1^{a}	2.4 ± 0.2^{b}	2.7 ± 0.2^{ab}	3.1 ± 0.4^{ab}	2.5 ± 0.1^{b}
Lipid	0.10 ± 0.03^{a}	0.16 ± 0.00^{a}	0.14 ± 0.02^{a}	0.12 ± 0.04^{a}	0.18 ± 0.00^{a}	0.16 ± 0.01^{a}
Ash	1.1 ± 0.0^{a}	1.1 ± 0.0^{ab}	1.0 ± 0.0^{c}	1.0 ± 0.0^{b}	1.1 ± 0.0^{a}	1.1 ± 0.0^{ab}
TDF	14.9 ± 0.1^{b}	16.0 ± 0.9^{ab}	15.6 ± 0.5^{ab}	13.6 ± 0.2^{b}	19.0 ± 2.0^{a}	16.0 ± 0.1^{ab}
IDF	10.6 ± 0.0^{b}	12.9 ± 1.2^{ab}	12.8 ± 0.4^{ab}	10.9 ± 0.3^{b}	15.5 ± 1.9^{a}	13.6 ± 0.3^{ab}
SDF	4.3 ± 0.1^{a}	3.1 ± 0.4^{bc}	2.8 ± 0.1^{bc}	2.7 ± 0.1^{bc}	3.6 ± 0.1^{ab}	2.4 ± 0.5^{c}
AIR	17.8 ± ND	18.5 ± ND	17.1 ± ND	16.9 ± 0.2	20.5 ± ND	17.7 ± ND
GalA[1]	5.9 ± 0.0^{ab}	5.3 ± 0.3^{ab}	5.1 ± 0.9^{ab}	5.9 ± 0.7^{ab}	7.4 ± 0.7^{a}	4.7 ± 0.9^{b}
DM (%)	41 ± 3^{a}	32 ± 4^{ab}	32 ± 5^{ab}	30 ± 5^{ab}	22 ± 2^{b}	31 ± 7^{ab}

Mean value ± standard deviation. Means within a line followed by the same letter (a–f) were not significantly different according to the Tukey's test (α = 0.05). SS/TA represents the sugar/acid ratio. TA: Titratable acidity (% citric acid). SS: Soluble solids (°Brix). TDF: Total dietary fiber. IDF: Insoluble dietary fiber. SDF: Soluble dietary fiber. AIR: Alcohol-insoluble residue. DM: Degree of methylation from AIR. ND: not determined. [1] Galacturonic acid from AIR, as an estimated of pectin content.

650 **Table 2.** AIR, fiber and pectin contents (g/100 g dry weight) and total phenolic content (g
651 gallic acid equivalent/100 g dry weight) of the heat-treated purees

Purees	AIR	TDF	IDF	SDF	GalA[1]	Total phenolic content
PaP	16.5 ± 0.7	13.2 ± 0.7	10.1 ± 0.4	3.1 ± 0.3	3.2 ± 0.5	1.39 ± 0.03
PaLTB+P	16.3 ± 1.0	12.5 ± 0.2	9.6 ± 0.3	2.8 ± 0.1	4.0 ± 0.7	1.31 ± 0.06
PoP	28.8 ± 0.8	21.8 ± 0.4	17.6 ± 0.5	4.2 ± 0.1	4.9 ± 0.2	1.36 ± 0.03
PoLTB+P	28.5 ± 0.7	21.3 ± 1.4	17.1 ± 1.3	4.3 ± 0.2	5.8 ± 1.2	1.32 ± 0.02
Cult	*	*	*	*	*	n.s
HT	n.s	n.s	n.s	n.s	n.s	n.s
Cult * HT	n.s	n.s	n.s	n.s	n.s	n.s

652 Mean value ± standard deviation. Results of the two-way ANOVAs (cultivar (Cult) and heat
653 treatment effects (HT), with interaction); n.s: not significant (P>0.05). Pa: Patriot. Po: Polaris.
654 P: pasteurization. LTB+P: low-temperature blanching + pasteurization. TDF: Total dietary
655 fiber. IDF: Insoluble dietary fiber. SDF: Soluble dietary fiber. AIR: Alcohol-insoluble residue.
656 [1] Galacturonic acid from AIR, as an estimated of pectin content.

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671 **Figures**

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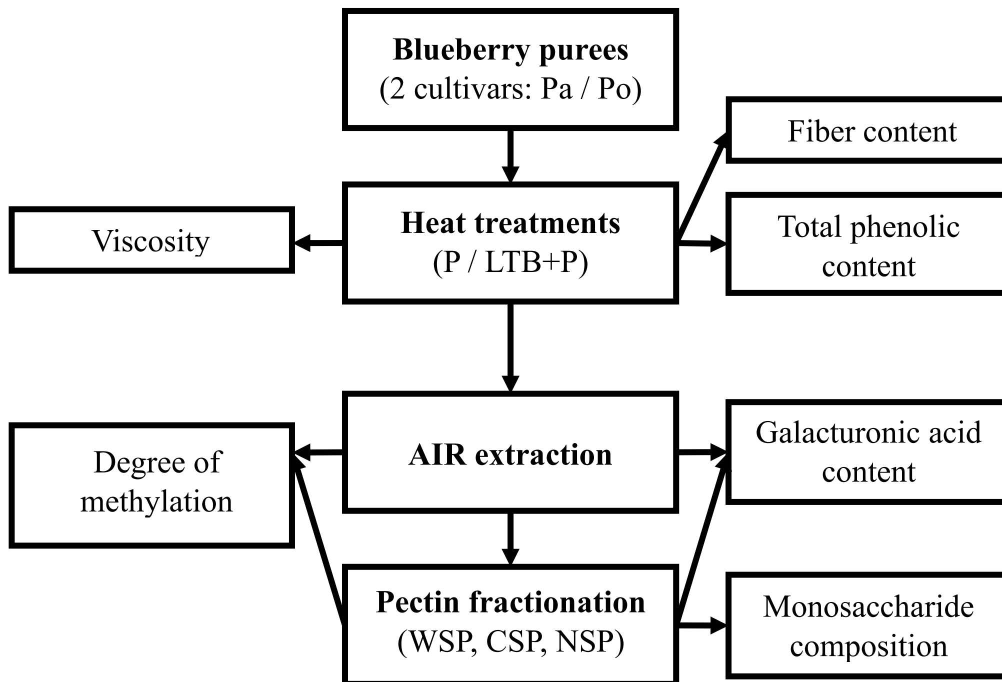

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683 **Figure 1. Schematic overview of the experimental setup**

684 Pa: Patriot; Po: Polaris; P: 90-95°C/5 min; LTB+P: 60°C/1 h + 90-95°C/5 min; AIR: alcohol-

685 insoluble residue; WSP: water-soluble pectin; CSP: chelator-soluble pectin; NSP: sodium

686 carbonate-soluble pectin.

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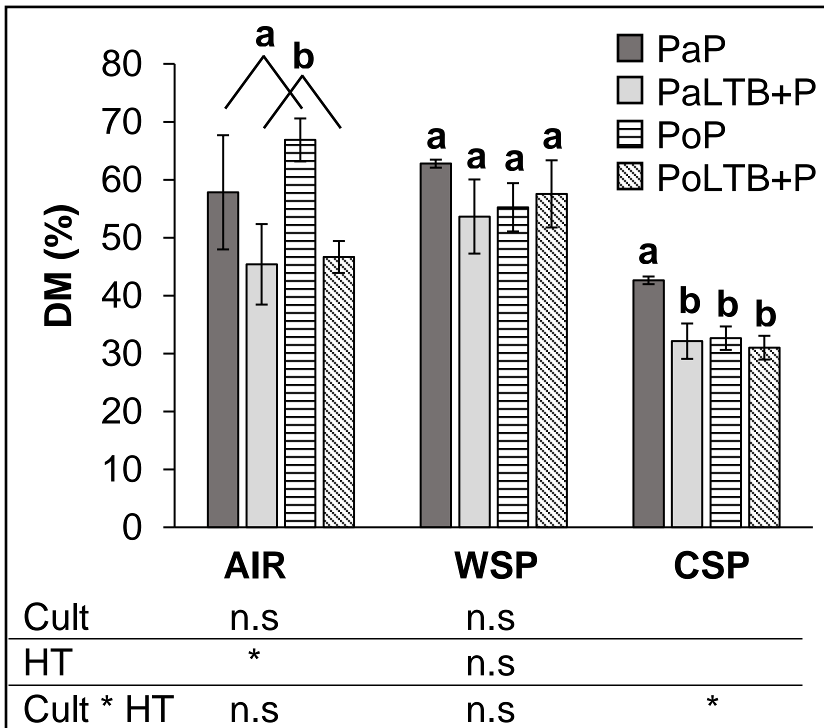
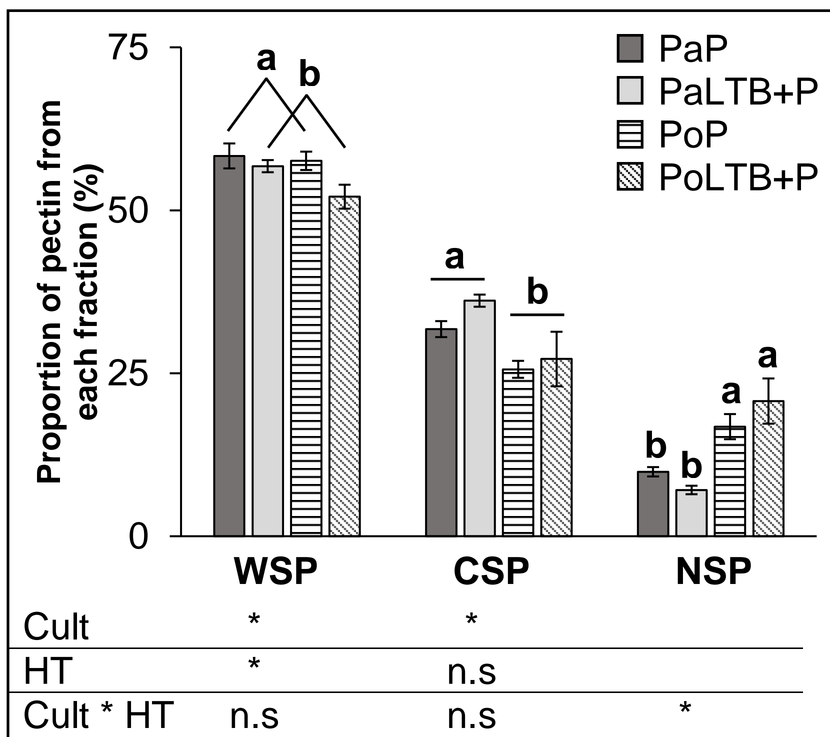


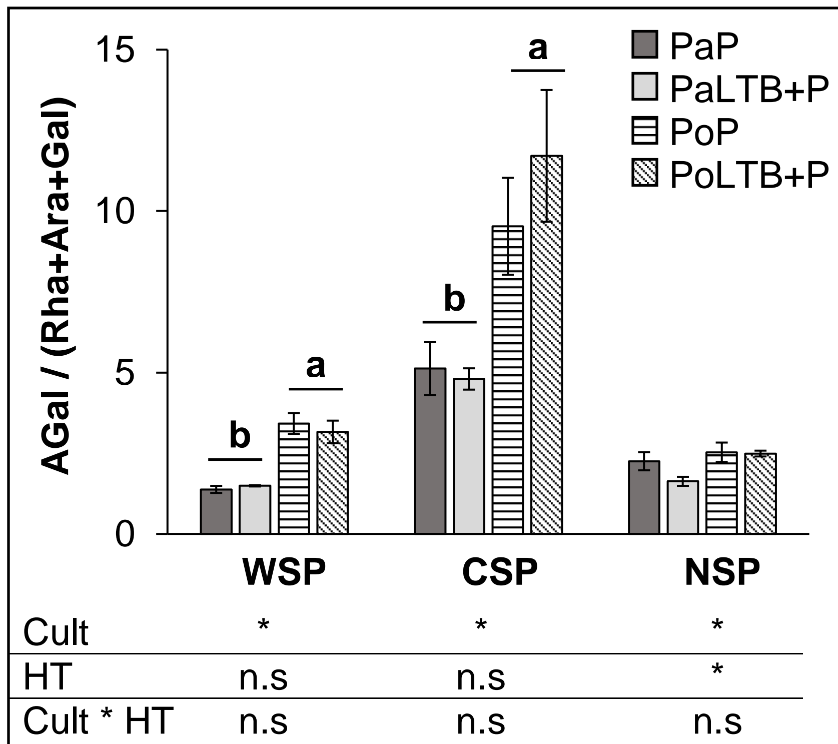
689 **Figure 2. Degree of methylation of AIR and the water- and chelator-soluble pectin**
690 **fractions (WSP and CSP) from the heat-treated purees**

691 Mean value ± standard deviation. Results of the two-way ANOVAs (cultivar (Cult) and heat
692 treatment effects (HT), with interaction); n.s: not significant (P>0.05). Means within a same
693 series followed by the same letter (a–b) were not significantly different according to Tukey's
694 test ($\alpha = 0.05$). For AIR, the mean of pasteurized cultivars (PaP + PoP) is significantly different
695 from the mean of blanched cultivars (PaLTB+P + PoLTB+P). Pa: Patriot. Po: Polaris. P:
696 pasteurization. LTB+P: low-temperature blanching + pasteurization. AIR: Alcohol-insoluble
697 residue.



698 **Figure 3. Relative amount of pectin-rich fractions**

699 Mean value ± standard deviation. GalA in the fraction/sum of the GalA amount of each fraction
700 x 100. For WSP, only the HT effect is represented: the mean of pasteurized cultivars (PaP +
701 PoP) is significantly different from the mean of blanched cultivars (PaLTB+P + PoLTB+P).
702 For CSP, the mean of Patriot cultivar (PaP + PaLTB+P) is significantly different from the mean
703 of Polaris cultivars (PoP + PoLTB+P). Pa: Patriot. Po: Polaris. P: pasteurization. LTB+P: low-
704 temperature blanching + pasteurization.



705 **Figure 4. Ratio of GalA to neutral monosaccharides in pectin-rich fractions**

706 Mean value ± standard deviation. All monosaccharides were measured as trimethylsilyl
707 derivatives after acidic methanolysis and subsequent GC analysis. For NSP, the mean of
708 pasteurized cultivars is significantly different from the mean of blanched cultivars, and the
709 mean of Patriot cultivar (PaP + PaLTB+P) is significantly different from the mean of Polaris
710 cultivars (PoP + PoLTB+P): not represented to avoid clutter. Rhamnose (Rha), arabinose (Ara),
711 and galactose (Gal) were the neutral monosaccharides considered. Pa: Patriot. Po: Polaris. P:
712 pasteurization. LTB+P: low-temperature blanching + pasteurization.

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714 **Supplementary table 1.** Physicochemical characteristics and composition (g/100 g dry weight)

715 of highbush blueberry cultivars in 2015

	Patriot	Polaris
Physicochemical characteristics		
pH	3.1 ± 0.1^{b}	3.6 ± 0.0^{a}
ST/TA	14.6 ± 0.1	24.1 ± 0.1
Composition		
Moisture	87.4 ± 0.0^{b}	88.8 ± 0.0^{a}
Protein	2.4 ± 0.0^{b}	4.2 ± 0.0^{a}
Lipid	0.53 ± 0.02^{b}	1.40 ± 0.01^{a}
Ash	0.9 ± 0.0^{b}	1.1 ± 0.0^{a}
TDF	11.8 ± 0.1^{b}	20.6 ± 0.0^{a}
IDF	9.0 ± 0.1^{b}	16.5 ± 0.3^{a}
SDF	2.8 ± 0.2^{b}	4.0 ± 0.3^{a}
AIR	14.0 ± ND	23.9 ± ND
GalA[1]	3.7 ± 0.2^{b}	5.9 ± 0.0^{a}
DM (%)	44 ± 2^{a}	41 ± 4^{a}

716 Mean value ± standard deviation. Means within a line followed by the same letter (a–b) were

717 not significantly different according to the Tukey's test (α = 0.05). SS/TA represents the

718 sugar/acid ratio. TA: Titratable acidity (% citric acid). SS: Soluble solids (°Brix). TDF: Total

719 dietary fiber. IDF: Insoluble dietary fiber. SDF: Soluble dietary fiber. AIR: Alcohol-insoluble

720 residue. DM: Degree of methylation from AIR. ND: not determined.

721 [1] Galacturonic acid from AIR, as an estimated of pectin content.

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729 **Supplementary table 2.** Rheological properties and mean particle size (D[4,3]) of the heat-
730 treated purees

Purees	Yield stress (Pa)	Viscosity (Pa.s)	Rate index	D[4,3] (µm)
PaP	2.8 ± 0.3	1.2 ± 0.0	0.52 ± 0.00	535 ± 37
PaLTB+P	2.5 ± 0.2	1.1 ± 0.1	0.54 ± 0.01	541 ± 49
PoP	5.1 ± 0.4	3.3 ± 0.2	0.48 ± 0.01	703 ± 58
PoLTB+P	4.9 ± 0.4	3.1 ± 0.5	0.48 ± 0.02	697 ± 44
Cult	*	*	*	*
HT	n.s	n.s	n.s	n.s
Cult * HT	n.s	n.s	n.s	n.s

731 Mean value ± standard deviation. Results of the two-way ANOVAs (cultivar (Cult) and heat
732 treatment effects (HT), with interaction); n.s: not significant (P>0.05). Yield stress, viscosity,
733 and rate index were obtained from the Herschel-Bulkley modelling of the up flow sweep. The
734 D[4,3] corresponded to the volume mean diameter. Pa: Patriot. Po: Polaris. P: pasteurization.
735 LTB+P: low-temperature blanching + pasteurization.

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745 **Supplementary table 3.** Abundant pectin-associated monosaccharide composition (%) of the
746 pectin-rich fractions (WSP, CSP, and NSP), measured as trimethylsilyl derivatives after acidic
747 methanolysis and subsequent GC analysis

Purees	Rha	Ara	Gal	GalA	GalA / Rha+Ara+Gal
WSP					
PaP	1.3 ± 0.1	15.6 ± 0.3	22.7 ± 1.9	54.9 ± 2.0	1.4 ± 0.1
PaLTB+P	1.3 ± 0.1	15.8 ± 1.4	21.0 ± 1.4	56.7 ± 0.4	1.5 ± 0.0
PoP	1.3 ± 0.2	11.1 ± 1.8	9.3 ± 0.7	74.1 ± 1.5	3.4 ± 0.3
PoLTB+P	1.3 ± 0.1	11.2 ± 0.6	10.6 ± 1.2	72.3 ± 2.2	3.2 ± 0.4
Cult	n.s	*	*	*	*
HT	n.s	n.s	n.s	n.s	n.s
Cult * HT	n.s	n.s	n.s	n.s	n.s
CSP					
PaP	0.9 ± 0.1	5.3 ± 1.1	9.9 ± 1.1	81.4 ± 2.3	5.1 ± 0.8
PaLTB+P	0.9 ± 0.1	5.6 ± 0.5	10.3 ± 0.6	80.3 ± 1.0	4.8 ± 0.3
PoP	0.8 ± 0.1	3.7 ± 0.8	4.9 ± 1.1	87.9 ± 1.1	9.5 ± 1.5
PoLTB+P	0.8 ± 0.2	3.4 ± 1.0	3.7 ± 0.1	89.9 ± 1.3	11.7 ± 2.0
Cult	n.s	*	*	*	*
HT	n.s	n.s	n.s	n.s	n.s
Cult * HT	n.s	n.s	n.s	n.s	n.s
NSP					
PaP	1.3 ± 0.1	9.6 ± 1.1	18.0 ± 1.5[b]	64.4 ± 2.9[b]	2.3 ± 0.3
PaLTB+P	1.4 ± 0.2	10.9 ± 2.0	22.5 ± 0.7[a]	56.6 ± 1.2[c]	1.6 ± 0.1
PoP	1.5 ± 0.2	8.4 ± 2.0	17.5 ± 0.8[b]	68.5 ± 2.5[a]	2.5 ± 0.3
PoLTB+P	1.5 ± 0.3	8.1 ± 1.5	18.0 ± 1.1[b]	68.8 ± 0.7[a]	2.5 ± 0.1
Cult	n.s	n.s			*
HT	n.s	n.s			*
Cult * HT	n.s	n.s	*	*	n.s

748 Mean value ± standard deviation. Results of the two-way ANOVAs (cultivar (Cult) and heat
749 treatment effects (HT), with interaction); n.s: not significant (P>0.05). When interaction is
750 significant (*), means within a column, for the same fraction, followed by the same letter (a–c)
751 were not significantly different according to Tukey's test (α = 0.05). WSP: water-soluble
752 fraction. CSP: chelator-soluble pectin. NSP: sodium carbonate-soluble pectin. Pa: Patriot. Po:
753 Polaris. P: pasteurization. LTB+P: low-temperature blanching + pasteurization. Rha:
754 Rhamnose. Ara: Arabinose. Gal: Galactose. GalA: Galacturonic acid.

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