1 **Insight into the evolution of flavor compounds during cooking of common beans utilizing a**
2 **headspace untargeted fingerprinting approach**

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11 **Abstract**

12 Beans age during storage leading to prolonged cooking times. Chemical reactions that occur
13 during cooking lead to volatile production and flavor generation. Whereas few studies profiled the
14 volatile fingerprint of either non-cooked beans or beans cooked for a specific time, this study
15 explored the evolution of volatiles through headspace fingerprinting of beans cooked at 95 °C to
16 different extents. The influence of aging of beans on this evolution was investigated. Cooking time
17 clearly influenced the evolution of volatiles for both fresh (non-aged) and aged beans. Aged beans
18 exhibited more discriminant compounds than fresh beans regardless of texture considerations due
19 to differences in pre-history of the beans. Strecker aldehydes, sulphur compounds and furan
20 compounds were identified as marker compounds and were linked to mainly lipid oxidation and
21 Maillard reactions. In conclusion, both aging prior to cooking and the cooking process itself largely
22 influence the evolution of volatile compounds during cooking.

23

24 **Keywords**

25 Beans; Aging; Cooking; Volatile compounds; Marker compounds; Maillard reactions

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27

28 **Highlights**

29 The evolution of volatile compounds during cooking of beans was investigated
30 Cooking time and aging greatly influenced the volatile profiles and their evolution
31 Strecker aldehydes, furan and sulphur compounds significantly changed during cooking
32 Formation/changes in volatiles were linked to lipid oxidation and Maillard reactions
33

34 **Chemical compounds studied in this article**

35 Sodium chloride (PubChem CID: 5234)

36 **1 Introduction**

37 Cooking of common beans (*Phaseolus vulgaris*) is performed to achieve palatability and to
38 inactivate phytohemagglutinin, a protein that can render beans poisonous when consumed either
39 raw or undercooked as revealed in a study by Rodhouse *et al.* (1990). The cooking process exerts
40 influence on sensory properties such as appearance, texture and flavor, which are usually
41 evaluated for quality determination. For long, the attribute of critical importance for cooking quality
42 of beans has been texture which can be assessed through both subjective (fingerpressing tests)
43 and objective (compression and cutting tests) hardness measurements. Compared with texture,
44 flavor perception has a much more complex multimodal nature that includes olfaction, taste,
45 somatosenses and various sensory elements that are processed by the brain (Reineccius, 2005).
46 The contribution of olfactory elements to flavor involves odor and aroma, which are perceived
47 through sniffing (orthonasal) and, mouth and respiratory system (retronasal), respectively (Van
48 Ruth, 2001). It is a challenge to differentiate volatile compounds that are perceived through
49 orthonasal means, giving rise to odor, those perceived through retronasal means, giving rise to
50 aroma and the flavor inactive volatiles. As such, all these compounds are herein collectively
51 referred to as volatile compounds.

52 To date, only a few studies are available on volatile compounds originating from *P. vulgaris* beans
53 during cooking (Ma, Boye, Azarnia, & Simpson, 2016; Mishra, Tripathi, Gupta, & Variyar, 2017;
54 Van Ruth, Dings, Aprea, & Otake, 2005). In addition, these studies are very limited in their scope.

55 First, the beans were either non-cooked or only a single cooking time was considered thereby
56 neglecting the evolution of volatiles and associated influence on bean flavor development during
57 the processing. However, in the current study, it was intended to gain insight into the evolution of
58 volatile compounds during the cooking process through analysis of beans cooked for different
59 times and furthermore, to investigate those compounds that change significantly during cooking,
60 using multi-variate data analysis (MVDA) techniques. Such compounds can then be used as
61 marker compounds for flavor generation during cooking of beans. Secondly, in all studies
62 mentioned above, only fresh beans were analyzed. It has been well established that storage
63 conditions, namely temperature and relative humidity, are important extrinsic factors that influence
64 the cooking quality of beans. Storage of beans under high temperature and high relative humidity
65 conditions induces development of a defect, hard-to-cook (HTC), that leads to prolonged cooking
66 times and consequences thereof (Paredes-López, Carabez-Trejo, Palma-Tirado, & Reyes-Moreno,
67 1991). It was hypothesised that HTC influences the cooking quality of beans and this has been
68 proven particularly for texture, leading to HTC being described as a textural defect. Therefore, in
69 this study, it was intended to evaluate this hypothesis with regards to flavor development. In this
70 context, the aim of the study was to investigate the evolution of volatile compounds during cooking
71 of beans and the extent to which storage-induced aging influences the volatile fingerprint after
72 storage and during subsequent cooking. Moreover, the kinetics of relevant markers for cooking
73 were determined as well as how they are influenced by the aging process prior to cooking.

74 Since volatile compounds indicate specific chemical reactions, volatile fingerprints are expected to
75 facilitate identification of chemical reactions occurring during cooking and the extent to which they
76 are influenced by presence of the HTC defect. This is interesting given that common beans are
77 nutritious legume seeds that are comprised of proteins, complex carbohydrates, vitamins and
78 several minerals (Reyes-Moreno, Paredes-López and Gonzalez, 1993), some of which can take
79 part in many chemical reactions.

80 **2 Materials and methods**

81 **2.1 Raw materials**

82 Canadian wonder common beans (*P. vulgaris*) bought from Thika Station (Kenya Agricultural and
83 Livestock Research Organization, Kenya) were sorted and cleaned. To facilitate development of
84 the HTC defect, part of the beans was incubated at 35 °C and 83% relative humidity for 3 months.
85 In order to achieve and maintain the relative humidity, the beans were incubated above a saturated
86 potassium chloride solution. The incubated and non-incubated beans are herein referred to as
87 'aged' and 'fresh' beans, respectively. All the aforementioned beans were stored in a freezer at
88 - 40 °C until use. The experimental approach of the current study is illustrated schematically in
89 Fig. 1.

90 **2.2 Determination of cooking profiles of beans**

91 Cooking profiles of fresh and aged beans were determined according to a procedure described by
92 Chigwedere *et al.* (2018). In a nutshell, fresh and aged beans were soaked and subsequently
93 cooked in demineralized water, with sampling of 20 seeds every 30 min. Soaked non-cooked
94 seeds were regarded as the control sample. The hardness of a cotyledon from each seed was
95 measured as an indicator for texture using a TA-X2i texture analyzer (Stable Microsystems,
96 Goldaming, England). A cylindrical flat head aluminum probe of diameter 25 mm was utilized at a
97 pre-test, test and post-test speed of 2, 1 and 2 mm/s, respectively. Averages of the measurements
98 and their associated standard deviations were plotted against cooking time to generate cooking
99 profiles.

100 **2.3 Untargeted fingerprinting of volatiles during cooking of beans**

101 **2.3.1 Sample preparation**

102 Based on the cooking profiles of both fresh and aged beans, a number of specific cooking times
103 were selected for investigation of the evolution of volatile compounds during cooking. From the
104 results obtained, the influence of differences in bean hardness on volatile compounds was
105 elucidated by comparing volatile profiles of fresh and aged beans cooked for the same time.
106 Moreover, cooking times for which fresh and aged beans attained similar hardness were selected

107 (based on cooking profiles) and their volatile profiles were compared in order to investigate if
108 similar hardness would imply similar volatile profiles.

109 Twenty bean seeds were soaked in demineralized water in a 1:4 (w/v) ratio, by placing the
110 samples in a water bath at 25 °C for 16 h. The soaked seeds and soaking water were transferred
111 into screw cap Pyrex tubes for cooking in a water bath at 95 °C for the selected cooking times
112 except for the control sample which consisted of soaked non-cooked beans. By cooking in screw
113 cap Pyrex tubes, the volatile compounds could be contained in the headspace thus preventing
114 their loss during cooking. Following that, the tubes were immersed in an ice bath for 30 min in
115 order to arrest the cooking process and condense the volatiles. Thereafter, the contents of each
116 tube were mixed using an IKA T25 digital ultra-turrax (Janke and Kunkel, Germany) at 8000 rpm
117 for 1 min. An ice bath was utilized to avoid heating-up of the sample which could promote escape
118 of many volatiles during mixing. The samples were frozen in liquid nitrogen and stored at - 40 °C
119 until use. Six repetitions were made for each sample.

120 **2.3.2 Analysis of volatiles from cooked beans**

121 Sample analysis was conducted using an untargeted fingerprinting approach following the method
122 described by Kebede *et al.* (2015) with modifications. Frozen samples were thawed in an ice bath
123 at 4 °C overnight, then 2.5 g was weighed into a 20 mL amber glass vial (VWR International,
124 Radnor, USA) to which 2.5 mL of saturated sodium chloride solution was added. Screw-caps with
125 silicon septum seals (Grace, Columbia, MD) were used to tightly close the vials before vortexing.
126 For profiling the volatiles, a headspace-solid phase microextraction-gas chromatography-mass
127 spectrometry (HS-SPME-GC-MS) system (Keysight Technologies, Diegem, Belgium) was utilized.
128 It was equipped with a CombiPAL autosampler (CTC Analytics, Zwingen, Switzerland) set at 4 °C
129 and fitted with a 30 m x 0.25 mm x 0.25 µm polar HP INNOWax capillary column (Agilent
130 Technologies, Santa Clara, California). Each sample was incubated at 40 °C for 20 min, with
131 agitation at 500 rpm after which a pre-conditioned (according to the manufacturer's instructions)
132 fiber with a 30/50 µm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) sorptive
133 coating (Stableflex, Supelco, Bellefonte, Pennsylvania) was used to extract a wide range of volatile

134 compounds from the headspace of the vial for 10 min. Extracted volatiles were desorbed in the
135 injection port at 230 °C for 2 min, then in splitless mode, they were injected onto the column for
136 separation with helium as the carrier gas at 1.5 mL/min. To facilitate separation and elution of the
137 injected volatile compounds, the GC oven was maintained at 40 °C for 2 min before the
138 temperature was ramped up to 80 °C at 5 °C/min and maintained for 1 min after which it was
139 ramped again to 220 °C at 6 °C/min. After a holding time of 2 min, a final ramp at 50 °C/min to 250
140 °C was made and immediately, the oven was cooled back to 40 °C. For the MS, the temperatures
141 of the quadrupole which was used at 70 eV, and the ion source were 150 °C and
142 230 °C, respectively while a mass-to-charge ratio scanning range of 35-550 at 3.8 scans/s was
143 employed. A standard, being a mixture of n-alkanes (C₈-C₂₂), was analyzed in order to obtain a
144 retention index standard (calibration data). Specifically, 10 µL of the alkane standard was mixed
145 with 5 mL of demineralized water in a 20 mL vial and analyzed as described above.

146 **2.3.3 Data analysis**

147 **2.3.3.1 Qualitative analysis of the headspace fractions of beans**

148 Data analysis was accomplished in five steps that include pre-processing, multivariate analysis,
149 marker selection and identification, and kinetic modeling of markers. The total ion chromatograms
150 obtained from HS-SPME-GC-MS analyses of the samples can be complex due to co-elution of
151 volatile compounds, leading to identification challenges. An automated mass deconvolution and
152 identification system (AMDIS) (Version 2.72, 2014, National Institute of Standards and Technology
153 (NIST), Gaithersburg, MD, USA) in which a retention index standard was established was used as
154 a first pre-processing step. Therefore, the chromatograms were deconvoluted using AMDIS in
155 order to obtain 'pure' component spectra. The spectra obtained were further subjected to peak
156 filtering and alignment utilizing a second pre-processing software, mass profiler professional (MPP)
157 (Version 12.0, 2012, Agilent Technologies, Diegem, Belgium). From MPP, a data table of samples
158 and peak areas of volatile compounds which are herein referred to as objects and variables,
159 respectively, was obtained. Hereafter, a manual verification of the pre-processing was performed
160 followed by data mean-centering and weighing of the variables by their standard deviations to

161 accord them equal variance. Multivariate data analysis which comprised different types of
162 statistical analyses including principal component analysis (PCA), partial least squares (PLS) and
163 partial least squares-discriminate analysis (PLS-DA) was conducted using Solo (Version 6.5, 2011,
164 Eigenvector Research, Wenatchee, WA, USA). The volatile compounds were considered as X-
165 variables whilst the different (classes of) objects were considered as Y-variables. First, PCA was
166 performed as an unsupervised exploratory classification technique with which potential outliers
167 could be detected for each class of objects. On the one hand, PLS was used to investigate the
168 influence of increased cooking time of both fresh and aged beans on evolution of volatile
169 compounds since cooking time is a continuous independent variable. On the other hand, PLS-DA
170 was used to compare the impact of different treatments of fresh and aged beans on their volatile
171 profiles. As such, volatiles from fresh and aged beans cooked either for the same time or to similar
172 hardness were analyzed using PLS-DA as these were considered categorical data. The selected
173 multivariate models (PLS and PLS-DA) were comprised of latent variables that contributed at least
174 2% of the Y-variance explained by the models.

175 A projection of variables on a coordinate system produces a loading plot. Loadings are
176 contributions of the original variables to selection of latent variables for determination of maximum
177 variance. Likewise, a projection of objects in variable space produces a score plot wherein the
178 proximity of classes of objects indicates similarities or differences in their volatile profiles.

179 Qualitative graphical presentations of scores and loadings simultaneously were facilitated by
180 construction of bi-plots using OriginPro 8 (Origin Lab Corporation, Northampton, MA, USA).

181 Following this, volatile compounds that showed either a clear change with increased cooking time
182 (for PLS) or significance for differentiation of treatments (for PLS-DA) were selected through
183 determination of variable identification (VID) coefficients. These VID coefficients, ranging between
184 -1 and +1, correspond to correlation coefficients between each original X-variable and the
185 Y-variable as predicted by the selected model. A threshold value of 0.8 was selected, hence
186 volatile compounds with absolute VIDs greater than 0.8 were considered important for the cooking
187 process or for distinguishing beans cooked for different times and are herein referred to as marker
188 and discriminant compounds, respectively. For identification of these compounds, deconvoluted

189 mass spectra were compared with ones from mass spectra libraries, NIST (NIST08, version 2.0,
190 Gaithersburg, MD, USA) and Wiley (Wiley2010, version 9, Hoboken, New York, USA). Moreover, a
191 visual inspection of spectral matching and confirmation of retention index were performed.

192 **2.3.3.2 Kinetic modeling of marker compounds**

193 Using the PROC NLIN procedure (SAS version 9.4, SAS Institute, Inc., Cary, NC, USA), the
194 evolution of marker compounds (from PLS analysis) during cooking was modeled following zero
195 order, first order or first order fractional conversion kinetics (the corresponding equations (1), (2)
196 and (3), respectively, as derived from differential equations are shown below, Van Boekel, 2008).

$$197 \quad C = C_0 - kt \quad (1)$$

$$198 \quad C = C_0 \cdot \exp(-kt) \quad (2)$$

$$199 \quad C = C_f + (C_0 - C_f) \cdot \exp(-kt) \quad (3)$$

200 Where C, C₀, C_f and k denote concentration (in our case, peak area) of a marker compound at any
201 time t, initial concentration at t=0, residual concentration and the reaction rate constant,
202 respectively. For zero order and first order fractional conversion models, the intercepts were not
203 estimated hence modeled data assumed average initial peak areas at t=0. The R²_{adjusted} which
204 indicates the degree of fit between measured and predicted (model-derived) data was calculated
205 as shown in Eq. (4) for assessment of suitability of a model in describing evolution of a marker
206 compound. A visual inspection of parity (measured vs predicted data) and residual plots (residuals
207 vs predicted data) were used in this assessment.

$$208 \quad R^2_{\text{adjusted}} = 1 - \left[\frac{(DF_{\text{total}} - 1) \left(1 - \frac{SS_{\text{model}}}{SS_{\text{total}}} \right)}{DF_{\text{error}}} \right] \quad (4)$$

209 Where SS and DF are sum of squares and degrees of freedom, respectively.

210 **3 Results and discussion**

211 **3.1 The volatile fingerprints of beans: from total ion chromatograms to bi-plots**

212 From cooking profiles, the hardness of both fresh and aged beans decreased with increased
213 cooking time although the former had a higher softening rate constant than the latter, indicating
214 faster cooking. These results are similar to the ones obtained in our previous work hence for a
215 detailed discussion, the reader is referred to Chigwedere *et al.* (2018). In the current work, the
216 profiles were useful for selection of cooking times for which evolution of volatiles would be
217 followed. In order to determine the evolution of volatile compounds during cooking of beans, fresh
218 beans were cooked for 30, 60, 120, 150, 180, 210 and 270 min whilst aged beans were cooked for
219 30, 60, 120, 150, 210, 270 and 330 min. Soaked non-cooked beans were considered as control
220 samples. Volatiles from fresh and aged beans cooked for the same time (for example, fresh and
221 aged beans cooked for 30 min) were compared. At any particular cooking time, aged beans are
222 harder than fresh beans due to the HTC defect. Fresh and aged beans cooked for 60 min and
223 210 min then 120 and 330 min, respectively in each case, were selected as having similar
224 hardness. This was to facilitate investigation of the influence of differences in the cooking times
225 (leading to the same hardness) on volatile compounds. Fig. 2 shows an example of the total ion
226 chromatograms obtained after analysis of headspace fractions of non-cooked (A) and cooked (B)
227 aged beans. The beans were cooked for 330 min, the longest cooking time explored in this study.
228 Labelled in each chromatogram are volatile compounds with notably high peak areas.

229 A combined graphical projection of score and loading plots results in bi-plots as depicted in Fig. 3.
230 A vector (arrow) whose length is a measure of the fit of the regression (PLS) model with the data
231 shows the direction of the response variable (cooking time). The longer it is, the better the fit. By
232 combining the score and loading plots, the contribution of each variable to explanation of the
233 response variable can be illustrated qualitatively. This is accomplished by including two ellipses in
234 the graph, an inner and outer one that represent correlation coefficients of 70 and 100%,
235 respectively. A variable that is projected between the ellipses has >70% of its variability explained
236 by the selected model whose first two latent variables (LVs) are included in the coordinate system
237 on which the objects and variables are projected. Therefore, the further from the origin a variable is

projected, the more it contributes to the correlation of volatile compounds and cooking time. The first LV (LV1) explains the highest variability in the data as compared to the second and subsequent LVs included in the model. Moreover, proximity of a variable to one class of objects reflects a higher correlation with it than with others. Variables that changed significantly with increased cooking time (marker compounds) can be distinguished from the rest; they are shown by circles with thick edges (Fig. 3A-D) and are labelled. These marker compounds were selected through correlation coefficients (VID) between each original X-variable and the Y-variable as predicted by the PLS model and, the minimum absolute VID threshold was 0.8.

3.2 Volatile compounds characteristic of non-cooked beans

Isolation of non-cooked fresh (Fig. 3A) and aged (Fig. 3C) samples from their corresponding cooked samples signifies remarkably different volatile profiles. During data analysis (from MPP tables), it was observed that some compounds were detected in non-cooked (fresh/aged) beans and not in their cooked counterparts. For fresh beans these were identified to be an alkene (1, 3-pentadiene), alcohols (1-pentanol, 2-penten-1-ol, 1-penten-3-ol and (Z)-3-hexen-1-ol), an aldehyde ((E)-2-hexenal), a ketone (2, 3-pentanedione) and an ester ((Z)-2-penten-1-ol acetate). For aged beans, they were an alkene (1, 3-pentadiene), alcohols ((Z)-2-penten-1-ol, 1-heptanol), aldehydes ((E)-2-butenal, (E)-2-pentenal, (Z)-2-hexenal, (E,E)-2,4-hexadienal, (E,E)-2-heptenal, (E,E)-2,4-heptadienal, and (E)-2-octenal), ketones (1-penten-3-one and (E,E)-3,5-octadien-2-one), an ester ((Z)-2-penten-1-ol acetate) and a furan compound (2-propylfuran). Some of these compounds were reported by Mishra *et al.* (2017) (1-pentanol, 1-penten-3-ol, 1-heptanol, 2-pentenal, 3-hexen-1-ol, 2-hexenal, 2, 4-hexadienal, 2, 4-heptadienal, 2-heptenal, 2-octenal) and Oomah, Liang, & Balasubramanian (2007) (2-hexenal and 2-heptenal) in non-cooked beans. Presence of unsaturated aliphatic aldehydes can be attributed to physical damages of raw beans in water (Oomah *et al.*, 2007). Formation of volatile compounds during or following physical degradation can be due to either autoxidation of metabolites or enzymatic activity since degradation results in breakdown of barriers separating enzymes and the metabolites (Christensen, Edelenbos and Kreutzmann, 2007). In general, some volatile compounds (esters,

265 alcohols, ketones and acids) can be generated from unsaturated fatty acids through β -oxidation
 266 which occurs during ripening, enzyme-catalyzed oxidation which yields largely one hydroperoxide
 267 that is further catabolized into volatile compounds, and autoxidation which yields several
 268 hydroperoxides and consequently much more volatile compounds (Christensen *et al.*, 2007).
 269 Aldehydes with six or more carbon atoms are typical lipid oxidation products (Stevenson & Chen,
 270 1996) and in the presence of air, aldehydes and alcohols among other electron-rich compounds
 271 are oxidized to acids through free radical reactions (Marteau *et al.*, 2013).

272 The pathway for volatile formation involving lipoxygenase was described by Salas, García-
 273 González, & Aparicio, (2006). Lipoxygenase stereoselectively oxygenates linoleic and α -linolenic
 274 fatty acids producing hydroperoxides that can be cleaved by either hydroperoxide lyase at the
 275 oxygenated function or lipoxygenase (as secondary reaction) leading to release of volatile
 276 aldehydes or alcohols derived from five carbon atoms, respectively. Alcohol dehydrogenase or
 277 cinnamic aldehyde dehydrogenase then reduces the aldehydes to alcohols which are then
 278 esterified by alcohol-acyl transferase. Such esters are characterized by sweet and fruity sensory
 279 notes. As was shown in our previous work (Chigwedere *et al.*, 2018), mechanical forces on non-
 280 cooked beans completely disintegrate cellular structure. In fact, this facilitates enzyme-substrate
 281 interaction. Therefore, there is a high possibility that lipoxygenase-catalyzed reactions could have
 282 occurred during analysis of non-cooked samples, that is, during incubation of the sample and
 283 subsequent extraction of the headspace components (Section 2.3.2).

284 It is interesting to note that the number of volatile compounds characteristic of non-cooked aged
 285 beans (absent in cooked aged beans) were double those of fresh beans. According to Christensen
 286 *et al.* (2007), the lipoxygenase pathway can be ripening-, physical damage- or stress-induced. El-
 287 Tabey Shehata (1992) reported lignification to be a stress-induced phenomenon and lipid oxidation
 288 to be closely related to seed aging. Lignification of cell walls and middle lamella, pectin
 289 crosslinking, degradation of lipids and, structural changes in proteins and starch have been
 290 associated with aging of beans as reviewed by Reyes-Moreno *et al.* (1993) and El-Tabey Shehata
 291 (1992). In a study on mung beans (*Vigna radiata*), Murthy and Sun (2000) found sugar hydrolysis

292 and lipid peroxidation to be associated with Maillard reactions (discussed in detail in Section 3.4).
293 This suggests that more volatile compounds and/or their precursors were possibly formed due to
294 biochemical changes (enzymatic and non-enzymatic) that occurred during aging of beans in the
295 current study, leading to more volatiles characteristic of non-cooked beans in aged than in fresh
296 beans. It confirms molecular mobility in the beans during the applied aging conditions. Since these
297 fresh and aged beans were subsequently cooked, the volatile profiles obtained thereof would be
298 generated from beans with different pre-history.

299 **3.3 Qualitative evolution of volatile fingerprints of fresh and aged beans during cooking**

300 Since the non-cooked beans had distinct profiles of volatile compounds compared to cooked
301 beans, it was interesting to visualize projections of objects from cooked beans only, as shown in
302 Fig. 3B and D. From the bi-plots, the outcome can be regarded as a 'zooming-in' effect. The
303 Y-variability explained by LV1 as well as the total variability explained by both LVs increased with
304 disregard of non-cooked objects, with a higher increase for aged beans. This confirms the
305 distinction between non-cooked and cooked samples since the chosen PLS models could explain
306 more variability for cooked beans only. With increased cooking time, the pattern of objects was
307 similar for both fresh and aged beans. In both cases, overlapping of some classes was observed,
308 highlighting some similarities in their volatile profiles. It is clear that volatile profiles of beans
309 cooked for 30 and 60 min were quite similar due to the close proximity of their corresponding
310 objects for both fresh and aged beans. Whilst the same marker compounds were identified when
311 data from fresh beans was analyzed with or without the non-cooked samples (Fig. 3A and B), aged
312 beans showed a few different markers (Fig. 3C and D). This means that disregarding the non-
313 cooked aged beans greatly influenced correlations between the original X variables and the Y
314 variance estimated by the chosen PLS model. As such, the correlation between volatile
315 compounds and cooking time was changed based on samples selected for analysis. In this
316 context, a comparison of results from fresh and aged beans indicates that overall differences
317 between non-cooked and cooked aged beans were greater than those between non-cooked and
318 cooked fresh beans. This notion is supported by the observed higher increase in total variability
319 explained by the model for aged beans as mentioned earlier. In addition, Supplementary Fig. 1

320 which is a plot of non-cooked fresh/aged beans and when cooked for the longest times explored in
321 this study confirms this since fresh samples are closer to each other than aged samples are.

322 **3.4 Volatile compounds identified in fingerprints of fresh and aged beans**

323 Whilst some volatiles were characteristic of non-cooked beans (absent in cooked beans) as
324 discussed earlier under Section 3.2, others either increased in concentration or were produced
325 during the cooking process. Moreover, some compounds were detected in either fresh or aged
326 beans whilst others were detected in both. In this section are discussed according to chemical
327 class, volatile compounds that were either increased in concentration or formed due to cooking of
328 fresh and aged beans. These volatiles are listed in Table 1 as discriminant compounds after
329 comparison of volatile fingerprints of fresh and aged samples using PLS-DA models.

330 *Aldehydes*

331 In the current study, 3-methylbutanal and benzaldehyde were identified in both non-cooked and
332 cooked beans. Ma *et al.* (2016) also identified these aldehydes in both non-cooked and cooked
333 beans. In addition, Mishra *et al.* (2017) reported presence of 3-methylbutanal in non-soaked non-
334 cooked beans. Benzaldehyde could have been produced from phenylalanine, an aromatic amino
335 acid reported by Murray *et al.* (1976) to be the likely precursor of aromatic aldehydes of pulses.
336 Aldehydes such as benzaldehyde are formed by a side reaction of the Maillard reaction (Adamiec,
337 Rössner, Velíšek, Cejpek, & Šavel, 2001). The Maillard reaction is a cascade of chemical reactions
338 involving reducing sugars and amino acids, and it is mainly influenced by temperature, time and
339 nature of the metabolites (Jaeger, Janositz, & Knorr, 2010; Jousse, Jongen, Agterof, Russell, &
340 Braat, 2002). Condensation of an amino acid and a reducing sugar results in an Amadori
341 rearrangement intermediate which can be cleaved into rearranged sugars. Among other reactions,
342 such sugars can be fragmented into dicarbonyl compounds which then react with amine groups
343 through a mechanism called Strecker degradation, yielding short-chain aliphatic aldehydes
344 (Strecker aldehydes) that are significant for flavor (Jousse *et al.*, 2002) such as 2-methylpropanal,
345 3-methylbutanal and 2-methylbutanal. Whilst 3-methylbutanal is formed from leucine, 2-
346 methylbutanal is formed from isoleucine (Cremer, Vollenbroeker, & Eichner, 2000; Estévez,

347 Ventanas, & Heinonen, 2011) and 2-methylpropanal is from valine (Cremer & Eichner, 2000).
348 Apart from carbohydrates, lipids and polyphenols can be involved in Strecker degradation leading
349 to uncommon flavor compounds as reviewed by Yaylayan (2003). Moreover, lipid oxidation
350 products such as alkadienals and ketodienes can take part in Strecker degradation-like reactions
351 (Zamora, Gallardo, & Hidalgo, 2007, 2008). This could explain the observed presence of the
352 Strecker aldehyde 3-methylbutanal in non-cooked beans.

353 In this study, nonanal and heptanal were detected in cooked aged beans. These two lipid-derived
354 aldehydes were reported in ground, non-soaked and non-cooked *P. vulgaris* beans by Oomah *et al.*
355 (2007). Hexanal was identified in both non-cooked and cooked beans in the current study. Ma
356 *et al.* (2016) and Oomah *et al.* (2007) identified hexanal as the chief aldehyde in non-cooked
357 and/or cooked beans. Galliard, Phillips, & Reynolds (1976) showed that hexanal is formed upon
358 cleavage of 13-hydroperoxylinoleic acid by lyases under anaerobic conditions. Common beans are
359 rich in polyunsaturated fatty acids (65-87% of total lipids) such as, in increasing abundance, oleic,
360 linoleic and linolenic acids (Reyes-Moreno *et al.*, 1993). Therefore, it is not surprising that hexanal
361 and (E,E)-2,4-heptadienal, the primary autoxidation products of linoleic and linolenic acids were
362 detected in this study. As can be noted above, a number of volatile compounds was detected in
363 both non-cooked and cooked beans. According to De Quirós, López-Hernández, González-Castro,
364 De la Cruz-García, & Simal-Lozano (2000), autoxidation processes are escalated by high
365 temperatures. Therefore, this explains the detection of some volatiles in both non-cooked and
366 cooked beans apart from other pathways such as the Maillard reactions that was mentioned
367 above.

368 *Alcohols*

369 In the current study, 1-, 2- and 3-hexanol were identified in non-cooked and/or cooked beans. Ma
370 *et al.* (2016) cooked red kidney beans for 40 min and identified 3-hexanol as the most abundant
371 volatile compound contributing to the flavor of beans. Alcohol oxidoreductase-catalyzed
372 transformation of n-hexanal leads to formation of n-hexanol compounds. According to Ma *et al.*
373 (2016), 1-penten-3-ol which was detected in aged beans in the current study, is formed through a

374 similar pathway as the n-hexanol compounds. Linolenic acid is oxidised with production of 16-
375 hydroperoxide which undergoes enzymatic isomerization, leading to formation of 1-penten-3-one.
376 Alcohol oxidoreductase-catalysed reduction of 1-penten-3-one results in formation of 1-penten-3-
377 ol. 1-Octen-3-ol which was suggested to be a linoleic oxidation product by Oomah *et al.* (2007) was
378 identified in non-cooked aged beans in the current study. Some branched alcohols and acids such
379 as 3-methylbutanol and 3-methylbutanoic acid which were identified in the current study in both
380 non-cooked and cooked beans, are formed upon breakdown of amino acids (Christensen *et al.*,
381 2007). 2-Methoxy-4-vinylphenol (p-vinylguaiacol) which was found in the volatile fingerprint of
382 cooked (fresh and aged) beans was also found in cooked beans by Mishra *et al.* (2017) and
383 Buttery, Seifert and Ling (1975). In the current study, ethanol was identified in all samples but was
384 more abundant in fresh than aged beans. Other alcohols that were detected in the current study
385 include 2-methylpropanol, 1-and 2-butanol, 3-pentanol, 2-ethylhexanol and benzyl alcohol.
386 Alcohols may be regarded as unimportant to development of bean flavor since their threshold
387 concentrations are relatively high.

388 *Ketones*

389 2-Heptanone was detected in cooked fresh and aged beans in the current study. According to Ma
390 *et al.* (2016) who also detected 2-heptanone in cooked beans, ketones are a product of
391 lipoyxygenase-catalyzed degradation of unsaturated fatty acid hydroperoxides. They identified
392 2-heptanone in cooked beans just as it was identified in cooked fresh and aged beans in the
393 current study. However, these researchers reported 2-butanone in non-cooked beans unlike in the
394 current study where it was found in cooked beans only. In addition, 3-hydroxy-2-butanone was
395 detected in cooked aged beans in the current study. 1-Penten-3-one which is produced by
396 enzymatic isomerization of 16-hydroperoxide as described before (for formation of 1-penten-3-ol)
397 was detected in aged beans in the current study. Whilst (E,E)-3,5-octadien-2-one was found in
398 non-cooked beans and 2-propanone in both cooked and non-cooked beans in this study, Oomah
399 *et al.* (2007) found both in their study on non-cooked beans. Buttery *et al.* (1975) also reported
400 3,5-octadien-2-one in cooked beans.

401 *Furan compounds*

402 Furan and a number of substituted alkylfurans namely, 2-methyl-, 3-methyl, 2-ethyl-, 2-propyl- and
403 2-pentylfuran were identified in non-cooked and/or cooked beans in this study. Ma *et al.* (2016)
404 reported 2-methylfuran in non-cooked beans and 2-ethylfuran in cooked beans whilst Oomah *et al.*
405 (2007) reported 2-ethyl- and 2-propylfuran in non-cooked beans. Oomah *et al.* (2007) reported
406 2-ethylfuran to be a lipid-derived volatile compound. However, these researchers suggested
407 negligible contribution of furan-compounds to the overall odor of beans. Furan and its alkyl
408 derivatives can be formed by cyclisation of rearranged sugars from the Maillard reaction (Jousse *et al.*, 2002),
409 from 4-hydroxy-2-butenal, a lipid peroxidation product (Perez Locas & Yaylayan, 2004)
410 and many other pathways as reviewed by Yaylayan (2006). Based on animal studies, furan is
411 assumed to be carcinogenic due to *cis*-2-butene-1,4-dial, a very reactive metabolite in the pathway
412 of furan formation. However, epidemiological studies to investigate this are lacking in literature
413 (Bakhiya & Appel, 2010).

414 *Sulphur compounds*

415 While dimethyl sulphide was detected in (non-cooked and cooked) fresh beans and cooked aged
416 beans, dimethyl disulphide was identified in cooked fresh and aged beans only. These two
417 compounds were reported in cooked beans by Mishra *et al.* (2017) among other sulphur
418 compounds and by Landaud, Helinck, & Bonnarme, (2008) in powders of dried legumes. Whilst
419 oxidation of the amino acid methionine to methional via Strecker degradation followed by oxidation
420 to methanethiol leads to dimethyl disulphide formation (Chin & Lindsay, 1994; Mishra *et al.*, 2017),
421 the pathway of formation of dimethyl sulphide from methionine is unclear (Mishra *et al.*, 2017).
422 Sulphur compounds have low odor threshold concentrations and hence greatly influence the flavor
423 of food (Landaud *et al.*, 2008).

424 *Alkanes*

425 A few alkanes including pentane, 2,4-dimethylheptane, octane, 6-ethyl-2-methyl octane, decane
426 and undecane were identified in non-cooked and/or cooked beans in the current study. Their
427 presence suggests occurrence of lipid oxidation from which they are derived (Oomah *et al.*, 2007).

428 More than alcohols, alkanes do not contribute to food flavors but rather influence volatility and
429 flavor-imparting characteristics of other volatile compounds as reported by Laye *et al.* (1995) cited
430 by Stevenson & Chen, (1996).

431 *Esters*

432 Octyl ester cyclopentanecarboxylic acid and methyl acetate as well as the cyclic esters
433 butyrolactone and 4-methyldihydro-2(3H)-furanone were detected. Methyl acetate was reported in
434 canned red kidney beans by Van Ruth *et al.* (2005). According to Christensen *et al.* (2007), fatty-
435 acid degradation through β -oxidation cycles can lead to formation of short- or medium-chain length
436 volatiles such as saturated or unsaturated lactones and esters among other carbonyl compounds.
437 Blank & Fay (1996) proposed a mechanism for formation of the aforementioned cyclic esters
438 (furanones/lactones) that involves the complex Maillard reactions. They proposed that
439 2,3-enolization of an Amadori compound followed by chain elongation utilizing Strecker aldehydes
440 with subsequent reduction of compounds formed thereof yields these cyclic esters.

441 *Benzopyran derivatives*

442 From results of the current study, we report 2H-1-benzopyran derivatives during cooking of beans,
443 for the first time. Benzopyran compounds are natural oxygenated heterocyclic plant-based
444 pigments whose structure is based on fused benzene and furan rings, for example flavonoids
445 (Delgado-Vargas, Jiménez, Paredes-López, & Francis, 2000; Zamora & Hidalgo, 2005). They can
446 be classified as either 1-benzopyran or 2-benzopyran depending on the position of the oxygen
447 atom on the furan ring. It could be that these compounds were produced from thermal degradation
448 of benzopyran compounds, specifically flavanones and isoflavones which are common in legumes
449 (Delgado-Vargas *et al.*, 2000). This is not surprising because bean seed coats are rich in such
450 compounds, some of which confer the color of colored seed coats and also exhibit health-related
451 functions (Reyes-Moreno *et al.*, 1993).

452 Based on relative quantities of volatile compounds and odor threshold considerations, Buttery *et al.*
453 (1975) established (Z)-3-hexen-1-ol and 1-octen-3-ol as the most important volatiles for aroma of
454 non-cooked beans. However, based on principal component scores and odor threshold

455 considerations, 1-penten-3-ol, benzaldehyde, hexanal and 3,5-octadien-2-one were considered as
456 the most authentic marker compounds for non-cooked beans by Oomah *et al.* (2007). Ma *et al.*
457 (2016) concluded that 3-hexanol, the most abundant volatile compound for both non-cooked and
458 cooked beans in their study, had a significant contribution to the flavor of beans. Dimethyl
459 disulphide was found to contribute to the aroma of cooked kidney beans in a study by Mishra *et al.*
460 (2017). According to Christensen *et al.* (2007), abundance of a volatile compound does not imply
461 importance for flavor but rather, its threshold concentration for olfaction does. However, the
462 contribution of volatile compounds generated due to severe cooking may become more dominant.
463 Therefore, as opposed to absolute quantities and for the first time in the analysis of volatiles from
464 beans, of importance in this study were compounds whose evolution significantly changed due to
465 cooking as highlighted in Fig 3 (A and C). The evolution of these markers is discussed in Section
466 3.6.

467 **3.5 Influence of hardening and extent of cooking on volatile profiles**

468 From comparison of volatile profiles of fresh and aged beans cooked for the same time using PLS-
469 DA models, similar results were obtained for all cooking times hence Fig. 3E and F are shown as
470 examples for beans cooked for 30 and 270 min, respectively. The discriminant compounds denote
471 volatile compounds that were either present in higher concentration (labeled in black) in one class
472 compared to the other or were not detected in the other class (labeled in maroon). It is clear from
473 the two bi-plots that the concentration of most of the volatile compounds was higher in aged than in
474 fresh beans irrespective of the extent of cooking. It can be concluded that there was an influence of
475 differences in pre-history of the samples on the volatile profiles as alluded to in Section 3.2 due to
476 the aging process of the beans.

477 When differences in bean hardness were overcome by cooking aged beans to a similar hardness
478 as fresh beans, a comparison of the obtained volatile profiles resulted in bi-plots similar to Fig. 3E
479 and F hence they are not shown. These results can be explained by the long cooking times for
480 aged beans that were required to attain similar hardness as fresh beans and possibly, the different
481 pre-history of the samples as already mentioned in Section 3.2. Table 1 shows the discriminant

482 compounds identified upon comparison of fresh and aged beans cooked for either the same time
483 or to similar texture level. These results clearly show that both cooking time and condition of beans
484 (fresh or aged) are of paramount importance for development of bean flavor during cooking.
485 Moreover, the majority of the volatile compounds identified in this study were also reported for
486 canned red kidney beans by Van Ruth, Dings, Buhr, & Posthumus (2004).

487 **3.6 Kinetics of marker compounds during cooking of fresh and aged beans**

488 The evolution of marker compounds with increased cooking time of fresh and aged beans was
489 illustrated by modeling the peak areas with cooking time and the results are shown in Fig. 4. The
490 reaction kinetics of these marker compounds followed zero order (2-propanone, 2-butanone,
491 2-ethylfuran, 2-methylfuran and ethyl acetate), first order (dimethyl disulphide and benzaldehyde)
492 or first order fractional conversion kinetics (benzopyran derivatives, 2-methylbutanal,
493 3-methylbutanal, dimethyl sulphide, 2-methylpropanal and 2-methoxy-4-vinylphenol). For marker
494 compounds such as benzopyran derivatives, dimethyl sulphide, 2-methylbutanal, 3-methylbutanal,
495 2-propanone and dimethyl disulphide that were selected for both fresh and aged beans, evolution
496 followed similar kinetic behavior. Whilst ethyl acetate, 2-ethylfuran, dimethyl sulphide and
497 3-methylbutanal were already detected in the non-cooked fresh beans, 3-methylbutanal,
498 2-propanone and 2-methylfuran were detected in non-cooked aged beans. Other marker
499 compounds were either below detection limit or were only formed upon cooking.

500 The mechanistic explanation for a reaction that follows zero order kinetics is that a compound
501 evolves independently of a reactant whose concentration is in excess and hence regarded as
502 constant (Van Boekel, 2008). Therefore, evolutions of 2-propanone, 2-butanone, 2-ethylfuran,
503 2-methylfuran and ethyl acetate were independent of precursor concentrations for the duration of
504 the cooking time of beans explored in this study. Since dimethyl disulphide and benzaldehyde
505 followed first order kinetics, they were most probably formed from a unimolecular reaction.
506 Evolutions of all other markers were best fit by a first order fractional conversion model which was
507 described by Van Boekel (2008) as an indicator for complex reactions characterized by a
508 combination of several mechanisms such as chain reactions. This is the case for Strecker

509 aldehydes (2-methylbutanal, 3-methylbutanal and 2-methylpropanal) which are produced from the
510 complex Maillard chemistry and were best fit by the first order fractional conversion model. For this
511 model, beyond a specific cooking time which can be marker-dependent, production of the marker
512 ceases.

513 From the estimated parameters (Table 2), the rate constant for evolution of 2-propanone was
514 significantly higher in aged beans compared to that for fresh beans whilst that for dimethyl
515 disulphide was significantly higher in fresh beans. However, there were no significant differences
516 between the rate constants for the rest of the markers that were selected in both fresh and aged
517 beans. This indicates a similar rate of formation and/or increase (since some were present in non-
518 cooked beans) of these volatile compounds during cooking. These results reveal that for most of
519 the volatile compounds, HTC did not influence their rate of increase or formation unlike in texture
520 evolution where it greatly influenced the rate of softening (Chigwedere *et al.*, 2018). Since the rate
521 of evolution of discriminant volatiles of fresh and aged beans was largely similar and yet their
522 volatile profiles were different, this confirms that differences in their volatile fingerprints emanated
523 from differences in their pre-history. In addition, differences between volatile fingerprints of beans
524 cooked to similar texture can then be attributed to a combination of differences in pre-history and
525 cooking time. These results support the hypothesis that HTC development that occurs during aging
526 of beans influences flavor development in the beans during cooking. Results from this study
527 necessitate absolute quantification of the marker compounds and subsequent evaluation on
528 sensorial consequences on flavor.

529 **4 Conclusion**

530 The evolution of volatile compounds during cooking was clearly influenced by cooking time as
531 observed by the apparent distinction between non-cooked and cooked beans, and by differences
532 in volatile profiles of beans cooked to different extents, for both fresh and aged beans. The
533 influence of aging on volatile profiles was demonstrated in non-cooked beans. Volatile compounds
534 characteristic of non-cooked aged beans were double those in non-cooked fresh beans,
535 suggesting their production or that of their precursors during aging. This notion is supported by

536 existence of several proposed biochemical changes reported to occur during aging of beans.
537 Discriminant compounds present in fresh and aged beans cooked for the same time exhibited
538 higher peak areas in the latter even though kinetic behavior showed that the rate of generation or
539 increase of these compounds was largely similar. This can be attributed to differences in the pre-
540 history of the fresh and aged beans as revealed by differences in volatile profiles of non-cooked
541 fresh and aged beans. Volatile profiles of fresh and aged beans cooked to a similar texture were
542 influenced by differences in pre-history and cooking time. In conclusion, pre-history and cooking
543 time had a large influence on volatile profiles of beans during cooking and discriminant compounds
544 were possibly due to mainly lipid oxidation and the Maillard reactions. These results support the
545 hypothesis that HTC development that occurs during aging of beans influences flavor development
546 in the beans during cooking. The practical relevance of these results is that process adaptation to
547 reach a similar texture for fresh and aged beans can lead to differences in flavor.

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665

Captions of Tables

Table 1. Discriminant volatiles with variable identification coefficient (VID) $>|0.80|$ for fresh and aged beans cooked for the same time thereby having different hardness and for different times to achieve similar hardness. F30' and A30' represent fresh and aged beans cooked for 30 min, respectively and, RI is the retention index.

¹ Listed under each class in decreasing order of VID are volatiles that had either higher peak areas in that class (positive VID) compared to the other (compounds not in bold) or volatiles that were not found in the other class (bolded and italicized). Volatile compounds that were present in lower concentration in a class thereby assuming negative VID values are not shown.

^{*2} These isomers had different elution times

^{*3} The benzopyran derivative was identified as 3,4,4a,5,6,8a-hexahydro-2,5,5,8a-tetramethyl-, (2 α ,4 α ,8 α)- 2H-1-benzopyran or , 3,4,4a,5,6,8a-hexahydro-2,5,5,8a-tetramethyl-, (2 α ,4 α ,8 α)- 2H-1-benzopyran or dihydroedulan II.

Table 2. Parameter estimates $\pm$ standard errors for evolution of marker compounds selected after partial least squares analysis of volatile profiles of fresh and aged beans cooked in demineralised water. PA₀ is the intercept (estimated for the first order model only), k is the rate constant and PA _{∞} is the infinite peak area (estimated for the first order fractional conversion model only).

¹ The rate constants (k) for evolution of marker compounds selected for both fresh and aged beans were compared to determine significant differences when modeled by the same kinetic model. Different superscript letters represent significant differences.

² The – indicates that the volatile compound under consideration was not selected as a marker for that class.

^{*3} The two benzopyran derivatives were identified as the same compound (3,4,4a,5,6,8a-hexahydro-2,5,5,8a-tetramethyl-, (2 α ,4 α ,8 α)- 2H-1-benzopyran or, 3,4,4a,5,6,8a-hexahydro-2,5,5,8a-tetramethyl-, (2 α ,4 α ,8 α)- 2H-1-benzopyran or dihydroedulan II) even though they were eluted at different times.

Captions of Figures

Fig. 1. A schematic illustration of the work involved in the current study

Fig. 2. Representative total ion chromatograms for the headspace fraction of non-cooked aged beans (A) and aged beans cooked for 330 min (B) as obtained after injection of volatiles extracted by a divinylbenzene/carboxen/polydimethylsiloxane fiber and their separation on an HP INNOWax polar column. For each chromatogram, a number of volatile compounds with notably high abundance are labelled.

Fig. 3. Bi-plots A to D depict the volatile profiles of fresh and aged beans after analyses of their volatile fractions using PLS models. A comparison of volatile profiles of fresh and aged beans cooked

for a short (30 min) and long (270 min) time using PLS-DA models resulted in biplots E and F, respectively. Symbols represent the samples (objects). Open circles represent all the volatile compounds in the headspace fraction but those with thicker edges represent discriminant compounds. These compounds were selected based on variable identification coefficient (VID) procedure ($VID > |0.80|$) and identified based on retention index and mass spectra. In the PLS-DA biplots (E and F), discriminant volatiles labeled in black had higher peak areas in the class close to their projection than the other class. Volatiles labeled in maroon were present only in the classes close to their projection.

*The two benzopyran derivatives in PLS biplots (A-D) were identified as the same compound (3,4,4a,5,6,8a-hexahydro-2,5,5,8a-tetramethyl-, (2 α ,4 α ,8 α)- 2H-1-benzopyran or , 3,4,4a,5,6,8a-hexahydro-2,5,5,8a-tetramethyl-, (2 α ,4 α ,8 α)- 2H-1-benzopyran or dihydroedulan II) regardless of different eluted times.

Fig. 4. Kinetic behavior for evolution of marker compounds during cooking of fresh and aged beans in demineralised water. The symbols represent experimental data and lines represent predicted data as modeled following zero order (A and B), first order (C and D) and first order fractional conversion kinetics (E and F).

Caption of Supplementary Figure

Supplementary Fig. 1. A bi-plot depicting volatile profiles of fresh and aged beans in a non-cooked state and when cooked for the longest time explored in this study. Fresh 0 means fresh beans cooked for 0 min (non-cooked).

721 **Tables**

722 **Table 1.** Discriminant volatiles with variable identification coefficient (VID) >|0.80| for fresh and aged beans cooked for the same time thereby having different
723 hardness and for different times to achieve similar hardness. F30' and A30' represent fresh and aged beans cooked for 30 min, respectively and, RI is the
724 retention index.

Samples	Fresh			Aged			Samples	Fresh			Aged			
	VID ¹	Marker	RI	VID ¹	Marker	RI		VID ¹	Marker	RI	VID ¹	Marker	RI	
Influence of differences in hardness between fresh and aged beans on volatile profiles														
Fresh non-cooked vs aged non-cooked				0.991	2,3 pentanedione	1061	F30' vs A30'	0.953	ethyl acetate	921	0.996	(Z)-3-hexen-1-ol	1392	
				0.987	1-penten-3-ol	1167		0.925	ethanol	952	0.991	2-pentanol	1122	
				0.987	2-pentanol	1122					0.989	2-methylpropanol	1095	
				0.981	(Z)-3-hexen-1-ol ²	1392					0.985	3-methylbutanal	941	
				0.981	pentanal	989					0.984	3-methylbutanol	1217	
				0.981	propanal	875					0.984	benzaldehyde	1552	
				0.981	1-pentanol	1261					0.982	2-methylpropanal	883	
				0.977	1-heptanol	1463					0.980	2-butanol	1025	
				0.976	(E,E)-2,4-heptadienal	1485					0.963	3-hexanol	1204	
				0.976	2-propylfuran	1515					0.959	2-methylbutanal	938	
				0.975	3-methylbutanol	1217					0.952	2-hexanol	1171	
				0.975	Pentane	829					0.940	octane	875	
				0.974	(E,E)-3,5-octadien-2-one	1538					0.927	2-ethylhexanol	1498	
				0.970	Heptanal	1197					0.899	2-ethylfuran	968	
				0.969	1-octen-3-ol	1457					0.892	dimethyl sulphide	861	
				0.969	2-octenal	1446					0.859	undecane	1030	
				0.967	2-hexenal	1235					0.858	benzyl alcohol	1896	
				0.965	3-methylbutanal	941					0.807	decane	998	
				0.964	1-hexanol	1362								
				0.955	hexanal	1084								
				0.951	(E)-2-pentenal	1139								

[illegible]

				0.874	2-methoxy-4-vinylphenol	2172					0.897	2-propanone	885			
				0.873	octane	875					0.874	2-ethylhexanol	1498			
				0.851	dimethyl sulphide	861					0.863	butyrolactone	1667			
				0.849	undecane	1030					0.796	2-butanol	1025			
				0.825	3-pentanol	1110										
				0.823	2,4-dimethylheptane	878										
				0.804	1,3-bis(1,1-dimethylethyl)-benzene	1438										
				0.800	3-methylfuran	927										
F150' vs A150'	0.993	ethyl acetate	921	0.995	3-methylbutanal	941	F210' vs A210'	0.977	ethyl acetate	921	0.995	2-methylpropanol	1095			
				0.992	2-methylbutanal	938					0.966	2-butanone	930	0.994	3-methylbutanal	941
				0.991	2-methylpropanol	1095						0.985	2-methylpropanal	883		
				0.990	2-methylpropanal	883						0.984	2-methylbutanal	938		
				0.988	2-pentanol	1122					0.981	2-pentanol	1122			
				0.984	(Z)-3-hexen-1-ol	1392					0.979	2-methylfuran	912			
				0.981	2-methoxy-4-vinylphenol	2172						0.974	2-butanol	1025		
				0.976	3-methylbutanol	1217						0.961	2-ethylfuran	968		
				0.970	2-methylfuran	912					0.957	(Z)-3-hexen-1-ol	1392			
				0.964	2-ethylfuran	968						0.956	dimethyl disulphide	1078		
				0.964	2-pentylfuran	1244						0.948	3-methylbutanol	1217		
				0.951	1-pentanol	1261						0.929	dimethyl sulphide	861		
				0.947	dimethyl sulphide	861						0.916	2-methoxy-4-vinylphenol	2172		
				0.945	pentane	829						0.899	2-pentylfuran	1244		
				0.919	3-methylfuran	927						0.893	2-heptanone	1193		
				0.918	dimethyl disulphide	1078						0.879	2-ethylhexanol	1498		
				0.916	octane	875						0.849	3-hexanol	1204		
				0.903	hexanal	1084						0.834	2-propanone	885		
				0.886	6-ethyl-2-methyl octane	1034						0.833	3-hydroxy-2-butanone	1308		
				0.870	benzyl alcohol	1896						0.832	octane	875		

				0.869	2-propanone	885					0.831	3-pentanol	1110
				0.846	1,3-bis(1,1-dimethylethyl)-benzene	1438					0.826	3-methyl butanoic acid	1691
				0.844	undecane	1030					0.819	1-butanol	1150
				0.809	2-butanol	1025							
F270' vs A270'	0.959	ethyl acetate	921	0.990	3-methylbutanal	941							
	0.866	ethanol	952	0.988	2-pentanol	1122							
	0.863	2-butanone	930	0.985	2-methylbutanal	938							
	0.814	octyl ester	1394	0.985	2-methylfuran	912							
		cyclopentanecarboxylic acid		0.980	2-methylpropanol	1095							
				0.973	(Z)-3-hexen-1-ol	1392							
				0.972	3-hexanol	1204							
				0.950	dimethyl sulphide	861							
				0.947	3-methylfuran	927							
				0.945	3-methylbutanol	1217							
				0.928	dimethyl disulphide	1078							
				0.902	4-methyldihydro-2(3H)-furanone	1650							
				0.900	2-ethylfuran	968							
				0.862	acetic acid	1476							
				0.844	2-ethylhexanol	1498							
				0.798	2-methylpropanal	883							
Influence of similarities in hardness between fresh and aged beans on volatile profiles													
F60' vs A210'	0.828	ethanol	952	0.995	2-methylpropanol	1095	F120' vs A330'	0.976	ethyl acetate	921	0.992	2-pentanol	1122
	0.798	ethyl acetate	921	0.995	3-methylbutanal	941		0.917	ethanol	952	0.989	2-methylpropanol	1095
				0.992	2-methylbutanal	938		0.914	nonanal	1403	0.988	dimethyl sulphide	861
				0.991	2-methylpropanal	883		0.873	1,2-dimethoxy	1745	0.988	dimethyl trisulphide	1402
				0.982	2-heptanone	1193			-benzene,		0.986	3-methylbutanol	1217
				0.980	2-butanol	1025					0.985	3-methylbutanal	941
				0.978	dimethyl disulphide	1078					0.984	2-methylpropanal	883

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0.978	2-methylfuran	912	0.982	2-methylfuran	912
0.976	2-pentanol	1122	0.980	2-methylbutanal	938
0.976	2-ethylfuran	968	0.978	2-propanone	885
0.968	2-propanone	885	0.974	2-methoxy-4-vinylphenol	2172
0.961	<i>benzaldehyde</i>	1552	0.971	disulphide dimethyl	1078
0.958	2-pentylfuran	1244	0.959	furan	877
0.957	dimethyl sulfide	861	0.948	acetic acid	1476
0.956	<i>(Z)-3-hexen-1-ol</i>	1392	0.941	<i>(Z)-3-hexen-1-ol</i>	1392
0.955	2-methoxy-4-vinylphenol	2172	0.874	2-ethylfuran	968
0.941	3-methylbutanol	1217	0.858	<i>2-butanol</i>	1025
0.903	pentane	829	0.849	octane	875
0.877	2H-1-benzopyran derivative ³	1542	0.824	<i>methyl acetate</i>	890
0.853	<i>3-hexanol</i>	1204	0.812	<i>3-hexanol</i>	1204
0.850	2-ethylhexanol	1498	0.809	<i>heptanal</i>	1197
0.839	<i>3-methyl butanoic acid</i>	1691			
0.835	acetic acid	1476			
0.810	octane	875			
0.803	undecane	1030			

¹ Listed under each class in decreasing order of VID are volatiles that had either higher peak areas in that class (positive VID) compared to the other (compounds not in bold) or volatiles that were not found in the other class (bolded and italicized). Volatile compounds that were present in lower concentration in a class thereby assuming negative VID values are not shown.

² These isomers had different elution times

³ The benzopyran derivative was identified as 3,4,4a,5,6,8a-hexahydro-2,5,5,8a-tetramethyl-, (2a,4aa,8aa)- 2H-1-benzopyran or , 3,4,4a,5,6,8a-hexahydro-2,5,5,8a-tetramethyl-, (2a,4aa,8aa)- 2H-1-benzopyran or dihydroedulan II.

Table 2. Parameter estimates $\pm$ standard errors for evolution of marker compounds selected after partial least squares analysis of volatile profiles of fresh and aged beans cooked in demineralised water. PA_0 is the intercept (estimated for the first order model only), k is the rate constant and PA_∞ is the infinite peak area (estimated for the first order fractional conversion model only).

Zero order		Fresh beans		Aged beans	
Markers	R^2 adjusted	k (area/min) ¹	R^2 adjusted	k (area/min) ¹	
2-propanone	0.99	$6.39E+04 \pm 1.25E+03^a$	0.92	$1.11E+05 \pm 5.24E+03^b$	
2-butanone	0.96	$1.07E+03 \pm 39.2E+00$	-. ²	-	
2-ethylfuran	0.94	$5.92E+03 \pm 2.45E+02$	-	-	
ethyl acetate	0.98	$4.52E+04 \pm 1.32E+03$	-	-	
2-methylfuran	-	-	0.96	$4.98E+03 \pm 1.51E+02$	

First order		Fresh beans		Aged beans		
Markers	R^2 adjusted	k (min ⁻¹) ¹	PA_0	R^2 adjusted	k (min ⁻¹) ¹	PA_0
dimethyl disulphide	0.89	$-0.01 \pm 1.22E-03^a$	$2.44E+05 \pm 7.26E+04$	0.95	$-0.007 \pm 5.37E-04^b$	$2.36E+06 \pm 3.70E+05$
benzaldehyde	0.86	$-0.01 \pm 1.02E-03$	$9.85E+04 \pm 2.24E+04$	-. ²	-	-

First order fractional conversion		Fresh beans		Aged beans		
Markers	R^2 adjusted	k (min ⁻¹) ¹	PA_∞	R^2 adjusted	k (min ⁻¹) ¹	PA_∞
2-methylbutanal	0.98	$1.51E-02 \pm 2.13E-03^a$	$4.71E+06 \pm 1.86E+05$	0.98	$1.05E-02 \pm 1.23E-03^a$	$1.57E+07 \pm 6.31E+05$
3-methylbutanal	0.98	$1.87E-02 \pm 2.4E-03^a$	$4.41E+06 \pm 1.46E+05$	0.98	$1.24E-02 \pm 1.76E-03^a$	$2.04E+07 \pm 6.69E+05$
dimethyl sulphide	0.97	$9.69E-03 \pm 2.20E-03^a$	$8.50E+05 \pm 6.82E+04$	0.97	$9.24E-03 \pm 1.33E-03^a$	$2.02E+06 \pm 1.08E+05$
2H-1-benzopyran a ³	0.98	$8.04E-03 \pm 1.20E-03^a$	$1.88E+06 \pm 1.28E+05$	0.97	$8.33E-03 \pm 1.32E-03^a$	$1.42E+06 \pm 8.94E+04$
2H-1-benzopyran b ³	0.97	$5.92E-03 \pm 1.51E-03^a$	$6.84E+05 \pm 9.56E+04$	0.96	$6.97E-03 \pm 1.50E-03^a$	$5.54E+05 \pm 5.29E+04$
2-methoxy-4-vinylphenol	-. ²	-	-	0.95	$9.24E-03 \pm 1.87E-03$	$7.27E+05 \pm 5.53E+04$
2-methylpropanal	-	-	-	0.98	$1.50E-02 \pm 1.62E-03$	$4.71E+06 \pm 1.43E+05$

¹ The rate constants (k) for evolution of marker compounds selected for both fresh and aged beans were compared to determine significant differences when modeled by the same kinetic model. Different superscript letters represent significant differences.

² The - indicates that the volatile compound under consideration was not selected as a marker for that class.

³ The two benzopyran derivatives were identified as the same compound (3,4,4a,5,6,8a-hexahydro-2,5,5,8a-tetramethyl-, (2a,4aa,8aa)- 2H-1-benzopyran or, 3,4,4a,5,6,8a-hexahydro-2,5,5,8a-tetramethyl-, (2a,4aa,8aa)- 2H-1-benzopyran or dihydroedulan II) even though they were eluted at different times.

Fig. 2.

Fig. 2.

Fig. 3.

Fresh beans

Aged beans

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772 Fig. 4.

773 **Supplementary figure**

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775 Supplementary Fig. 2. A bi-plot depicting volatile profiles of fresh and aged beans in a non-cooked state and when cooked for the longest time explored in
 776 this study. Fresh 0 means fresh beans cooked for 0 min (non-cooked).

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