1 Thermal inactivation of pectin methylesterase from different 2 potato cultivars (*Solanum tuberosum* L.)

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27 **Journal:** LWT – Food Science and Technology

28 **Submitted:** August 17, 2020

29 **Revision 1 submitted:** November 16, 2020

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31 **List of abbreviations**

32 A residual absolute enzyme activity

33 A_0 initial absolute enzyme activity

34 A_r residual relative enzyme activity

35 $A_{r,0}$ initial relative enzyme activity

36 DM degree of methylesterification (%)

37 E_a activation energy (J/mol)

38 eq. equation

39 GalA galacturonic acid

40 HG homogalacturonan

41 HTST high temperature short time

42 j number of model parameters

43 k_T inactivation rate constant at temperature T (s^{-1})

44 l thermolabile PME fraction

45	LTST	low temperature long time
46	n	number of observations
47	PG	polygalacturonase
48	PME	pectin methylesterase
49	R	universal gas constant (8.314 J/mol*K)
50	R^2_{corr}	corrected correlation coefficient
51	RG-I	rhamnogalacturonan I
52	RG-II	rhamnogalacturonan II
53	s	thermostable PME fraction
54	SSR	sum of squares due to regression
55	SST	total sum of squares
56	T	temperature (K or °C)
57	t	time (s)
58	T_{ref}	reference temperature (K or °C)

59

60 Abstract

61 Pectin methylesterase (PME) is an important enzyme that can alter the texture of potato products during
62 processing and storage. To manage the textural properties of potato based products, low and/or high
63 temperature blanching can be used to promote or inactivate PME, respectively. However, the many potato
64 cultivars being used for processing may contain PMEs with a different thermal stability. Therefore, the thermal
65 inactivation of PME extract from three different potato cultivars (Fontane, Royal and Innovator) was studied.
66 A thermostable and thermolabile enzyme fraction were found in all three cultivars, being inactivated at
67 temperatures from 45 °C and 57.5 °C on, respectively. Innovator may contain a lower level of thermostable
68 PME than the other cultivars tested. The inactivation rate constant of the thermostable PME fraction was lower

69 than the rate constant of the labile fraction at a reference temperature of 55 °C, but no significant differences
70 between the respective rate constants of the different cultivars was found. The thermal inactivation kinetics of
71 PME extract from Fontane were investigated in detail and represented by a first order model that allows the
72 calculation of temperature-time combinations to reach a target residual PME activity.

73

74 **Keywords:** pectin methylesterase; thermal inactivation; potato cultivar; inactivation kinetics; enzyme
75 inactivation

76

77 1. Introduction

78 Potatoes (*Solanum tuberosum* L.) belong to the four most important vegetable crops in the world, and provide
79 the human diet with carbohydrates, vitamins, minerals, and proteins (De Haan & Rodriguez, 2016; Miranda &
80 Aguilera, 2006; Tian et al., 2016). Mainly processed potato products are consumed, such as French fries, chips,
81 and other dehydrated, frozen or canned food products (Camire et al., 2009; Miranda & Aguilera, 2006). More
82 than 4000 potato cultivars are grown, with their intended use depending on characteristics such as potato
83 composition and storage properties (Camire et al., 2009).

84 Texture is one of the main quality attributes of fruit and vegetable based food products in general (Barrett et
85 al., 2010; Waldron et al., 2003), and potato products in particular (Moyano et al., 2007). Texture of plant
86 tissues is mainly determined by the turgor pressure and the composition of the cell wall and the middle lamella,
87 that acts as a glue between the different cells (Barrett et al., 2010). Pectin is an important polysaccharide in the
88 cell wall and the middle lamella and provides plasticity. It mainly consists of the linear subdomain
89 homogalacturonan (HG), and the branched subdomains rhamnogalacturonan I (RG-I) and rhamnogalacturonan
90 II (RG-II), of which HG is the most abundant (approximately 60%). HG consists of linked galacturonic acid
91 (GalA) residues that can be methylesterified on the C-6 carboxyl group, depending on the origin of the polymer
92 (Christiaens et al., 2016; Mohnen, 2008; Ridley et al., 2001; Voragen et al., 2009; Willats et al., 2006). The
93 degree of methylesterification (DM) and pattern of methylesterification determine to a large extent the
94 functionality of this polymer (Christiaens et al., 2016; Ridley et al., 2001; Willats et al., 2006). HG can be
95 demethylesterified enzymatically by pectin methylesterase (PME, E.C. 3.1.11), that can be found in bacteria,

96 fungi and higher plants (Andersson et al., 1994; Christiaens et al., 2016; Markovič et al., 2002; McMillan &
97 Pérombelon, 1995; Pelloux et al., 2007). Most plant PME's are bound to the cell wall by electrostatic interaction
98 (Christiaens et al., 2016; Ly Nguyen et al., 2002) and demethylesterify HG in a blockwise way, resulting in
99 blocks of negatively charged demethylesterified GalA residues. Divalent cations such as Ca^{2+} are able to bind
100 blocks of negatively charged GalA residues, forming intra- and intermolecular pectin crosslinks. This
101 assemblage of HG domains is referred to as the egg-box model and improves cell adhesion and tissue firmness
102 (Caffall & Mohnen, 2009; Christiaens et al., 2016). Moreover, the demethylesterification and ionic
103 crosslinking of pectin slow down the chemical depolymerisation of HG by beta-elimination, resulting in a
104 slower texture degradation during thermal processing at temperatures higher than 80 °C (Andersson et al.,
105 1994; González-Martínez et al., 2004; Sila et al., 2006). Additionally, low methylesterified HG is the substrate
106 for polygalacturonase (PG), a pectolytic enzyme that catalyses the hydrolysis of the glycosidic bounds between
107 demethylesterified GalA residues, softening the texture (Christiaens et al., 2016; Puri et al., 1981). Given the
108 above, PME can be considered as an important texture determining enzyme.

109 In comparison with other fruits and vegetables, pectin from potatoes has a relatively low HG to RG-I ratio
110 (Ryden & Selvendran, 1990; Vincken et al., 2000) and the presence of RG-II in potato cell walls has not been
111 demonstrated yet (Ralet et al., 2016; Vincken et al., 2000). Nevertheless, HG represents a significant part of
112 potato pectin (about 25%) (Oomen et al., 2003; Vincken et al., 2000), with a DM of 45-50% (Huang et al.,
113 2016, 2017; Ralet et al., 2016; Van Marle et al., 1997). Multiple studies demonstrate the existence of PME in
114 potatoes (Anthon & Barrett, 2002; McMillan & Pérombelon, 1995; Puri et al., 1982; Roeb & Stegemann, 1975;
115 Tijssens et al., 1997), with an optimal activity of PME extract being 55 °C (Puri et al., 1982). Five different
116 potato PME isozymes were already found using gel electrophoresis (Roeb & Stegemann, 1975). PME activity
117 may facilitate the depolymerisation of HG by PG. In potatoes, only exo-PG has been found. This enzyme
118 removes single GalA units from pectin, which may only have a small effect on the pectin structure and potato
119 texture. However, the cleavage of terminal GalA monomers that are linked to other cell wall components may
120 have an effect on cell wall integrity (Puri et al., 1981). Given the texture determining role of PME in potatoes,
121 its activity needs to be controlled to manage product texture during processing and storage.

122 Blanching is a thermal treatment used in the potato processing industry to inactivate enzymes, to promote the
123 leaching of reducing sugars to reduce Maillard reactions and acrylamide formation during frying, to remove

124 air from the tissue, and to reduce fat uptake during frying (Andersson et al., 1994; Anthon & Barrett, 2002;
125 Moyano et al., 2007). Depending on the purpose of the treatment, the temperature and duration of the blanching
126 step may vary. As the leaching out of reducing sugars requires long times, low temperatures (50-70 °C) are
127 used to prevent extensive tissue softening. Moreover, these low temperature long time (LTLT) blanching steps
128 can improve tissue firmness as this temperature range is optimal for PME activity. In contrast, high temperature
129 (> 70° C) short time (HTST) blanching steps are used to inactivate enzymes that may deteriorate the quality
130 of the product during further processing or storage. Often, a LTLT blanching step is followed by a HTST step
131 (Andersson et al., 1994; Miranda & Aguilera, 2006; Moyano et al., 2007). As high temperatures may also be
132 detrimental for quality attributes such as colour, flavour and nutritional value, the HTST should be sufficient
133 enough to completely inactivate the enzymes, but minimal to prevent quality loss. That is why the thermal
134 stability of the enzyme intended for inactivation should be known (Anthon & Barrett, 2002). This paper focuses
135 on the thermal stability of PME in different potato cultivars.

136 In general, the activity of enzymes enhances with increasing temperature, until a maximal activity is reached
137 at the optimal temperature. Further increasing the temperature results in a steep decline of activity due to
138 enzyme denaturation, with the activity decreasing exponentially with time at a constant temperature,
139 suggesting first order inactivation kinetics (Tijssens et al., 1997). Therefore, thermal inactivation of enzymes
140 is often described by a first order kinetic model, characterised by the rate constant of inactivation k_T at a certain
141 temperature, and by the activation energy E_a as a measure for the temperature dependency of the rate constant.
142 Knowledge of these two parameters allows the calculation of enzyme inactivation rates and residual enzyme
143 activities at any temperature-time conditions (Anthon & Barrett, 2002; Espachs-Barroso et al., 2006; Indrawati
144 et al., 1999). The inactivation of PME from potatoes shows a biphasic course, indicating the presence of a
145 thermolabile and thermostable enzyme fraction (Anthon & Barrett, 2002). Therefore, in case both fractions are
146 inactivated by first order kinetics and independently from each other, two inactivation rate constants and
147 activation energies need to be estimated. The residual enzyme activity can then be calculated as the sum of the
148 residual activities of both fractions (Anthon & Barrett, 2002; Espachs-Barroso et al., 2006; Indrawati et al.,
149 1999).

150 Although the thermal inactivation of PME and the presence of two fractions with different thermal stability
151 has already been studied for some potato cultivars, e.g. Russet Burbank (Anthon & Barrett, 2002) and Bintje

152 (Tijssens et al., 1997), there is all together very few data on the inactivation kinetics of PME in different potato
153 cultivars, especially those that are currently used in the potato processing industry. Moreover, the use of
154 different PME extraction methods in the available literature data complicates the comparison of thermal
155 inactivation kinetics between cultivars. Therefore, we consider it valuable and important to compare potato
156 PME inactivation kinetics of different and relevant potato cultivars within one study. This will contribute not
157 only to a better scientific understanding of PME behaviour during thermal processing, but also to the control
158 of PME activity in an industrial context, where processing needs to balance the enzyme inactivation while
159 ensuring the desired product quality.

160 Therefore, in this paper the thermal inactivation of PME from the potato cultivars Fontane, Royal and
161 Innovator was studied. These potato cultivars are commonly used in the production process of frozen French
162 fries in Belgium. Firstly, the potato cultivars were characterised by their composition and PME activity.
163 Secondly, the thermal stability of PME from the different cultivars was compared. The thermal treatments
164 were performed on enzyme extracts to minimise the negative effects of starch gelatinisation on PME
165 extraction. Thirdly, the detailed thermal inactivation kinetics of PME were determined for the cultivar with the
166 most thermostable PME.

167

168 **2. Materials and methods**

169 **2.1 Plant materials and chemicals**

170 Potatoes (*Solanum tuberosum* L.) of the cultivars Fontane, Royal and Innovator were grown and harvested by
171 local farmers (Fontane: Carolushoeve, Hoegaarden, Belgium; Royal and Innovator: Coopérative de l'Anglée
172 scrl, Tourinnes Saint Lambert, Belgium). They were retrieved right after harvest and stored for maximum one
173 week in a fridge at 8 °C. Twenty randomly chosen potatoes of each cultivar were manually peeled and cut into
174 cubes of approximately 1 x 1 cm at 4 °C (Anthon & Barrett, 2002). After vacuum packing (A 300/16, Multivac,
175 Wolfertschwenden, Germany), the cubes were frozen and stored at -40 °C.

176 Sodium bisulfite (99%) and apple pectin were retrieved from Sigma Aldrich Chemie GmbH (Steinheim,
177 Germany). Tris(hydroxymethyl)aminomethane (99.8%), NaCl (> 99.5%), ammonium sulfate (99.5%) and
178 KOH (85%, extra pure, flakes) were retrieved from Acros Organics (Geel, Belgium). Concentrated volumetric

179 NaOH solutions (convol normadose, 0.01M), ethanol (99%), NaOH (p.a.), and glacial acetic acid were
180 retrieved from VWR Chemicals (Leuven, Belgium). The total starch assay kit (AA/AMG) was retrieved from
181 Megazyme Inc. (Bray, Ireland). Unless mentioned otherwise, all chemicals were analytical grade.

182 2.2 Characterisation of potato cultivars

183 2.2.1 Dry matter content

184 The frozen potato cubes were mixed (Grindomixer GM200, Retsch, Haan, Germany) and thawed in a 20 °C
185 water bath (DC30 and W13, Haake, Karlsruhe, Germany). The puree was dried in triplicate in porcelain
186 crucibles, placed in a vacuum oven (1445-2, UniEquip, Planegg, Germany) at 70 °C and 80 kPa pressure. The
187 pressure was reduced to 20 kPa with pressure reduction steps of 20 kPa every hour. Finally, the puree was
188 dried overnight at 40 °C. The dry matter content was calculated as the mass of dried puree divided by the mass
189 of wet puree (Moens et al., 2020; Nguyen et al., 2016).

190 2.2.2 Starch content

191 The starch and protein measurements were performed on potato powder. Therefore, the frozen cubes were
192 mixed (Grindomixer GM200, Retsch, Haan, Germany) and freeze-dried for 22 h (2-4 LSC, Christ Alpha,
193 Osterode, Germany). Subsequently, the dried puree was milled (Ball mill MM400, Retsch, Haan, Germany)
194 until a fine powder was obtained.

195 The starch content was determined using the Total Starch Assay kit (AA/AMG, Megazyme Inc., Bray, Ireland).
196 According to the manufacturer's protocol, the starch was gelatinised, hydrolysed by α -amylase and
197 amyloglucosidase, and the glucose concentration of the hydrolysate was measured spectrophotometrically
198 (UV-1800, Shimadzu, Wommel, Belgium). This concentration was converted to the starch content (Gwala et
199 al., 2020). For every cultivar, starch was hydrolysed twice and the glucose concentration of each hydrolysate
200 was measured in duplicate.

201 2.2.3 Protein content

202 Two mg of the potato powder was weighted in tin capsules and burnt as a whole. The nitrogen content of the
203 ash was determined using a CHNS-O Elemental analyser (CE instrument, Thermofischer Scientific, Waltham,

204 USA) and converted to the protein content using a 6.25 conversion factor (Gwala et al., 2020). The protein
205 content was measured in duplicate for every cultivar.

206 2.2.4 Pectin methylesterase extraction and activity measurement

207 The extraction method of pectin methylesterase (PME) was based on the extraction procedure of Ly-Nguyen
208 et al. (2003) with some adjustments. Puree, obtained after homogenisation of the frozen potato tissue
209 (Grindomixer GM200, Retsch, Haan, Germany), was immediately mixed with 0.05% cold sodium bisulfite
210 (0.6 L/kg puree) to prevent enzymatic browning. The mixture was centrifuged 30 min at 10 000g at 4 °C (J2-
211 HS, Beckman Coulter, Brea, CA) and the pellet was washed once again with cold sodium bisulfite. A
212 preliminary experiment showed that the PME activity in these supernatants was below detection limit of the
213 activity measurement method used. PME was extracted by adding 0.2 M tris(hydroxymethyl)aminomethane
214 buffer at pH 8.0 containing 1M NaCl to the washed pellet (1.3 L/kg puree), rotating the mixture end-over-end
215 at 4 °C overnight, and centrifuging the mixture at 4 °C for 30 min at 18 000g. Next, large proteins were
216 precipitated by adding ammonium sulfate to the supernatant to a 30% concentration, rotating the mixture end-
217 over-end at 4 °C for 30 min, and centrifuging the mixture at 4 °C for 1 h at 18 000g. Finally, PME was
218 precipitated by increasing the ammonium sulfate concentration of the supernatant to 80% and precipitating the
219 enzyme analogously. The obtained pellet was dissolved in 0.02M tris(hydroxymethyl)aminomethane buffer at
220 pH 7.0 (0.5 L/kg puree) and stored at -40 °C until activity measurement. The extraction procedure was
221 performed twice for every cultivar and the PME activity of every extract was measured twice.

222 One unit PME activity was defined as the amount of enzyme necessary to generate 1 $\mu\text{mol COO}^-$ groups per
223 minute at 22 °C and pH 7 (Ly-Nguyen et al., 2003). Therefore, 0.5 mL of PME extract was added to 30 mL
224 0.35 % (w/v) apple pectin (DM 70.3%) solution with 0.117 M NaCl at pH 6.5, that acted as substrate for PME.
225 The pH of the mixture was monitored and kept at 7 for 1200 s by adding 0.01 M NaOH using an automatic pH
226 stat titrator (718 STAT Titrino, Metrohm, Herisau, Switzerland) with cryostat (DC, Haake, Karlsruhe,
227 Germany) at 22 °C. The rate of 0.01M NaOH addition was calculated to obtain the PME activity (Ly-Nguyen
228 et al., 2003).

229

230 2.3 Temperature range for inactivation of pectin methylesterase

231 The temperature range for inactivation of PME was determined using a single enzyme extract obtained from
232 each cultivar (Section 2.2.4). The extract was thawed in a water bath at 20 °C (DC30 and W13, Haake,
233 Karlsruhe, Germany), contained in capillaries of 200 µL (internal diameter 1.4 mm, external diameter 2 mm,
234 length 125 mm, Blaubrand, Wertheim, Germany) without air bubbles, and stored in an ice bath for maximum
235 2 h. Four capillaries were submitted 5 min to a constant temperature between 35 and 80 °C in a temperature-
236 controlled water bath (Julabo MP-5, Julabo GmbH, Seelbach, Germany) (Indrawati et al., 1999), of which the
237 temperature was verified using a calibrated mercury thermometer (Anthon & Barrett, 2002). After 5 min
238 heating, the capillaries were immediately cooled and stored in an ice bath for maximum 4 h until activity
239 measurement. The thermal treatments were performed once for every tested temperature and the activity of the
240 thermally treated enzyme extract was measured after mixing the content of the four capillaries submitted to
241 the same temperature. The activity of extract from four untreated capillaries was measured as a blanc. The
242 residual relative activity A_r was calculated as A/A_0 , with A the activity of the (partially) inactivated extract and
243 A_0 the activity of the untreated extract.

244

245 2.4 Detailed thermal inactivation kinetics of pectin methylesterase

246 The detailed inactivation kinetics of PME from one cultivar were determined by measuring the enzyme activity
247 after submitting the extract to different temperature-time combinations in a temperature-controlled water bath
248 (Julabo MP-5, Julabo GmbH, Seelbach, Germany). The experiment was designed based on the results obtained
249 in Section 2.3. Six relevant temperatures were selected based on the temperature range for PME inactivation
250 (Section 2.3). The data from the determination of the temperature range for PME inactivation were modelled
251 to a biphasic first order model (Section 2.5.2), allowing the calculation of thermal inactivation rate constants
252 and relevant heating times at the selected temperatures. Four capillaries, prepared as in Section 2.3, were
253 submitted once to a relevant temperature-time combination, and immediately cooled and stored in an icebath.
254 The content of the four capillaries that underwent the same treatment was mixed and the activity was measured.
255 Again, a blanc was generated and the residual relative activity A_r was defined as A/A_0 .

256

257 2.5 Statistical data analysis

2.5.1 Characterisation of cultivars

Significant differences in dry matter content, starch content, protein content, and PME activity between cultivars were investigated using the Tukey honestly significant difference (HSD) test with significance level 5% using JMP Pro software (version 14.2.0, SAS Institute, Tervuren, Belgium).

2.5.2 Thermal inactivation of pectin methylesterase

Thermal inactivation of quality related enzymes at a constant temperature can be described by the first-order model

$$A_r = A_{r,0} * \exp(-k_T * t) \text{ (eq. 1)}$$

with A_r the residual relative activity at heating time t , $A_{r,0}$ the initial relative enzyme activity and k_T the inactivation rate constant at temperature T (s^{-1}) (Anthon & Barrett, 2002; Indrawati et al., 1999). It was already found that potato PME shows a biphasic thermal inactivation, indicating the presence of a thermolabile and a thermostable PME fraction (Anthon & Barrett, 2002). In this case, the total residual activity decay at temperature T is the sum of the activity decays of both fractions, described by

$$A_r = A_{r,0,l} * \exp(-k_{T,l} * t) + A_{r,0,s} * \exp(-k_{T,s} * t) \text{ (eq. 2)}$$

with A_r the total residual relative activity at heating time t , and the subscripts l and s referring to the thermolabile and thermostable PME fraction, respectively (Indrawati et al., 1999). The temperature dependency of the inactivation rate constants can be described by the equation of Arrhenius, being

$$k_T = k_{T_{ref}} * \exp((E_A/R) * (1/T_{ref} - 1/T)) \text{ (eq. 3)}$$

with $k_{T_{ref}}$ the inactivation rate constant at a reference temperature T_{ref} (s^{-1}), E_A the activation energy (J/mol), R the universal gas constant (8.314 J/mol*K) and T_{ref} a reference temperature (K) (Indrawati et al., 1999). In this case, the reference temperature was set at 55 °C or 328.15 K, near the middle of the tested temperature range of 35-80 °C (Anthon & Barrett, 2002).

Hence, the total PME activity as a function of time and temperature can be modelled using the following equation (equation 3 substituted in equation 2):

$$A_r = A_{r,0,l} * \exp(-k_{T_{ref},l} * \exp((E_{A,l}/R) * (1/T_{ref} - 1/T)) * t) + A_{r,0,s} * \exp(-k_{T_{ref},s} * \exp((E_{A,s}/R) * (1/T_{ref} - 1/T)) * t) \text{ (eq. 4)}$$

with the subscripts l and s referring to the thermolabile and thermostable fraction, respectively.

In case of the experiment determining the temperature range for PME inactivation (Section 2.3), the temperature was changed (35-80 °C) and the heating time was fixed at 5 min or 300 s. $A_{r,0}$, k_{Tref} and E_a of both fractions were estimated using the nonlinear regression procedure in SAS software (version 9.4, SAS Institute, Tervuren, Belgium). The model parameters were compared between cultivars and enzyme fractions using their 95% confidence intervals. The corrected correlation coefficient was calculated as $R^2_{corr} = 1 - (n-1)(1 - SSR/SST)/(n-j)$ with n = number of observations, j = number of model parameters, SSR = sum of squares due to regression and SST = total sum of squares (De Roeck et al., 2010).

For the experiment determining the detailed kinetics (Section 2.4), the estimates of $k_{Tref,l}$, $k_{Tref,s}$, $E_{a,l}$ and $E_{a,s}$ of the chosen cultivar (Section 3.2) were used to calculate $k_{T,l}$ and $k_{T,s}$ for six relevant temperatures using eq. 3. These rate constants together with the estimates of $A_{r,0,l}$ and $A_{r,0,s}$ were used to calculate relevant heating times at the selected temperatures (eq. 2). The resulting temperature-time combinations were used in Section 2.4 for the determination of the thermal inactivation kinetics of PME. The measured total residual PME activities, together with their corresponding temperature-time combinations were used to reestimate $A_{r,0,l}$, $A_{r,0,s}$, $k_{Tref,l}$, $k_{Tref,s}$, $E_{a,l}$ and $E_{a,s}$ with reference temperature 55 °C or 328.15 K (eq. 4). In this case, not only the temperature but also the heating time was varied. Therefore, these parameter estimates were considered to be more precise than the ones estimated before. Significant differences between parameter estimates from the different enzyme fractions were determined by comparing their 95% confidence intervals. Again, the corrected correlation coefficient R^2_{corr} was calculated.

3. Results and discussion

3.1 Characterisation of potato cultivars

The potato cultivars studied are, among others, commonly used in the potato processing industry in Belgium for the production of frozen French fries. They were compared by their dry matter content, starch content, protein content and PME activity (**Table 1**). Potatoes of the cultivar Innovator have a higher dry matter content compared to the other cultivars. This may be explained by the higher starch content of Innovator. Fontane potatoes have the lowest starch content. Although a slightly higher protein content was observed for Royal,

310 Fontane and Innovator have a higher PME activity than Royal. Differences in PME activity may be important
311 when selecting temperature-time combinations for blanching, as potatoes with identical PME inactivation
312 kinetics but a higher initial absolute enzyme activity may require longer heating times to obtain a target residual
313 absolute enzyme activity.

314

315 3.2 Temperature range for inactivation of pectin methylesterase from different potato cultivars

316 The total residual relative activity of PME from three different potato cultivars was measured at 22 °C after
317 heating the enzyme extracts 5 min at temperatures between 35 and 80 °C (**Figure 1**). Temperatures higher than
318 45 °C inactivated the enzyme, with the inactivation being complete after 5 min heating at 80 °C. The activity
319 decay followed a superposition of two sigmoidal curves, suggesting a biphasic inactivation of PME. Therefore,
320 the datapoints were fitted to a first order inactivation model with two fractions (eq. 4), describing the total
321 residual relative activity decay as a function of temperature after 5 min heating (**Figure 1** and **Table 2**).

322 The corrected correlation coefficients indicate that the model fitted the data well (**Table 2**). The inactivation
323 rate constant of the stable fraction was for all three cultivars lower than the inactivation rate constant of the
324 labile fraction, indicating a slower inactivation of the stable fraction at 55 °C compared to the labile fraction.
325 However, no significant differences between inactivation rate constants of the labile or stable fractions from
326 the different cultivars were observed. PME from Fontane and Royal consisted of 50% thermostable and 50%
327 thermolabile fraction (**Table 2**). In contrast, the labile fraction was more abundant (almost 70%) in the PME
328 extract from Innovator. No clear differences in activation energy were observed between fractions and cultivars
329 (**Table 2**). However, the uncertainty on the estimated parameters was quite high. Therefore, a detailed study
330 of the thermal inactivation kinetics is needed to confirm these observations.

331 In order to ensure total inactivation of PME during blanching, the inactivation kinetics of the most thermostable
332 PME were determined. The temperature-time combinations leading to inactivation of this PME will
333 automatically result in inactivation of PME from the other cultivars. Given the smaller stable enzyme fraction
334 in Innovator (**Table 2**), determination of the inactivation kinetics of PME from Fontane and Royal seemed
335 more relevant. Additionally, PME from Fontane may be more thermostable than PME from Royal, regarding

the seemingly lower rate constants of PME from Fontane (**Table 2**). For these reasons, the detailed thermal inactivation kinetics of PME from Fontane were investigated.

338

339 3.3 Detailed inactivation kinetics of Fontane pectin methylesterase

340 The PME extract of Fontane was heated at six different temperatures (55-72.5 °C) for different heating times
341 (0-80 min) and the residual relative activity was measured (**Figure 2**). The data were modelled using the first
342 order inactivation model (eq. 4) describing the residual relative activity as a function of heating time and
343 temperature (**Figure 2** and **Table 3**). A single step approach considering the dataset as a whole was used.
344 According to the corrected correlation coefficient, the model fitted the data well.

345 At temperatures below 57.5 °C, increasing the heating time didn't result in total activity loss (**Figure 2**). The
346 plateau value after prolonged heating confirms the presence of a thermostable fraction, with an estimated initial
347 activity of 36% (**Table 3**). In contrast, Anthon & Barrett (2002) found a thermostable PME fraction from
348 potatoes of the cultivar Russet Burbank of 20%. Possibly, different potato cultivars contain different
349 proportions of the thermostable and thermolabile fractions, as was already indicated in **Table 2**. However,
350 studying the thermal inactivation of PME from the different cultivars in a more detailed kinetic study (**Table**
351 **3**) may result in more precise estimates of these proportions. Up to now, the thermostable fraction seems less
352 abundant than the thermolabile fraction.

353 At temperatures higher than 57.5 °C, also the thermostable fraction was inactivated. Therefore, PME activity
354 will be completely lost after prolonged heating. It can also be observed that enzyme inactivation was faster at
355 higher temperatures (**Figure 2**).

356 The inactivation rate constant of the stable fraction at the reference temperature was lower than the rate
357 constant of the labile fraction at the same temperature (**Table 3**), indicating the faster denaturation of the labile
358 fraction at 55 °C. Although inactivation of the stable fraction only occurred at higher temperatures, the
359 activation energy was similar for both fractions (**Table 3**), meaning both inactivation rate constants show a
360 similar temperature dependency.

361 The curve of Fontane in **Figure 1** represents an intersection of the curves in **Figure 2** at 5 min heating time.
 362 While the dataset from the first experiment (Section 3.2) covers a two dimensional surface with dimensions
 363 temperature and residual relative activity after 5 min heating, the dataset from the second experiment (Section
 364 3.3) covers a three dimensional space with dimensions temperature, heating time, and residual relative activity.
 365 Therefore, the parameter estimates of the detailed inactivation kinetics of PME from Fontane (Section 3.3) can
 366 also be used to recalculate the curve of Fontane in **Figure 1**. This is illustrated in **Figure 3**, where the
 367 inactivation of PME from Fontane as a function of temperature after heating 5 min was calculated using eq. 4
 368 and the parameter estimates from both the temperature range experiment in **Table 2** (dashed line) and the
 369 detailed kinetics experiment in **Table 3** (full line). Small differences between the curves exist. As more data
 370 points were used to estimate the model parameters in **Table 3**, the errors on these estimates are smaller than
 371 the ones in **Table 2**, and the full curve is considered to be more precise than the dashed curve. This is illustrated
 372 by the smaller error range of the full curve that was calculated using error propagation.

373 The estimates of the rate constants at the reference temperature and the activation energy in **Table 3** allow the
 374 calculation of the inactivation rate constant of both fractions at any temperature, using the equation of
 375 Arrhenius (eq. 3). These inactivation rate constants, together with the initial relative PME activity of the
 376 fractions, can be used to calculate the residual relative enzyme activity at any time during heating (eq. 4), and
 377 thus to calculate different temperature-time combinations to inactivate the enzyme to a target residual relative
 378 activity (Anthon & Barrett, 2002). As this study demonstrates that the PME fractions from the different
 379 cultivars have a similar thermal stability (**Table 2**), this model may be useful for all studied cultivars.

380 However, the residual relative enzyme activity is a relative value, based on the absolute initial PME activity.
 381 Therefore, blanching conditions leading to a target residual relative activity will result in a higher absolute
 382 PME activity if potatoes with a relatively high initial absolute PME activity are processed. Consequently,
 383 potatoes with a higher initial absolute PME activity, such as Fontane (**Table 1**), may need longer heating times
 384 to obtain a target absolute enzyme activity. This should be taken into consideration when looking for suitable
 385 blanching conditions.

386 Finally, these parameter estimates apply for the inactivation of PME extracts and may be different for the
 387 enzyme *in situ*, as most enzymes are less vulnerable to thermal denaturation if embedded in a tissue matrix
 388 compared to the purified enzyme (Balogh et al., 2004; Christiaens et al., 2016; Sila et al., 2007). Therefore,

389 studying the effect of the potato matrix on heat transport and on thermal inactivation kinetics could help to
390 ensure the target enzyme activity is reached during processing of different potato cultivars.

391

392 **4. Conclusion**

393 In this paper the thermal inactivation of PME from different potato cultivars (Fontane, Royal, Innovator) was
394 studied. A thermostable and thermolabile enzyme fraction were found in all three cultivars, with the
395 thermolabile fraction being inactivated at temperatures higher than 45 °C and the thermostable fraction
396 requiring temperatures higher than 57.5 °C for inactivation. Consequently, the stable fraction had a lower
397 inactivation rate constant at the reference temperature of 55 °C compared to the labile fraction. However, no
398 difference in rate constants of the same fractions could be found between cultivars. Also the activation energy
399 was barely different between fractions and cultivars. On the other hand, PME from Innovator may contain
400 smaller amounts of the thermostable enzyme compared to the other cultivars.

401 Blanching conditions should be sufficient enough to inactivate the more thermostable PME, while minimising
402 the detrimental effects of heating on product quality. Therefore, the detailed inactivation kinetics of PME from
403 Fontane were determined. The estimated parameters allow the calculation of the enzyme inactivation rate
404 constant and the time required to inactivate the enzyme to a target residual relative activity at any temperature.
405 However, the initial absolute enzyme activity is required to calculate conditions to reach a target residual
406 absolute activity. Further research is necessary to transfer these temperature-time conditions for inactivation
407 of PME extract to inactivation of the enzyme *in situ*.

408

409 **Declaration of competing interest**

410 The authors declare no conflict of interest.

411

412 **Acknowledgement**

413 This research was financially supported by VLAIO (Flanders Innovation and Entrepreneurship) in the context
414 of a Baekeland mandate (HBC.2016.0591).

415

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Figure 1: Total residual relative activity (A_r) of pectin methylesterase from three potato cultivars (● Fontane, ▲ Royal, ■ Innovator) after 5 minutes heating at temperatures between 35 and 80 °C. The enzyme activity as a function of temperature was measured once for each cultivar and was fitted to a first order inactivation model (full lines) with two fractions and reference temperature 55 °C using non linear regression.

Figure 2: Total residual relative activity (A_r) of pectin methylesterase from Fontane after heating the extract for different heating times (0-80 min) at different temperatures (○ 55 °C, ● 57.5 °C, □ 60 °C, ■ 62.5 °C, ◇ 67.5 °C, ◆ 72.5 °C). Each temperature-time combination was performed once and all data points were fitted to a first order inactivation model with two fractions and reference temperature 55 °C using a single step approach.

Figure 3: Total residual relative activity (A_r) of pectin methylesterase from Fontane that was measured once after 5 minutes heating at temperatures between 35 and 80 °C (●). The black curves represent the total residual relative activity as a function of temperature after 5 minutes heating, and were calculated using eq. 4 and the parameter estimates of the first order inactivation models that were found for the first (Table 2, dashed line) and second dataset (Table 3, full line). The grey lines represent the standard errors on the black curves (dashed lines: first dataset, full lines: second dataset), calculated using error propagation.

	Fontane	Royal	Innovator
Dry matter content (%)	25.2 ± 0.2 ^B	25.0 ± 0.1 ^B	26.7 ± 0.1 ^A
Starch content (% dm)	66.8 ± 0.2 ^C	68.0 ± 0.2 ^B	70.5 ± 0.2 ^A
Protein content (% dm)	9.2 ± 0.1 ^B	9.7 ± 0.1 ^A	9.1 ± 0.1 ^B
PME activity (U/g puree)	0.88 ± 0.01 ^A	0.73 ± 0.02 ^B	0.82 ± 0.02 ^A

Table 1: Dry matter content ($n=3$), starch content ($n=4$), protein content ($n=2$) and pectin methylesterase (PME) activity ($n=4$) of the potato cultivars Fontane, Royal and Innovator with their standard error. Significant differences between cultivars are indicated in superscript, based on the results of a Tukey HSD test with significance level 5%. dm=dry matter

	Fontane	Royal	Innovator
$k_{Tref,l}$ (10^{-3} s^{-1})	2.66 ± 0.38^A	4.38 ± 1.00^A	3.34 ± 0.17^A
$k_{Tref,s}$ (10^{-3} s^{-1})	0.34 ± 0.39^B	0.48 ± 0.70^B	0.11 ± 0.08^B
$E_{a,l}$ (kJ/mol)	346.0 ± 85.6^{AB}	320.4 ± 83.0^{AB}	360.1 ± 16.7^A
$E_{a,s}$ (kJ/mol)	163.3 ± 60.8^B	172.2 ± 86.9^{AB}	224.6 ± 43.3^{AB}
$A_{r,0,l}$	0.49 ± 0.13^{AB}	0.55 ± 0.17^{AB}	0.69 ± 0.02^A
$A_{r,0,s}$	0.51 ± 0.13^{AB}	0.45 ± 0.17^{AB}	0.31 ± 0.02^B
R^2_{corr}	0.9986	0.9982	0.9998

Table 2: Parameter estimates with their standard errors for the inactivation kinetics of potato pectin methylesterase from the cultivars Fontane, Royal and Innovator after submitting extracts for 5 min at temperatures between 35 and 80 °C. The residual relative enzyme activity after 5 min heating was modelled as a function of temperature using a first order inactivation model with two fractions and reference temperature T_{ref} 55 °C by nonlinear regression. Significant differences between parameter estimates of different fractions or cultivars are indicated with different letters in superscript, based on the overlap of their 95% confidence intervals. $k_{Tref,l}$: inactivation rate constant of the labile fraction at T_{ref} , $k_{Tref,s}$: inactivation rate constant of the stable fraction at T_{ref} , $E_{a,l}$: activation energy of the labile fraction, $E_{a,s}$: activation energy of the stable fraction, $A_{r,0,l}$: initial relative enzyme activity of the labile fraction, $A_{r,0,s}$: initial relative enzyme activity of the stable fraction, R^2_{corr} : corrected correlation coefficient.

$k_{T_{ref},l} (10^{-3} \text{ s}^{-1})$	2.66 ± 0.15^A
$k_{T_{ref},s} (10^{-3} \text{ s}^{-1})$	0.01 ± 0.01^B
$E_{a,l} (\text{kJ/mol})$	360.7 ± 15.2^A
$E_{a,s} (\text{kJ/mol})$	342.5 ± 14.8^A
$A_{r,0,l}$	0.64 ± 0.01^A
$A_{r,0,s}$	0.36 ± 0.01^B
R^2_{corr}	0.9971

640

641 **Table 3:** Parameter estimates with their standard errors for the inactivation kinetics of pectin methylesterase
642 from Fontane. The residual relative enzyme activity as a function of time was modelled using a first order
643 inactivation model with two fractions and reference temperature of 55 °C by nonlinear regression. Significant
644 differences between parameter estimates of different fractions are indicated with different letters in superscript,
645 based on the overlap of their 95% confidence intervals. $k_{T_{ref},l}$: inactivation rate constant of the labile fraction at
646 T_{ref} , $k_{T_{ref},s}$: inactivation rate constant of the stable fraction at T_{ref} , $E_{a,l}$: activation energy of the labile fraction,
647 $E_{a,s}$: activation energy of the stable fraction, $A_{r,0,l}$: initial relative enzyme activity of the labile fraction, $A_{r,0,s}$:
648 initial relative enzyme activity of the stable fraction, R^2_{corr} : corrected correlation coefficient.

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