Kinetics of Strecker aldehyde formation during thermal and high pressure high
temperature processing of carrot puree

*Biniam Kebede***, Tara Grauwet, Tewodros Andargie, Geoffery Sempiri, Stijn Palmers, Marc Hendrickx,

*Ann Van Loey**

All authors are affiliated to:

Laboratory of Food Technology

Leuven Food Science and Nutrition Research Center (LForCe)

Department of Microbial and Molecular Systems (M²S)

KU Leuven

Kasteelpark Arenberg 22 box 2457

B-3001 Heverlee

Belgium

<http://www.biw.kuleuven.be/m2s/clmt/lmt>

*Author to whom correspondence should be addressed, **on post publication**

Fax: +32-16-32.19.60

Telephone: +32-16-32.15.67

E-mail: ann.vanloey@biw.kuleuven.be

Author to whom correspondence should be addressed **during submission process

Fax: +32 16 32 19 60

Telephone: +32 16 37 67 63

E-mail: BiniamTamiru.Kebede@biw.kuleuven.be

25 **Abstract**

26 Strecker aldehydes have been negatively associated to flavor of heat sterilized plant-based foods. The
27 present study demonstrated the importance of processing conditions (temperature, pressure and
28 time) as a strong means for the control of Strecker aldehyde formation in vegetables purees. A kinetic
29 study was set up (at isothermal and isothermal-isobaric conditions) to quantify the effects of single
30 process parameters on the changes of 3-methylbutanal (3-MB) in carrot puree as a case study. The
31 increase in 3-MB concentrations was best described by an empirical, logistic model. During the
32 isothermal treatment at atmospheric pressure, the maximum reaction rate constant of 3-MB
33 formation was increased as a function of processing temperature. However, the formation rate was
34 clearly slower at high pressure (600 MPa) compared to the process at 0.1 MPa. Hence, the reduced
35 formation of Strecker aldehydes under high pressure could open a new possibility for process control
36 and optimization of the formation of these compounds.

37

38

39 **Keywords**

40 Strecker aldehydes; kinetics; thermal processing; high pressure high temperature processing; carrot
41 puree.

42

43 **1 Introduction**

44 Since the year 2000, the use of high pressure high temperature (HPHT) processing for sterilization of
45 foods has been the focus of intensive studies (although without industrial implementation so far)
46 (Hoogland et al., 2001; Krebbers et al., 2002; Matser et al., 2004; Wilson et al., 2008). HPHT is a
47 relatively young technology and its effect on important quality related attributes is not as well
48 understood as is the case for conventional thermal processing. Compared to conventional thermal
49 processing, HPHT has an extra process variable, pressure, which can have a different effect on the rate
50 constants of quality related chemical reactions. Depending on the thermodynamic properties
51 (activation volume) of the constituting reactions, high pressure might have an overall slowing-down or
52 accelerating effect on chemical reactions (Cheftel, 1995). In addition, HPHT processing takes advantage
53 of compression heating and decompression cooling to achieve faster heating and cooling rates, which
54 can result in shorter processing times as compared to conventional thermal processing (Barbosa-
55 Canovas & Juliano, 2008; Wilson et al., 2008). For low-acid, conduction heating food products, the
56 integrated effect of pressure, temperature and time can result in a better preservation of original
57 quality attributes (Barbosa-Canovas & Rodriguez, 2005; Rastogi et al., 2007). Therefore, there is a need
58 for comparative studies to identify the different effects of HPHT processing on quality aspects
59 compared to conventional thermal processing (Oey et al., 2008b; Oey et al., 2008a; Van der Plancken
60 et al., 2012). One of the key challenges is the development of an effective analytical and process
61 engineering strategy to identify and study quality-related chemical reactions that are differently
62 affected by this novel sterilization technique in comparison to thermal processing. From analytical
63 point of view, food quality investigations have evolved from targeted single response studies towards
64 a targeted multiresponse and untargeted multivariate fingerprinting approaches. In recent years,
65 chemical fingerprinting emerged as an interesting approach to study food quality changes at the
66 molecular level (Hall, 2011). In contrast to targeted approaches, in which focus is given to

67 predetermined particular compounds of interest (which is a hypothesis-based approach),
68 fingerprinting opens the possibility to uncover unexpected and unknown compound changes. As
69 clearly outlined by Grauwet et al. (2014) fingerprinting could be used as a hypothesis-free starting
70 point to screen for key quality changes in particular food extracts. Once the discriminating compounds
71 and reactions are identified, a kinetic study can be set-up to understand and quantify the effect of
72 individual processing variables. From a process engineering point of view, either the integrated impact
73 of the different set of processing variables or the effect of a single process variable, typically with a
74 kinetic study, on quality changes can be investigated.

75

76 In our previous study, the potential of headspace fingerprinting as a fast screening tool for quality
77 differences among thermal and HPHT sterilized carrot purees was demonstrated (Kebede et al., 2014).
78 Aiming for a fair comparison, the processing conditions of both treatments were selected targeting at
79 an equivalent microbial inactivation ($F_0 = 5$ min). Using the appropriate multivariate data analysis,
80 chemical fingerprinting enabled the selection of markers, compounds of which the detected quantities
81 are clearly different in the volatile food fraction of differently processed vegetables. In the carrot
82 purees, Strecker aldehydes, such as 3-methylbutanal (3-MB), were detected in a significantly higher
83 amount after microbial equivalent thermal sterilization compared to HPHT sterilization processes
84 (Kebede et al., 2014). These volatiles are reaction products of the Strecker degradation. The reaction
85 involves oxidative deamination and decarboxylation of α -amino acids (e.g. valine, isoleucine and
86 leucine) in the presence of α -dicarbonyl compounds which are formed during Maillard reaction
87 (Cremer & Eichner, 2000; Kerler et al., 2010). The observed reduction by HPHT processing was
88 interesting because the Strecker aldehydes are often linked to off-flavour development in processed
89 plant-based foods (Yaylayan, 2003; Rizzi, 2008; Cremer & Eichner, 2000). Designing a process from an
90 equivalent point of view (e.g. microbial impact, enzyme inactivation) is needed for an unbiased

comparison between different processing technologies and allows insight only into the integrated effect of the different set of processing variables. However, since the effect of each processing variable is not investigated individually with this experimental set-up, it is not possible to link the observed differences in the formation of Strecker aldehydes to one of the process variables (e.g. pressure, temperature and time) in particular. In addition, given the fact that with the fingerprinting approach only relative differences could be determined, the absolute quantification was not a direct research output. Therefore, there is a need for a quantitative kinetic study.

In this work, a kinetic study was set-up for 3-MB to obtain quantitative insight into the separate effects of the process parameters pressure, temperature and time. This is an important research step towards process control and optimization of the formation of these reaction products. As a case study, an orange carrot puree was selected. To the best of our knowledge, even though the formation of Strecker aldehydes in foods and food-related model systems has been studied, the present work is the first in literature to report the kinetics of their formation during HPHT processing.

2 Materials and methods

2.1 Preparation of the carrot purees

A single batch of freshly harvested orange carrots (cv. *Nerac*) was purchased at a local market. The carrots were carefully washed, peeled and cut into standardized cylindrical pieces of approximately 1 cm thickness. The carrot pieces were packed into low-density polyethylene bags. To prevent enzymatic reactions during processing, storage and thawing, the packaged carrots were blanched at 95 °C for 8 min in a water bath (Haake W15 DC-10, Clausthal-Zellerfeld, Germany). The blanching conditions were validated using a qualitative and quantitative peroxidase test. After blanching, the plastic bags were immediately cooled in ice water for 10 min, frozen in liquid nitrogen and stored in a freezer at -40 °C

114 until processing. Prior to processing, the samples were thawed overnight at 4 °C. In order to prepare
115 the puree, deionized water was added to the blanched carrot (1/1 w/w), blended for 1 min using a
116 Buchi mixer (B-400, BUCHI, Switzerland) and further homogenized by high pressure homogenization
117 (at 1000 bar while temperature maintained < 4 °C) (Panda 2K, Gea Niro Soavi, Mechelen, Belgium). The
118 pH of the sample was 6.1.

119 **2.2 Isothermal treatment**

120 For the thermal treatments, stainless steel tubes (13 mm inner diameter, 16 mm outer diameter, 150
121 mm length) were completely filled with carrot puree (no headspace), tightly closed and immersed in
122 an oil bath (Grant Instruments, UK) preset at the desired process temperature. Treatments were
123 performed at three different temperatures, 118 (a similar processing temperature as our previous
124 work, (Kebede et al., 2014)), 124 and 130 °C, for maximum holding times between 60 and 80 min at
125 isothermal conditions. During the treatments, the temperature of the purees was monitored using an
126 Ellab E-val temperature registration system (Ellab, Denmark) and thermocouples (type T, Thermo
127 Electric Benelux, Belgium).

128 **2.3 Isothermal-isobaric treatment**

129 For the HPHT treatments, Teflon (polytetrafluoroethylene) sample holders (diameter 12 mm, length
130 85 mm) were selected. The tubes were completely filled with carrot puree (no headspace), closed with
131 a movable stopper and vacuum-packed with double plastic bags. The treatments were performed in a
132 laboratory-scale 6-vessel (diameter vessel 2 cm, volume 43 cm³) high pressure equipment (Resato,
133 Netherlands). This equipment was provided with computer-controlled pressure build-up and data
134 logging software for pressure and temperature. Propylene glycol (PG fluid, Resato, Netherlands) was
135 used as a pressure medium. The HPHT processes were performed at 600 MPa combined with a process
136 temperature of 118 °C, for maximum holding times up to 180 min at isothermal-isobaric conditions. A
137 higher temperature (i.e. 124 and 130 °C) could not be selected because the isothermal-isobaric

138 conditions, desired for kinetic modeling, were difficult to maintain at these high temperatures. Using
139 only compression heating, product temperatures cannot be raised to the desired process
140 temperatures. Therefore, after loading the teflon sample holders into the pre-heated high pressure
141 vessels, the samples were allowed to heat up to an experimentally determined initial temperature of
142 75 °C. When this temperature was reached, the pressure build-up started. For pressure increase, the
143 equipment consists of a pressure prefill pump which builds up the pressure to 150 MPa with a single
144 piston displacement after which a high pressure intensifier can further built up the pressure at a
145 particular selected pressure build-up rate (10 MPa/sec was used in this work). After reaching 600 MPa,
146 the individual vessels were isolated, and an equilibration time of 1 min and 30 sec was taken into
147 account. At the selected holding times, the pressure was released from the vessels, which was
148 accompanied by a fast temperature drop inside the product (decompression cooling).

149 **2.4 Post treatment sample handling**

150 Following both treatments, samples were immediately transferred to ice water to further cool the
151 product. Afterwards, treated samples were emptied in a cooling room and transferred to a small
152 volume (10 ml) polyethylene terephthalate tubes with a polyethylene cap. Hereafter, the tubes were
153 frozen in liquid nitrogen and stored at -40 °C until analysis.

154 **2.5 Quantification of Strecker aldehydes**

155 3-Methylbutanal (3-MB) was analyzed with solid phase microextraction coupled to gas
156 chromatography-mass spectrometry (SPME-GC-MS). Because of the high volatility of this compound,
157 precautions had to be taken to obtain reliable and reproducible results. Standard stock solution (ca. 10
158 mg/ml in methanol) and working solution (ca. 1 µg/ml in deionized water) was obtained by making a
159 serial dilution of 3-MB (≥ 98 %, Sigma Aldrich, USA) in closed headspace amber glass vials (10 ml, VWR
160 International, Radnor, PA, USA) with a PTFE/silicone septum seal. 3-MB was added to the solvent with
161 a gastight syringe through the septum of the vials. Both the amount of solvent and the amount of

162 solution added were determined by differential weighing and the 3-MB concentration was calculated
163 from the respective masses. The solutions were prepared daily and stored on ice.

164 Quantitative determination of the aldehyde was carried out according to the standard addition
165 method. Samples were thawed overnight in the cooling room (4 °C). Each sample was divided into four
166 portions, 2.5 g each, and added into a 10 ml amber glass vial. To each vial, 2.5 ml saturated NaCl
167 solution was added and the vials were sealed with a septum. Next, a different volume (0, 100, 200 and
168 300 µl) of stock solution is added into the four vials. To obtain a standard total volume, a different
169 amount of deionized water was added into the vials. The samples were stored at a temperature of 10
170 °C until analysis.

171 The analyses were carried out on a gas chromatography (GC) system (7890N, Agilent Technologies,
172 Diegem, Belgium) coupled to a mass selective detector (MSD) (5977N, Agilent Technologies, Diegem,
173 Belgium) and equipped with a CTC Combi PAL autosampler (CTC Analytics, Switzerland). Targeting a
174 good signal, the HS-SPME-GC-MS method of analysis was optimized beforehand. The SPME fiber
175 (Supelco, USA) had a 85 µm carboxen/polydimethylsiloxane (CAR/PDMS) sorptive coating, which was
176 exposed to the headspace of the samples for 15 min at 40 °C. After headspace extraction, the fiber was
177 transferred to the GC injection port, where the adsorbed compounds were thermally desorbed for 2
178 min at 230 °C. After each run, the fiber was thermally cleaned for 5 min at
179 300 °C in the conditioning station of the autosampler. The volatiles were injected in split (1/5) mode
180 and subsequently separated on HP-5MS capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness,
181 Keysight Technologies, USA), using helium as the carrier gas at a constant flow rate of 1.2 ml/min. The
182 GC oven temperature was programmed from a starting temperature of 40 °C, which was retained for
183 5 min, to 100 °C at 4 °C/min, then ramped to 300 °C at 100 °C/min and kept constant at 300 °C for 2 min
184 before cooling back to the initial temperature. Mass spectra were obtained by electron ionisation (EI)

185 at 70 eV, in the combined SCAN and SIM mode. The scanning range extended m/z 35-400. The selected
186 ions monitored were m/z 57 (quantifier) and 41 (qualifier). MS ion source and quadrupole
187 temperatures were 230 and 150 °C, respectively. The dwell time was 10 seconds. The identity of the 3-
188 MB was confirmed by comparison with retention times of standards and by calculating the response
189 ratio of the qualifier ions and the quantifier ions, as analogous to the identification of the aldehyde.

190 **2.6 Analysis of the kinetic data**

191 3-Methylbutanal (3-MB) formation was kinetically modeled as a function of processing conditions. For
192 a detailed discussion on the general principles of kinetic modeling, the reader is referred to the work
193 of van Boekel (2008). In general, chemical changes in foods are often reported to follow zero- or first-
194 order kinetics, which basically means that the formation or the degradation of the compound under
195 consideration is (apparently) independent of the reactant concentration or the result of a
196 monomolecular reaction. This observation might hold for a relatively short period of time. However, as
197 the chemical reaction proceeds, the reaction conditions will start to change (e.g. limiting reactant
198 concentrations, pH shift) and the mechanistic basis for the selection of these kinetic models might
199 become blurred. In such a case, any empirical model that provides an acceptable fit of the data might
200 as well describe the proceeding of the chemical reaction. Given the complexity of the reaction
201 pathways of the Strecker degradation (cf. Introduction) and to comprehensively describe the reaction
202 stages, an empirical, logistic model was selected in the present study (Eq. (1)):

$$203 \quad A = \frac{A_s}{1 + \exp\left[\frac{4 * k_{max}}{A_s}(\lambda - t) + 2\right]} \quad (1)$$

204

205 where A (µg/g puree) is representing the compound concentration at processing time t (min), A_s (µg/g
206 puree) a plateau concentration at long processing times, k_{max} (µg/g puree/min) the maximum reaction
207 rate constant at the at the inflection point, and λ (min) the duration of the lag phase, for a given

208 processing condition. This logistic model is often used for modeling isothermal growth in microbiology,
209 because the kinetic parameters provide an excellent means for the characterization of sigmoidal
210 curves. The temperature dependence of the kinetic parameters can sometimes be described by
211 theoretically derived or empirical relationships. In the present study, the temperature dependence of
212 the maximum reaction rate constant $k_{\max}$ followed the Arrhenius equation (Eq. (2)), whereas the
213 duration of the lag phase could be described as a linear function of the processing temperature (Eq.
214 (3)):

$$215 \quad k_T = k_{\text{ref}} \cdot \exp\left(\frac{-E_a}{R} \left(\frac{1}{T} - \frac{1}{T_{\text{ref}}}\right)\right) \quad (2)$$

$$216 \quad \lambda_T = \lambda_{\text{ref}} + b_T(T - T_{\text{ref}}) \quad (3)$$

217

218 where the activation energy E_a (KJ/mol) and parameter b_T (min/K) represent a quantitative measure
219 for the temperature-dependencies of the maximum reaction rate constant $k_{\max}$ and the lag time λ ,
220 respectively. Both temperature relationships were derived empirically, although the Arrhenius
221 equation (Arrhenius, 1889) was afterwards put in a theoretical perspective. For statistical reasons, the
222 processing temperature T (K) was reparametrized against a reference temperature T_{ref} (K). R is the
223 universal gas constant (8.314 J/K mol).

224 The kinetic modeling of the 3-MB concentrations in the carrot puree was performed in two steps. First,
225 a suitable kinetic model was selected by visual inspection of the concentration, parity, and residual
226 plots, and by the calculation of R^2_{adjusted} . Second, the corresponding kinetic parameters were estimated
227 using nonlinear regression (SAS version 9.4, Cary, North Carolina).

228 3 Results and discussion

229 3.1 Strecker aldehyde formation during isothermal treatments

230 In order to quantify the effects of the process parameters temperature and time on 3-methylbutanal
231 (3-MB) formation in vegetable-based food systems, a kinetic study was set up in carrot puree as a case
232 study. Carrot puree was thermally treated at three different isothermal temperatures (118, 124 and
233 130 °C) and the formation of aldehyde was followed as a function of time (only during the isothermal
234 phase). The formation of the 3-MB seems to follow a sequence of steps (**Fig. 1A**). During the beginning
235 of the isothermal treatment, after t_{iso} (the time the isothermal condition is reached), the formation of
236 3-MB appears to have an induction phase, in which the compound concentrations remained
237 approximately constant at their initial level. After this lag time, the 3-MB concentration started to
238 increase as a function of processing time. A comparable trend was also observed by Cremer et al.
239 (2000), when investigating the reaction kinetics for the formation of Strecker aldehydes in model
240 systems (containing glucose and leucine). The authors hypothesized that the induction phase can be
241 due to a need for an intermediate Maillard reaction product to be formed before further
242 decomposition into the reactive α -dicarbonyl compounds (that undergo the Strecker degradation). In
243 the present work, both the lag time and the rate of 3-MB formation seem to be affected by the
244 processing temperature (**Fig. 1A**). At all temperatures, the increase in 3-MB concentration continued
245 for most of the processing times. However, at the end, the rate of 3-MB formation slowly decreased,
246 which might suggest that for long processing times, the 3-MB concentration was moving toward a
247 maximum or plateau concentration.

248 For a quantitative discussion of the 3-MB formation during processing of the carrot puree, the results
249 were analyzed using kinetic modeling. As discussed above, the duration of the lag phase and the
250 formation rate seem to be dependent on the processing temperature. As can be seen from **Fig. 1A**, at
251 high processing temperatures, the lag phase was shorter and the rate of 3-MB formation was clearly

252 higher. Similar to the approach followed by Palmers et al (2015), by combining the expressions for the
 253 temperature relationship of both kinetic parameters (Eqs. (2) and (3), respectively) with the three-
 254 parameter logistic model (Eq. (1)), an overall kinetic model was obtained that described the 3-MB
 255 formation in carrot puree as a function of the processing temperature and time.

256
 257 **Fig. 1.** (A) Concentration of 3-MB as a function of heating time at a pressure of 0.1 MPa and a temperature of 118 (♦), 124 (■) and 130
 258 (▲) °C. The full lines represent the 3-MB concentrations estimated by an overall kinetic model, which is obtained by combining the
 259 expressions for the temperature relationship of both kinetic parameters (Eqs. (2) and (3), respectively) with the three-parameter logistic
 260 model (Eq. (1)), while the measured concentrations are represented by the symbols. The formation of the compounds was followed
 261 during the isothermal phase (starting from $t_{iso} = 0$). (B) Parity plot of the estimated versus the measured 3-MB concentrations.

262
 263 The overall logistic model consisted of five kinetic parameters, expressed against a reference
 264 temperature of 124 °C. The kinetic parameters were estimated by means of nonlinear one-step

265 regression and represented in **Table 1**, together with their explanation. Visual inspection of the parity
 266 (**Fig. 1B**) and residual plots (graphs not shown) and the calculation of R^2_{adjusted} (0.976) all indicated a
 267 good fit of the overall logistic model. In the present processing experiment, there were no data points
 268 that prove the actual existence of a plateau concentration for 3-MB.

269 **Table 1**

270 Parameter estimates with 95% confidence intervals for the five-parameter logistic model (i.e. combination of Eqs. (1)–(3))
 271 describing 3-MB formation in the in carrot puree at three process temperatures, 118, 124 and 130 °C. The kinetic
 272 parameters were estimated by means of nonlinear one-step regression, using a reference temperature of 124 °C.

Kinetic parameters	Parameter estimate	Explanation	273
A_s (µg/g puree)	1.031 ± 0.061	Hypothetical plateau concentration	
K_{ref} (µg/g puree/min)	0.019 ± 0.002	Maximum reaction rate constant at $T_{\text{ref}} = 124^\circ\text{C}$	274
E_a (KJ/mol)	127.344 ± 32.123	Activation energy	
λ_{ref} (min)	46.596 ± 2.536	Duration of the lag phase at $T_{\text{ref}} = 124^\circ\text{C}$	275
b_T (min/K)	-2.741 ± 0.477	Temperature-dependency of the lag phase	276

277

278 Therefore, it is not possible to attach much significance to the parameter estimates for the plateau
 279 concentration. The maximum reaction rate constant (at the inflection point of the sigmoidal curve) and
 280 the lag time for the overall model were estimated at the reference temperature of 124 °C. The overall
 281 logistic model also provided a quantitative measure for the temperature-dependencies of the
 282 maximum reaction rate constant (activation energy of 127.344 ± 32.123 KJ/mol) and the lag time
 283 (decrease of 2.741 ± 0.477 min/K). In general, in the present work, both the lag time and the rate of 3-
 284 MB formation were clearly affected by the processing temperature. At high processing temperatures,
 285 the lag phase was shorter and the rate of 3-MB formation was clearly higher. The logistic models of the
 286 present study can be a tool for design and optimization of the processing conditions, but the kinetic
 287 parameters should always be established specifically for the product under consideration.

288 3.2 Strecker aldehyde formation during isothermal-isobaric treatments

289 To investigate the effect of the process parameter pressure on the rate of 3-MB formation, the carrot
290 puree was treated at 0.1 and 600 MPa at a process temperature of 118 °C, and the concentration was
291 followed as a function of time. No HPHT treatment was performed at 124 and 130 °C, because the
292 isothermal-isobaric conditions, desired for kinetic modeling, were difficult to maintain at these high
293 temperatures. Similar to the isothermal treatments (section 3.1), the formation of 3-MB can be
294 described by an induction phase (at the beginning of the isothermal-isobaric treatment, after t_{iso} (**Fig.**
295 **2A**))) followed by an increasing formation phase. However, at the end, the rate of 3-MB formation
296 slowly decreased, which might suggest that for long processing times, the 3-MB concentration was
297 moving toward a maximum or plateau concentration. This trend was clearly observed at 0.1 MPa than
298 at 600 MPa. The increase in 3-MB concentrations was best described by an empirical, logistic model
299 with three kinetic parameters (Eq. (1)). At each processing condition, the kinetic parameters were
300 estimated by means of nonlinear regression and represented in **Table 2**, together with the
301 corresponding value of $R^2_{adjusted}$.

Fig. 2. (A) Concentration of 3-MB as a function of process time at a temperature of 118 °C and a pressure of 0.1 MPa (♦) and 600 MPa (■). The full lines represent the 3-MB concentrations estimated by an empirical, three-parameter logistic model (Eq. (1)), while the measured concentrations are represented by the symbols. The formation of the compounds was followed during the isothermal-isobaric phase (starting from $t_{iso} = 0$). (B) Parity plot of the estimated versus the measured 3-MB concentrations.

Fig. 2A shows the fit for the three-parameter logistic models describing the 3-MB formation at 0.1 and 600 MPa processing condition. Next to $R^2_{adjusted}$, model evaluation was based on visual inspection of the parity plot (**Fig. 2B**) and a plot of the residuals (not shown), which all indicated a good fit of the selected models. In the present processing experiment, the plateau concentration was more than 2 times higher at 0.1 MPa compared to the processing at 600 MPa. Furthermore, the maximum reaction rate constant was more than 10 times higher and the lag time was by half shorter for the process at 0.1 MPa compared to at 600 MPa. Therefore, the formation rate was significantly reduced at higher pressure (opposite to the effect of temperature).

315 **Table 2**

316 Parameter estimates with 95 % confidence interval for the empirical, three-parameter logistic model (Eq. 1) describing 3-
 317 MB formation during thermal (0.1 MPa) and HPHT (600 MPa) processing at a temperature of 118 °C.

Kinetic parameters	Parameter estimate		Explanation
	Thermal	HPHT	
A_s (µg/g puree)	0.171 ± 0.007	0.064 ± 0.034	Hypothetical plateau concentration
$K_{\max}$ (µg/g puree/min)	0.00453 ± 0.000483	0.000347 ± 0.00009	Maximum reaction rate constant
λ (min)	31.766 ± 2.189	69.044 ± 25.706	Duration of the lag phase
R^2_{adj}	0.99	0.97	Adjusted R^2

318

319 In the next step, the 3-MB concentration was compared at ambient and high pressure conditions in
 320 the industrially relevant area. At 118 °C, the target sterilization condition, inactivation of spores of
 321 *Clostridium botulinum* ($F_{121.1^\circ\text{C}}^{10^\circ\text{C}}(F_0) = 5$ min), was achieved after 10 min during the isothermal phase
 322 (afte t_{iso}). At this industrially relevant condition, the concentration of 3-MB reached 0.01 µg/g puree at
 323 0.1 MPa, this was more than 2.5 times as compared to at 600 MPa. Considering the fact that the
 324 Strecker aldehydes are low-odor threshold compounds (e.g. 0.2 µg/L of water of 3-MB (Mayer et al.,
 325 2008)), the concentration reached after thermal processing (at 0.1 MPa) might affect the flavor of
 326 vegetable-based foods. Therefore, HPHT processing is an interesting alternative technology to
 327 minimize formation of these malty/off-odor compounds compared to the conventional thermal
 328 processing. Broadly speaking, the use of HPHT processing to reduce the negative impact of the
 329 Maillard and its side reactions on some food production could become more and more relevant.

330 In order to put the effect of HPHT processing on the overall quality into perspective, it is essential to
 331 discuss its impact on other relevant quality attributes (e.g color degradation, texture and viscosity
 332 changes) (Van der Plancken et al., 2012). Vervoort et al. (2012) compared the impact of different high
 333 pressure and thermal treatments at intensities ranging from mild pasteurization to sterilization

334 conditions on a wide range of quality attributes, including specific (micro)nutrients, process-induced
335 contaminants, enzyme activities and other relevant quality aspects, such as texture, dry matter
336 content and color. This study demonstrated that the potential benefit of high pressure over thermal
337 processing of carrots is largely dependent on the processing intensity applied. In general, thermal
338 sterilization affected carrot quality the most compared to HPHT sterilization. Nevertheless, future
339 studies should strive to fill the knowledge gaps in the context of process uniformity and impact on
340 food safety and quality attributes.

341 **4 Conclusions**

342 The present study demonstrated the importance of processing conditions (temperature, pressure and
343 time) as a strong means for the control of Strecker aldehyde formation in vegetables purees. A kinetic
344 study was set up to quantify the effects of single process parameters on the changes of 3-
345 methylbutanal (3-MB) in carrot puree as a case study. The formation of 3-MB can be described by an
346 induction phase followed by an increasing formation phase. However, at the end, the rate of 3-MB
347 formation slowly decreased, which might suggest that for long processing times, the 3-MB
348 concentration was moving toward a maximum or plateau concentration. The increase in 3-MB
349 concentrations was best described by an empirical, logistic model. During the isothermal treatment at
350 atmospheric pressure, the maximum reaction rate constant of 3-MB formation was increased as a
351 function of processing temperature. However, the formation rate was more than 10 times slower at
352 high pressure (600 MPa) compared to at 0.1 MPa. Considering the fact that these low-odor threshold
353 volatiles have been negatively associated to flavor of thermally sterilized plant-based food products,
354 their reduced formation under high pressure could open a new possibility for process control and
355 optimization of the formation of these compounds. Strecker aldehydes formation is the result of many
356 interacting, complex reaction pathways rather than a single conversion step. As a result, the observed
357 reaction rate constant is a composite one, strongly influenced by intrinsic matrix properties (e.g.

precursors, pH). The empirical nature of the logistic models did not allow to obtain any mechanistic insight into these complex reaction pathways during processing. For unambiguous identification of the main Strecker aldehyde precursors during processing, a specific experimental design should be set up (e.g. using labeled precursors or addition of known precursor concentrations). However, the results of this section demonstrated that the processing conditions (temperature, pressure and time) provide a strong means for the control of Strecker aldehyde formation in vegetables purees. Furthermore, since quality related chemical reactions change during storage after processing, it would be interesting to investigate the kinetics of the change in Strecker aldehyde concentration during storage.

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372

373 6 REFERENCE LIST

374

375 Arrhenius, A. (1889). Über die Reaktionsgeschwindigkeit bei der Inversion von Rohrzucker durch Säuren. *Zeitschrift für*
376 *physikalische Chemie*, 4, 227-248.

377 Barbosa-Canovas, G. V. & Juliano, P. (2008). Food sterilization by combining high pressure and thermal energy. In *Food*
378 *Engineering: Integrated Approaches* (pp. 9-46). New York: Springer.

379 Barbosa-Canovas, G. V. & Rodriguez, J. J. (2005). Thermodynamic Aspects of High Hydrostatic Pressure Food Processing. In
380 G.V.Ed.Barbosa-Canovas (Ed.), (pp. 183-205). Florida, USA: CRC Press.

381 Cheftel, J. C. (1995). Review: High-pressure, microbial inactivation and food preservation. *Food Science and Technology*
382 *International*, 1, 75-90.

383 Cremer, D. R. & Eichner, K. (2000). The reaction kinetics for the formation of Strecker aldehydes in low moisture model
384 systems and in plant powders. *Food Chemistry*, 71, 37-43.

385 Grauwet, T., Vervoort, L., Colle, I., Van Loey, A., & Hendrickx, M. (2014). From fingerprinting to kinetics in evaluating food
386 quality changes. *Trends in Biotechnology*, 32, 125-131.

387 Hall, R. D. (2011). Plant Metabolomics in a Nutshell: Potential and Future Challenges. In *Annual Plant Reviews Volume 43*
388 (pp. 1-24). Wiley-Blackwell.

389 Hoogland, H., de Heij, W., & Van Schepdael, L. (2001). High pressure sterilization: novel technology, new products, new
390 opportunities. *New Food*, 21-26.

391 Kebede, B. T., Grauwet, T., Palmers, S., Vervoort, L., Carle, R., Hendrickx, M. et al. (2014). Effect of high pressure high
392 temperature processing on the volatile fraction of differently coloured carrots. *Food Chemistry*, 153, 340-352.

393 Kerler, J., Winkel, C., Davidek, T., & Blank, I. (2010). Basic chemistry and process conditions for reaction flavours with
394 particular focus on Maillard-type reactions. In A.J.Taylor & R. S. T. Linforth (Eds.), *Food Flavour Technology* (2 ed.,
395 pp. 51-81). Blackwell Publishing Ltd.

396 Krebbers, B., Matser, A. M., Koets, M., Bartels, P., & Van den Berg, R. (2002). High pressure-temperature processing as an
397 alternative for preserving basil. *High Pressure Research*, 22, 711-714.

398 Matser, A. M., Krebbers, B., van den Berg, R. W., & Bartels, P. V. (2004). Advantages of high pressure sterilisation on quality
399 of food products. *Trends in Food Science & Technology*, 15, 79-85.

400 Mayer, F., Takeoka, G. R., Buttery, R. G., Whitehand, L. C., Naim, M., & Rabinowitch, H. D. (2008). Studies on the Aroma of
401 Five Fresh Tomato Cultivars and the Precursors of cis- and trans-4,5-Epoxy-(E)-2-Decenals and Methional. *Journal*
402 *of Agricultural and Food Chemistry*, 56, 3749-3757.

403 Oey, I., Lille, M., Van Loey, A., & Hendrickx, M. (2008a). Effect of high-pressure processing on colour, texture and flavour of
404 fruit- and vegetable-based food products: a review. *Trends in Food Science & Technology*, 19, 320-328.

405 Oey, I., Van der Plancken, I., Van Loey, A., & Hendrickx, M. (2008b). Does high pressure processing influence nutritional
406 aspects of plant based food systems? *Trends in Food Science & Technology*, 19, 300-308.

407 Palmers, S., Grauwet, T., Celus, M., Wibowo, S., Kebede, B. T., Hendrickx, M. E. et al. (2015). A kinetic study of furan
408 formation during storage of shelf-stable fruit juices. *Journal of Food Engineering*, 165, 74-81.

409 Rastogi, N. K., Raghavarao, K. S. M. S., Balasubramaniam, V. M., Niranjana, K., & Knorr, D. (2007). Opportunities and
410 challenges in high pressure processing of foods. *Critical Reviews in Food Science and Nutrition*, 47, 69-112.

411 Rizzi, G. P. (2008). The strecker degradation of amino acids: Newer avenues for flavor formation. *Food Reviews*
412 *International*, 24, 416-435.

413 Van Boekel, M. A. J. S. (2008). Kinetic Modeling of Food Quality: A Critical Review. *Comprehensive Reviews in Food Science*
414 *and Food Safety*, 7, 144-158.

415 Van der Plancken, I., Verbeyst, L., De Vleeschouwer, K., Grauwet, T., Heinio, R. L., Husband, F. A. et al. (2012). (Bio)chemical
416 reactions during high pressure/high temperature processing affect safety and quality of plant-based foods. *Trends*
417 *in Food Science & Technology*, 23, 28-38.

418 Vervoort, L., Van der Plancken, L., Grauwet, T., Verlinde, P., Matser, A., Hendrickx, M. et al. (2012). Thermal versus high
419 pressure processing of carrots: A comparative pilot-scale study on equivalent basis. *Innovative Food Science &*
420 *Emerging Technologies*, 15, 1-13.

421 Wilson, D. R., Dabrowski, L., Stringer, S., Moezelaar, R., & Brocklehurst, T. F. (2008). High pressure in combination with
422 elevated temperature as a method for the sterilisation of food. *Trends in Food Science & Technology*, 19, 289-299.

423 Yaylayan, V. A. (2003). Recent Advances in the Chemistry of Strecker Degradation and Amadori Rearrangement:
424 Implications to Aroma and Color Formation. *Food Science and Technology Research*, 9, 1-6.

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7 List of figures

Fig. 1. (A) Concentration of 3-MB as a function of heating time at a pressure of 0.1 MPa and a temperature of 118 (◆), 124 (■) and 130 (▲) °C. The full lines represent the 3-MB concentrations estimated by an overall kinetic model, which is obtained by combining the expressions for the temperature relationship of both kinetic parameters (Eqs. (2) and (3), respectively) with the three-parameter logistic model (Eq. (1)), while the measured concentrations are represented by the symbols. The formation of the compounds was followed during the isothermal phase (starting from $t_{\text{iso}} = 0$). (B) Parity plot of the estimated versus the measured 3-MB concentrations.

Fig. 2. (A) Concentration of 3-MB as a function of process time at a temperature of 118 °C and a pressure of 0.1 MPa (◆) and 600 MPa (■). The full lines represent the 3-MB concentrations estimated by an empirical, three-parameter logistic model (Eq. (1)), while the measured concentrations are represented by the symbols. The formation of the compounds was followed during the isothermal-isobaric phase (starting from $t_{\text{iso}} = 0$). (B) Parity plot of the estimated versus the measured 3-MB concentrations.

443 **8 List of tables**

444

445 **Table 1**

446 Parameter estimates with 95% confidence intervals for the five-parameter logistic model (i.e. combination of Eqs. (1)–(3))
447 describing 3-MB formation in the carrot puree at three process temperatures, 118, 124 and 130 °C. The kinetic parameters
448 were estimated by means of nonlinear one-step regression, using a reference temperature of 124 °C.

449

450 **Table 2**

451 Parameter estimates with 95 % confidence interval for the empirical, three-parameter logistic model (Eq. 1) describing 3-
452 MB formation during thermal (0.1 MPa) and HPHT (600 MPa) processing at a temperature of 118 °C.

453