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Manuscripts

Effect of Bread Crumb and Crust Structure on the *in vivo* Release of Volatiles and the Dynamics of Aroma Perception

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1 **Abstract**

2 This study examined the effects of bread crumb and crust structure on volatile release
3 and aroma perception during oral processing. French baguettes with different crumb
4 structures were procured from a supermarket or local bakeries (n=6) or produced in the
5 laboratory via par baking (n=3). Eight study participants consumed crumb-only and
6 crumb-and-crust samples, and the resulting volatile release was measured *in vivo* using
7 proton transfer reaction-mass spectrometry. A statistical model was then used to
8 examine the contributions of volatile compounds to target ion production (i.e., crumb or
9 crust markers). Utilizing the three laboratory-produced breads, chewing behavior and
10 aroma perception were measured via electromyography and the temporal dominance of
11 sensations method, respectively. The results revealed that the initial levels of crumb
12 markers as well as crumb firmness affected crumb markers release. Crust markers were
13 released more quickly than crumb markers, leading to different perception dynamics.

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16

17 **Keywords**

18 Proton transfer reaction-mass spectrometry (PTR-MS); Aroma release; Bread; Temporal
19 dominance of sensations (TDS); Oral processing

20

21 Introduction

22 When a food is being eaten, its aroma compounds are released into the consumer's oral
23 cavity; there, the compounds can interact with olfactory receptors, leading to retronasal
24 aroma perception.¹⁻³ Release dynamics depend on food-related physicochemical
25 processes and on consumer-related physiological parameters. Together, these factors
26 determine the quantity and kinetics of the volatile organic compounds (VOCs) released in
27 the oral cavity and thus contribute to how consumers perceive the food they are eating.^{4,5}
28 Therefore it is important to study volatile release during food consumption to better
29 understand aroma perception and its dynamics, the ultimate goal being to create
30 products that meet consumer expectations and match consumer preferences.

31 In this context, nose-space analyses such as atmospheric pressure chemical ionization-
32 mass spectrometry (APCI-MS)⁶ or proton transfer reaction-mass spectrometry (PTR-
33 MS)⁷ are very useful tools to monitor the release of aroma compounds in the air exhaled
34 by a subject consuming food. They have been extensively employed over the past twenty
35 years to study VOC release by different food types.⁸ PTR-MS is a soft chemical ionization
36 technique in which protons are transferred from the protonated reagent, H_3O^+ , to VOCs
37 that have a greater affinity for protons than does H_2O .⁷ Although the ionization mainly
38 produces the protonated molecular ion MH^+ , smaller fragments can also result, especially
39 from alcohols.⁹ For this reason, and because there is no separation step, it is often
40 difficult to identify the fragments yielded by foods with complex volatile profiles. However,
41 despite this limitation, PTR-MS has been described as an efficient method for
42 quantitating the relationship between the volatile fingerprints and sensory characteristics
43 of foods.¹⁰⁻¹²

44 In particular, PTR-MS has been used to examine differences in VOC release among
45 liquid, semi-solid, and solid foods. Food structure has been found to play an important

46 role. For example, liquid foods such as orange or carrot juice tend to release aromas
47 post-swallow, while solid foods such as peanuts or carrot pieces display pre-swallow
48 release.¹³ These results, which are focused on large structural differences, show that it is
49 important to study the impact of structure on VOC release. Furthermore, nose-space
50 analyses have highlighted that there is a large degree of interindividual variability in VOC
51 release profiles, which could be linked to differences in oral processing—including
52 mastication, salivation, and velum opening—that then lead to differences in aroma
53 perception among individuals.^{14–17} This variation must thus be accounted to understand
54 aroma perception.

55 Bread is a good tool to study the impact of structure on VOC release because of its
56 structural complexity: it is composed of a soft porous crumb surrounded by a rigid crust.
57 In a recent study, the volatile profiles of bread boli collected at three stages of oral
58 processing were analyzed *in vitro* using PTR-MS.¹⁰ The results showed that the
59 incorporation of saliva into the boli impeded the release of VOCs. Furthermore, the
60 presence of crust increased the quantity of released ion fragments likely responsible for
61 perceived notes of “roasted cereals” or “cardboard”. However, *in vitro* studies cannot
62 reveal the natural dynamics of VOC release from breads. To date, only one study has
63 followed the *in vivo* release of VOCs during bread consumption.¹⁸ It showed that release
64 largely occurred post swallowing. A major constraint is that only one bread type and one
65 subject were used.

66 The objective of this study was therefore to gain insight into the effect of bread crumb
67 and crust structure on VOC release and aroma perception over the course of oral
68 processing. To this end, a statistical model was used that revealed the contribution of
69 VOCs to the production of key ion fragments observed during nose-space analysis.

70

71 **Material and Methods**

72 **Characterizing bread and sample types**

73 Nine types of French baguettes were used in this study; all had the same composition
74 (white flour, water, yeast, and salt). For these breads, pictures and texture measurements
75 were preliminary performed at different times of purchase before the study to control the
76 stability of production. One came from a supermarket (S), and five came from three local
77 bakeries (b1, b2, and b3). These baguettes fell into two categories: (i) ordinary breads
78 (O), produced using short fermentation times and possibly containing additives, and (ii)
79 traditional breads (T), produced using long fermentation times and containing no
80 additives.¹⁹ Three other baguettes were produced in the laboratory run by Lesaffre
81 International (B1, B2, and B3; Marcq-en-Baroeul, France), as described in previous
82 studies^{20,21}; they were par baked and frozen until usage. Two sample types were studied:
83 crumb only (CO) and crumb with crust (CC). The supermarket/bakery baguettes were
84 used within 4 hours of purchase (which occurred in the morning), and the laboratory-
85 produced baguettes were used no more than 2 hours after the cooling phase had ended.
86 The nine baguette types were characterized based on their density, crumb water content,
87 and crumb elasticity as performed in Jourden *et al.* (2016).²⁰ Bread density was
88 measured using the rapeseed displacement method. Crumb water content was
89 determined by weighing samples before and after oven drying. Crumb elasticity (i.e.,
90 Young's modulus) was estimated using compression tests performed on crumb cylinders
91 (h: 2.5 cm; d: 3.0 cm) with a TA.XT Plus Texture Analyzer (Stable Micro System, UK). All
92 measurements were performed in triplicate (Table 1).

93 **Monitoring *in vivo* aroma compound release via PTR-MS**

94 **Preliminary *in vitro* studies: selection and identification of target ions.** An *in vitro*
95 study was conducted to help select the ions to be monitored by PTR-MS.¹⁰ The ions were

96 selected based on (i) their ability to be detected by *in vivo* measurements (determined
97 using pretests involving equipment sensitivity) and (ii) their ability to represent sensory
98 differences (Table 2).

99 To identify these target ions, the VOCs released by the crumbs of 40 French baguettes
100 (the 9 baguette types described above plus 31 additional baguettes obtained from
101 supermarkets and bakeries) were characterized. The 31 additional baguettes were
102 mainly composed of white flour, water, yeast, and salt; they did not contain any
103 sourdough, fat, or sugar. Their *in vitro* VOC profiles were quantified using two methods:
104 (i) gas chromatography-mass spectrometry (GC-MS) coupled with purge-and-trap
105 extraction and (ii) proton transfer reaction-mass spectrometry (PTR-MS). For both the
106 GC-MS and PTR-MS analyses, the crumbs of each baguette (n=40) were cut into 1-cm
107 squares and frozen at -80°C in glass vials. No replicates were performed in this
108 preliminary study.

109 In the **GC-MS analyses**, 5 g of bread crumb (defrosted at 4°C for 15 h) were mixed with
110 10 mL of Milli-Q water (Merck Milipore, Merck KGaA, Germany) at 4°C. Five mL of the
111 mixture, homogenized using a Polytron[®] PT 2100 (VWR, Radnor, USA), were introduced
112 into the sampling tube of a purge-and-trap system, which was heated to 37°C. A helium
113 purge was run for 20 min at a flow rate of 20 mL/min, during which time VOCs were first
114 stripped out from the sample and then retained in a TENAX trap at 40°C. The trap was
115 subsequently heated to 250°C and kept there for 2 min to induce desorption. The VOCs
116 were concentrated in the cryomodule at 150°C, just before the injection step at 180°C.

117 The VOCs were then separated in the chromatography column, ionized by electron
118 impact using GC-MS (6890A Agilent, Santa Clara, USA) and identified with database; the
119 apparatus was equipped with a splitless injector kept at 180°C and a CP-Wax 57CB polar
120 capillary column (50 m x 0.25 mm; film thickness of 0.2 µm; Agilent, Santa Clara, USA).

121 Helium was the carrier gas (constant flow rate of 1.2 mL/min). Oven temperature
122 increased from 40°C to 200°C at a rate of 4°C/min and then remained at 200°C for 9 min.
123 In the **PTR-MS analyses**, 5 g of crumb (frozen at -20°C for 30 min) were stored at 20°C
124 for one hour in 100-mL Schott flasks that were equipped with valved caps (GL 45, Duran
125 Group, Wertheim, Germany). A highly sensitive PTR-MS apparatus (Ionicon Analytik,
126 Innsbruck, Austria) was used in SCAN mode over a mass range of m/z 20 to 200; dwell
127 time was 100 ms per peak. Five cycles (90 s) were dedicated to measuring the ambient
128 air (i.e., the background signal), and 15 cycles (290 s) were dedicated to measuring the
129 sample headspace. The gas above the samples was introduced into the system through
130 a capillary line heated to 110°C at a flow rate of 100 mL/min. The PTR-MS settings were
131 as follows: H₂O flow rate of 7.0 mL/min; drift tube pressure of 200 Pa; drift tube
132 temperature of 60°C; and drift voltage of 600 V ($E/N = 153$ Td).

133 The PTR-MS dataset was then statistically linked to the GC-MS dataset: the goal was to
134 associate the specific ion fragments detected by PTR-MS with the VOCs identified by
135 GC-MS (see the **Data analyses** section for more details).

136 ***In vivo* study.** A panel of eight volunteers (six women and two men between the ages of
137 23 and 34) was recruited from the staff of the Grignon center of the French National
138 Institute for Agricultural Research (INRA) to participate in the *in vivo* PTR-MS study. They
139 gave their free and informed consent and received compensation for their participation.
140 They were asked not to drink or eat for at least one hour before the sessions. They took
141 part in nine 30-min PTR-MS sessions. One session was dedicated to each baguette type.
142 During the sessions, participants were asked to eat naturally pre-cut CO samples
143 (cylinder: h: 2.5 cm; d: 3.0 cm) and CC samples (half-cylinder: h: 2.5 cm; r: 3.0 cm).
144 Samples were cut from a vertical slice that was 2.5 cm thick; no more than one minute

145 passed before the samples were given to the participants.²⁰ The participants were asked
146 to rinse their mouths with mineral water (Evian, Danone, France) between sample types.

147 **Measuring *in vivo* dynamics.** The *in vivo* release of aroma compounds was
148 characterized using the multiple ion detection (MID) mode of the PTR-MS apparatus.
149 Three monitoring ions (m/z 21, 37, and 59) and eight target ions (m/z 45, 47, 57, 71, 73,
150 87, 95, and 97) were studied. Two series of measurements were performed (Table 2). As
151 a result, the cumulative dwell time of a given series was 0.55 s, which did not exceed the
152 expiration time of the participants.²² Three replicates were performed for each series.

153 In each series, levels of m/z 21, 37, and 59 were quantified to verify measurement
154 conditions. The m/z 21 ion, which corresponds to $\text{H}_3^{18}\text{O}^+$, is the isotopolog of the m/z 19
155 reagent ion. It is measured instead of m/z 19 to protect the PTR-MS apparatus from
156 excessively high levels of the latter (natural isotopic ratio of $^{18}\text{O}/^{16}\text{O} \approx 0.2$).⁷ Humidity
157 levels were checked by monitoring the abundance of the m/z 37 ion, which must
158 represent less than 2% of reagent ion signal intensity to limit the background signal. The
159 relative amounts of the m/z 30 (NO^+) and 32 (O_2^+) ions were considered to be low
160 enough that they did not contribute to ionization. The m/z 59 ion (resulting from propan-2-
161 one protonation) was monitored to trace participant breath.²²

162 Each *in vivo* analysis began with a 10-s measurement of ambient air. Then, the breath
163 expired via the participants' noses was measured for 30 s, and they subsequently
164 introduced the samples into their mouths. For each participant, nose air space was
165 sampled by inserting the two inlets of a stainless steel nosepiece into the nostrils. The
166 system was affixed to eyeglasses so that the participants could eat relatively normally.
167 The gas produced by the samples was introduced into the PTR-MS apparatus through a
168 capillary line heated to 110°C at a mean flow rate of 85 mL/min. The PTR-MS settings

169 were as follows: H₂O flow rate of 6.0 mL/min; drift tube pressure of 200 Pa; drift tube
170 temperature of 60°C; drift voltage of 600 V (E/N = 152 Td).

171 **Characterizing aroma perception dynamics using the temporal dominance of**
172 **sensations (TDS) method**

173 **Panel.** A panel of 14 volunteers was formed (ten women and four men between the ages
174 of 23 and 60) by recruiting participants from the PTR-MS study and additional staff from
175 INRA-Grignon. Individuals were invited to join based on their motivation for participating
176 and their aroma discrimination abilities. They gave their free and informed consent and
177 received compensation for their participation. They were asked not to eat or drink for at
178 least one hour before the sessions.

179 **Sensory assessment.** All sensory analyses were carried out in individual booths under
180 white light in an air-conditioned room (20°C). Participants were presented with pre-cut
181 vertical slices of bread (2.5 cm thick) placed in plastic boxes labeled with randomly
182 selected three-digit numbers. They were asked to cut a CO or CC sample from the
183 vertical slice using the same protocol as in the PTR-MS sessions; they were also
184 instructed to be consistent in their sample cutting. This approach was employed to
185 maximize sample freshness.

186 After four sessions dedicated to the generation and selection of aroma attributes, a
187 training session was devoted to the temporal dominance of sensations (TDS) test. The
188 participants were introduced to the notion of dominance: a dominant sensation is the
189 sensation that triggers the most attention at a given point in time.²³ Only six samples (CO
190 and CC samples of B1, B2, and B3) were tested using TDS, which was implemented
191 using Fizz Acquisition software (Version 2.47A, Biosystemes, France). All six samples
192 were tested during a single session; three replicates were performed. The samples were
193 presented in randomized order to the different participants to avoid a bias in the results.

194 Participants were instructed to click on the "start" button when they introduced the
195 sample into their mouths. Then, they were asked to select its dominant attributes until
196 they could no longer perceive anything, at which point they were to click on the "stop"
197 button. They indicated when they had swallowed the sample by clicking on the "I'm
198 swallowing" button. Four aroma attributes ("wet flour", "fermented", "wheat", and "butter")
199 were used in the assessment of the CO samples, and seven ("wet flour", "fermented",
200 "wheat", "roasted cereals", "cardboard", "toasted", and "grilled") were used in the
201 assessment of the CC samples.¹⁰ The list of attributes was presented in a randomized
202 order to the participants, but the order was always the same for a given participant. To
203 prepare participants for the test, a warm-up sample was introduced at the beginning of
204 each session.

205 **Chewing behavior quantified by electromyography**

206 Three participants (#1, #3, and #8) were chosen, and their chewing behavior during
207 natural bread consumption was characterized. The same samples were used as in the
208 TDS tests (CO and CC samples of B1, B2, and B3).

209 The activity of the muscles of mastication (the superficial masseter and the anterior
210 temporalis), which are located on both sides of the face, was recorded using surface
211 electromyography (EMG). Two surface electrodes (F-E5GH model, Astro-Med, USA)
212 were placed 1.5 cm apart along the length of each of the muscles, which were located via
213 palpation. To minimize electrical background noise, an ear clip electrode (model F-
214 E34DG, Astro-Med, USA) was placed on the participant's ear lobe.

215 The six samples were assessed in triplicate during the same session to avoid any bias
216 linked to electrode positioning. The participants ate pre-cut CO and CC samples first and
217 second, respectively; they were asked to chew as naturally as possible. Sample
218 dimensions were the same as in the PTR-MS study.

219 **Data analyses**

220 Statistical analyses were carried out with Fizz Traitement software (Version 2.47A,
221 Biosystemes, France) for the sensory data and XLStat software (Version 2010.4.02,
222 Addinsoft, France) for other data.

223 **Bread properties.** A one-way analysis of variance (ANOVA, $p < 0.05$) followed by a
224 multiple comparisons test (Fisher's LSD test, $p < 0.05$) was used to assess differences in
225 properties among bread types. A multiple factor analysis (MFA) was used to examine
226 bread structural properties (density, water content, and Young's modulus) and the initial
227 volatile fingerprints (*in vitro* PTR-MS data) for CO samples for the nine bread types.

228 **Relative contribution of VOCs to target ions.** To follow VOCs release and not only
229 fragments, it was necessary to relate with statistical models VOCs and ions. For that, we
230 selected precursors VOCs using the literature and the results of the GC-MS
231 analysis.^{7,9,24–28} The m/z 47 and 97 ions can only be produced by the fragmentation of
232 ethanol and furfural, respectively. However, the other ions can result from several
233 different VOCs (ranging from three to ten). Therefore, to identify the VOC that most
234 contributed to producing a given ion, it was assumed that the contribution depended on
235 the amount of the VOC in the sample, VOC volatility, and the VOC's proportion of
236 fragmentation into the target ion. We estimated VOC volatility using Henry's law constant
237 ($\text{atm}\cdot\text{m}^3/\text{mole}$), which was calculated with the Bond contribution method in 25°C water
238 (EPI Suite, US Environmental Protection Agency, USA). We used the VOC's proportion
239 of fragmentation observed in pure solution.^{7,9,24–28} When no F was found in literature, we
240 choose to maximize the contribution of this molecule to 100. The respective contribution
241 of VOC x to ion y is given by the equation (1):

$$242 \quad \% \text{ contribution } (x, y) = 100 \times \frac{AUC_x \times V_x \times F_{x,y}}{\sum_1^N AUC_i \times V_i \times F_{i,y}} \quad (1)$$

243 where N is the number of molecules, AUC is the area under the curve obtained by GC-
244 MS, V is volatility, and F is the proportion of fragmentation of VOC x to ion y .

245 To confirm that this value was consistent, a simple linear regression was performed
246 between the intensity of ion y and X , the sum of the corrected AUCs (equation (2)).

$$247 \quad X = \sum_1^N AUC_i \times V_i \times F_{i,y} \quad (2)$$

248 A linear regression was performed using the data on the 40 bread types, by
249 bootstrapping 30 randomized lines (1000 iterations). The R^2 distributions of these linear
250 regressions were plotted, and the mean R^2 was calculated.

251 The % contribution was weighted by the percentage of Y explained by X as expressed by
252 the R^2 value (when mean $R^2 > 0.5$) according to the following equation (3):

$$253 \quad \% \text{ contribution } (x, y) = 100 \times R^2 \times \frac{AUC_x \times V_x \times F_{x,y}}{\sum_1^N AUC_i \times V_i \times F_{i,y}} \quad (3)$$

254 This value was calculated for each bread type. The means and standard deviations for
255 the nine bread types used in the *in vivo* study are presented in Table 3.

256 **Kinetics of ion release.** Ion release curves were drawn using PTR-MS software (Ionicon
257 Analytik, Innsbruck, Austria). The background noise was removed by subtracting the
258 mean signal for the ambient air and the mean signal for the participant's breath from the
259 signal obtained during oral processing. Since the objective was to compare ion release
260 kinetics among bread types, arbitrary units (intensity in ion counts per second [cps]) were
261 used. The following variables were calculated from each individual release curve and for
262 each ion: maximum intensity (I_{max}), the time at which maximum intensity occurred
263 (T_{max}), and the total area under the curve (AUC). A three-way ANOVA followed by a
264 multiple comparisons test was performed using the PTR-MS data ($p < 0.05$, Fisher's LSD
265 test). The model included bread type (the nine breads), sample type (CO vs. CC),
266 participant identity (ID), and the following interactions: bread type*sample type, bread

267 type*participant ID, and sample type*participant ID. Since the differences between the
268 CO and CC samples were highly significant, we also performed two-way ANOVAs on the
269 CO and CC datasets separately to study the differences among bread types; the models
270 included bread type, participant ID, and bread type*participant ID.

271 **TDS data.** Sensation dominance, expressed as a percentage, was calculated at each
272 point in time: it was the relative frequency of a given attribute being described as
273 dominant by the participants. These percentages were smoothed using the Bézier
274 procedure in Fizz Traitement software and were plotted against standardized time (0 =
275 start of mastication and 100 = end of perceived sensations). The curves for all the
276 attributes were plotted on the same graph, along with the “chance level” line and the
277 “significance level” line.²⁹

278 **EMG data.** The following variables were calculated from the EMG curves: number of
279 bites, chewing frequency (s^{-1}), mean burst duration (ms), mean EMG activity (mean of
280 the peak areas per masticatory cycle averaged across the four muscles; $\mu V.s$) and total
281 EMG activity (sum of the peak areas for all the masticatory cycles averaged across the
282 four muscles; $mV.s$).³⁰ A three-way ANOVA followed by a multiple comparisons test ($p <$
283 0.05, Fisher's LSD test) was performed; the model included bread type, sample type,
284 participant ID, and all possible interactions.

285

286 **Results**

287 **Bread crumb properties**

288 The nine breads differed in the structural properties and VOC profiles of their crumbs
289 (Table 1 and Figure 1). Figure 1 displays the results of the MFA, notably the variable
290 correlation circle (Figure 1.a) and bread position along axes 1 and 2 (Figure 1.b). Bread
291 types B1, B2, and B3 had significantly higher crumb water content than the other bread

292 types. They had also larger amounts of the main VOC ion fragments. Bread type b3_T, a
293 traditional bread from a bakery (b3), had the firmest, densest crumb. Breads B2 and S_O
294 had the least dense crumb. On average, the crumb of the three ordinary breads (b1_O1,
295 b1_O2, and S_O) was less dense and more dry than that of the three traditional breads
296 (b1_T, b2_T, and b3_T). Moreover, the ordinary breads had very similar crumb structures
297 and VOC profiles; the traditional breads showed greater differentiation. Among the par-
298 baked breads, breads B1 and B3 were similar in crumb structure, while breads B2 and
299 B3 had similar crumb VOC profiles.

300 **Relative VOC contributions to target ions**

301 To identify the ions to monitor, the contributions of each VOC to ion production were
302 determined based on the linear regression between the amount of a given ion, as
303 measured by PTR-MS, and the sum of the amount of contributing VOCs, as measured by
304 GC-MS (Table 3). The calculations were based on data for the crumb samples of the nine
305 bread types. As expected, m/z 47 was closely associated with ethanol ($R^2 = 0.78$);
306 indeed, it is the main ion produced by the protonation of ethanol during PTR-MS.^{9,22} This
307 result confirms that it is possible to relate the GC-MS data to the PTR-MS data. The R^2
308 values of the linear regressions for m/z 57 and 87 were significantly higher than 0.5,
309 suggesting that each set of VOCs explained most of the variability in each ion's
310 abundance. For example, m/z 57 could result from any one of 10 VOCs found in bread
311 crumb, but in this study, it was mainly related (58%) to 2-methyl-1-propanol, which was
312 found in the nine bread types used. This VOC is produced during fermentation, occurs in
313 large quantities in bread crumb, and is associated with sensory notes such as "glue",
314 "alcoholic", "wine-like", and "malty".³¹ The m/z 87 ion seems to result from 2-
315 methylbutanal (34%) and 3-methylbutanal (15%). In the crumb, these compounds are
316 mainly produced during fermentation (via the Ehrlich pathway); they are also known as

317 Maillard compounds.³² They have been identified as key odorants in baguette crusts with
318 “malty”, “almond”, and “roasted” notes.^{31,33} For m/z 45 and 73, the R^2 values were higher
319 than 0.5 but not significantly so. Some hypotheses may nonetheless be proposed. The
320 m/z 45 ion could mainly be produced by acetaldehyde, as observed in other studies.^{7,34,35}
321 The m/z 73 ion could be generated by 2-butanone and 2-methylpropanal, compounds
322 produced by fermentation or Maillard reactions. Finally, the regression results were not
323 significant for m/z 71, 95, and 97.

324 ***In vivo* release**

325 **Differences in ion release dynamics.** In Figure 2, an example of the mean release
326 curves for the different ions across all the participants is provided; the data come from
327 the B1 CO and CC samples. Ion release kinetics differed for the different bread types.
328 The intensities of the m/z 45, 47, 57, and 71 ions rose progressively from the time the
329 sample was introduced into the mouth until it was swallowed; they peaked at swallowing
330 for both sample types. Since release dynamics were the same for the CO and CC
331 samples, it appears that the presence of crust did not impact their release. Consequently,
332 these ions can be seen as crumb markers. In contrast, m/z 73 and 87 release differed
333 between the CO and CC samples: it was close to nine times higher when crust was
334 present. Moreover, the ions' intensities peaked at the beginning of mastication, just after
335 the sample entered the mouth. Then, they decreased slightly and were not impacted by
336 swallowing. These ions can thus be seen as crust markers. The m/z 95 and 97 ions
337 occurred at very low levels in both the CO and CC samples ($RSB < 1.5$), so their results
338 were not analyzed further.

339 **Differences in VOC release among bread types and sample types.** Maximum ion
340 release intensities were determined for each participant for all the bread types and
341 sample types (Figure 3). We observed differences in release profiles among breads for

342 all the ions detected in the CO and/or CC samples. In the CO samples, the par-baked
343 breads, especially B1 and B3, had the highest maximum intensities for the crumb
344 markers (m/z 45, 47, 57, and 71). This result makes sense, given that the initial VOC
345 profiles for the CO samples of these breads were rich in m/z 45, 47, and 57 (Figure 1). In
346 the CC samples, the par-baked breads had the lowest maximum intensities for the crust
347 markers (m/z 73 and 87). Their crusts were probably poorer in Maillard compounds than
348 those of the other breads. The intensities of m/z 73 and 87 were highest in bread b1_O2,
349 suggesting, for example, it was subject to more intensive baking than the others. Finally,
350 there was no clear distinction between ion intensities in ordinary versus traditional breads
351 for either the CO or CC samples.

352 **Differences in ion release among participants.** Individuals differed in their ion release
353 dynamics (Figure 4). Participants #1 and #7 had low maximum intensities for all the ions
354 (except m/z 87 with medium intensity), whereas the opposite was true for participants #2,
355 #3, #4, and #5. Participants #6 and #8 had intermediate maximum intensities across the
356 panel, but the highest intensity of m/z 87 for participant #6.

357 **Chewing behavior dynamics**

358 Using EMG, the chewing behavior of three participants (#1, #3, and #8) was
359 characterized as they consumed CO and CC samples of the three par-baked breads (B1,
360 B2, and B3) (Table 4). As expected, some differences were observed between the CO
361 and CC samples. When crust was present, the mean number of bites increased 1.25 to
362 1.5 times, and the mean activity required to break down the CC versus the CO samples
363 was 2-fold higher. The three par-baked breads also showed significant differences, which
364 seemed to be driven by bread density and crumb firmness. The densest and firmest
365 bread—B1—was associated with the greatest number of bites, the lowest chewing
366 frequency, the highest mean burst duration, and the highest mean and total EMG activity

367 for both the CO and CC samples (data not shown). Finally, the participants differed in
368 their chewing behavior. Participant #1 was a slow eater and displayed an intermediate
369 level of EMG activity. It appears that this individual did not adapt her chewing behavior to
370 sample type because she showed no difference in chewing frequency or mean burst
371 duration for the CO versus the CC samples. Participant #3 was also a slow eater but
372 displayed a high level of EMG activity. Participant #8 consumed all the samples faster
373 than did the two others and had a low level of EMG activity. His mean burst duration was
374 higher than that of the other two, which was suggestive of a different oral management
375 strategy.

376 **Perception dynamics for the three par-baked breads**

377 The dynamics of the dominant sensations associated with the CO and CC samples of the
378 three par-baked breads (B1, B2, and B3) were characterized using the TDS method
379 (Figure 5). These three breads were used because they are well characterized,
380 especially with regards to their bolus properties.^{10,20,21} Therefore, clarifying how these
381 breads break down during oral processing could enhance our understanding of
382 perception dynamics. The results show that the different bread types and sample types
383 resulted in different sensations over time.

384 When participants consumed the CO samples, they perceived all three breads as having
385 dominant “wheat” notes just before swallowing. Furthermore, bread B2 conveyed a
386 dominant “fermented” sensation at the beginning of consumption. Otherwise, there was
387 no agreement among participants with regards to the dominant sensations experienced
388 until the intermediate phase of crumb consumption. After swallowing, “wet flour” and
389 “wheat” notes persisted in association with breads B2 and B3, respectively.

390 When participants consumed the CC samples, they attributed a greater number of
391 dominant attributes to the breads. At the beginning of consumption, they perceived the

392 three breads as having dominant “toasted” notes. As oral processing proceeded, “roasted
393 cereals” and “cardboard” notes emerged for breads B1 and B2, respectively. For breads
394 B1 and B3, the dominance of “wheat” emerged prior to swallowing, as for the CO
395 samples. In contrast, there were no commonalities between B2 CO and CC samples.

396

397 **Discussion**

398 **VOC contribution to ion fragments**

399 To our knowledge, this study is the first to statistically relate ion fragment data obtained
400 using PTR-MS to VOC data obtained using GC-MS with the purpose of quantitating the
401 contributions of VOCs to ion fragments. An important caveat is that the relationships
402 described here are only applicable to the study food: French baguette crumb. For m/z 71,
403 95, and 97, the linear regressions were not significant. There could be different
404 explanations for this finding: (i) all the VOCs capable of generating these ions were not
405 included in the statistical models; (ii) background noise (i.e., excessive variability) could
406 be obscuring the relationship given that both the ions and the VOCs occurred at low
407 levels; and/or (iii) some of the VOCs could display a lesser affinity for the purge-and-trap
408 sorbent (i.e., Tenax[®]), skewing their abundance and ratios relative to their actual
409 occurrence in bread.³⁶ However, for five of the target ions, the coefficients of
410 determination were high enough that a percentage contribution could be calculated. The
411 results are consistent with those found with data on baker’s yeast starters obtained via
412 PTR-MS utilizing a time-of-flight mass analyzer³⁴; however, the latter study focused
413 exclusively on VOCs produced during fermentation. In this study, the VOCs produced
414 during both fermentation and baking were examined. This statistical analyses have
415 helped clarify the dynamics behind the ion fragments generated by complex food

416 products such as bread. It has also shown that ion fragments are not always related to a
417 VOC's protonated ion.

418 **Impact of bread structure and composition on *in vivo* aroma release**

419 As expected, the release of crumb markers (i.e., m/z 45, 47, 57, and 73) during crumb
420 consumption was influenced by the crumb's initial VOC profile. In breads B1, B2, B3, and
421 b1_T, both the initial content and intensity of crumb markers were high. Furthermore, the
422 release of crumb markers was also impacted by crumb firmness. Indeed, breads with
423 high Young's modulus values, such as b3_T or b1_O1, also displayed high intensity
424 values for crumb markers, even though these markers were present at intermediate or
425 low levels. The greater release of crumb markers in breads with firm crumb could be
426 explained by the fact that firm breads induced more intense chewing activity. This result
427 concurs with results found for model cheeses varying in hardness.¹⁶ So, the high degree
428 of muscular activity associated with firm crumbs³⁷ could lead to a more thorough
429 breakdown of the food product and thus result in greater ion release. When crust was
430 present, there was a large release of crust markers (m/z 73 and 87). Indeed, crust
431 markers had a greater rate of release than did crumb markers. Structural differences
432 between the crumb and the crust could be at the origin of this observation. The soft and
433 elastic crumb is surrounded by the rigid and brittle crust.^{38,39} During consumption, the
434 crust is probably broken down more rapidly than the crumb due to its placement on the
435 bread's surface and its brittle structure, thus leading to a faster release of crust markers.
436 To conclude, in addition to initial VOC profile, crumb firmness and, in all likelihood, crust
437 brittleness are important factors that can impact the *in vivo* release of VOCs. They should
438 therefore be accounted for when designing the aromatic properties of breads.

439 **Impact of chewing behavior on *in vivo* aroma release**

440 It is well known that individuals vary in their oral processing and thus release aromas
441 differently.^{17,40} Mastication, saliva volume, and saliva composition all have a major
442 impact.⁴¹ To delve further into aroma release dynamics, we studied the chewing behavior
443 of three of the participants in the *in vivo* study. The results revealed that greater muscular
444 activity appears to release greater amounts of VOCs. However, although a previous *in*
445 *vitro* study¹⁰ had found that the volume of saliva added to the bolus plays a key role in
446 limiting aroma release, the links were not obvious here. In fact, contrary to expectations,
447 participant #3, who had a more hydrated bolus than did participants #1 and #8, also
448 showed more intense chewing activity and greater levels of ion release (bolus data come
449 from a previous study involving the same three subjects²⁰). Thus, under *in vivo*
450 conditions, mastication appears to have a greater effect on aroma release than does
451 salivation. However, this result must be confirmed with a greater number of subjects. In
452 summary, participant physiology affects VOC release. More specifically, intense chewing
453 activity releases greater amounts of VOCs in the oronasal cavity. However, the
454 differences in VOC release were more dramatic among bread types than among
455 participants. A study involving a larger number of subjects would be required to validate
456 these results.

457 **Impact of *in vivo* aroma release on perception dynamics**

458 Differences in the release of crumb and crust markers could explain the perception
459 dynamics observed via TDS. It was difficult to link a specific marker with a specific
460 attribute, because a perceived aroma is the result of a combination of several VOCs, and
461 all the compounds responsible for generating aroma perceptions were not monitored.
462 Nonetheless, some relationships could be established at the level of sample type. Crumb
463 markers such as acetaldehyde (m/z 45), ethanol (m/z 47), and 2-methyl-1-propanol (m/z
464 57) were progressively released until a sample was swallowed, which is when they

465 reached maximum intensity. They could thus contribute to dominant sensations at the
466 end of the mastication phase and post swallowing. These sensations were associated
467 with crumb-specific attributes such as “wheat”, “wet flour”, or “fermented”. In contrast,
468 when crust was present, crust markers such as Strecker aldehydes (2- and 3-
469 methylbutanal [m/z 87] and 2-methylpropanal [m/z 73]) were released in larger quantities
470 from the beginning of mastication. They likely contributed to initial dominant sensations
471 such as “toasted”, “roasted cereals”, or “cardboard”. However, although the release of
472 crumb markers was generally similar between CO and CC samples, the B2 CC sample
473 was not perceived as having dominant “wheat” and “wet flour” notes like the B1 and B3
474 CC samples at the end of mastication. For B2, this absence could have resulted from the
475 bread’s low-density, elastic crumb structure, rapidly broken crust, or a masking effect of
476 the crust’s “cardboard” note.

477 In conclusion, crumb and crust markers were released at different rates, which apparently
478 led to different sequences of sensation dominance between crumb and crust attributes.
479 This study revealed that the initial levels of crumb markers as well as crumb firmness
480 affected crumb markers release, leading to different perception dynamics. Overall, this
481 study underscores that characterizing bread texture is essential for a better
482 understanding volatile release dynamics and, thus, the way in which bread aroma is
483 perceived. These results could help inform the development of new bread types (e.g., via
484 yeast selection or customizing specific steps in the bread-making process) that better
485 target consumer needs and desires. To go further with the project, the effect of crumb
486 and crust structure could have been investigated in *in vitro* conditions (e.g. using a model
487 –mouth and applying different break forces), in order to get more insights and to
488 eliminate subject variation in chewing. The ion/flavor release during consumption could

489 have been analyzed with and without chewing, to gain information of static and dynamic
490 flavor release including the effect of saliva and the formation of Strecker Aldehydes.

491

492 **Abbreviations**

493 APCI-MS, atmospheric pressure chemical ionization-mass spectrometry; PTR-MS,
494 proton transfer reaction-mass spectrometry; VOC, volatile organic compound; TDS,
495 temporal dominance of sensations; EMG, electromyography; ANOVA, analysis of
496 variance; CO, crumb only; CC, crumb with crust.

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505

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- 627

628

629 **Figure captions:**

630 Figure 1: Correlation circle (a) and the graphical representation of bread type features (b)
631 along axes 1 and 2 in the multiple factor analysis (MFA) performed on structural data (in
632 red) and PTR-MS data (in blue) for the crumb of the nine bread types. The target ions
633 from the *in vivo* analyses are represented with open symbols.

634 Figure 2: Example mean release curves for the eight target ions; they were generated
635 using data for crumb-only (CO) and crumb-with-crust (CC) samples of bread type B1.
636 Intensity (in counts per seconds [cps]) was plotted against consumption time (s). The
637 mean swallowing time (ST) is indicated by the vertical line.

638 Figure 3: Mean maximum intensities (I_{max} , in cps) and the associated standard errors
639 ($n=24$) for the nine bread types (for crumb-only [CO] or crumb-with-crust [CC] samples).
640 The letters a through e indicate when means differed significantly among bread types
641 (Fisher's LSD test; $p < 0.05$).

642 Figure 4: Mean maximum intensities (I_{max} , in cps) and the associated standard errors
643 ($n=54$) for the eight study participants. The letters a through e indicate when means
644 differed significantly among participants (Fisher's LSD test; $p < 0.05$).

645 Figure 5: Temporal patterns of sensation dominance for crumb-only (CO) samples (on
646 the left) and crumb-with-crust (CC) samples (on the right) during consumption. Time was
647 standardized (% of time available for perception). SL = significance line; CL = chance
648 line; ST = swallowing time.

Tables

Table 1: Physical properties of the nine studied breads assessed in triplicates. Product effect was determined by a one-way ANOVA. Letters a to g indicate means that significantly differ between products at $p < 0.05$ (Fisher's LSD test).

Bread code name	Origin	Type	Density (-)	Water content of crumb (g per 100 g of crumb)	Young's modulus of crumb (kPa)
B1	Laboratory	Par-baked	0.273 ± 0.001 <i>a</i>	51.5 ± 0.2 <i>a</i>	15.0 ± 4.1 <i>b</i>
B2	Laboratory	Par-baked	0.164 ± 0.001 <i>g</i>	49.3 ± 0.1 <i>b</i>	3.8 ± 0.9 <i>e</i>
B3	Laboratory	Par-baked	0.220 ± 0.003 <i>c</i>	50.6 ± 0.1 <i>a</i>	12.5 ± 0.6 <i>bc</i>
S_O	Supermarket	Ordinary	0.164 ± 0.001 <i>g</i>	45.4 ± 0.3 <i>e</i>	7.8 ± 0.9 <i>cde</i>
b1_O1	Local bakery 1	Ordinary	0.180 ± 0.002 <i>e</i>	46.6 ± 1.8 <i>d</i>	11.0 ± 4.2 <i>bcd</i>
b1_O2	Local bakery 1	Ordinary	0.171 ± 0.001 <i>f</i>	47.1 ± 0.3 <i>cd</i>	7.1 ± 4.6 <i>de</i>
b1_T	Local bakery 1	Traditional	0.216 ± 0.002 <i>c</i>	48.2 ± 0.5 <i>bc</i>	11.5 ± 3.5 <i>bcd</i>
b2_T	Local bakery 2	Traditional	0.192 ± 0.001 <i>d</i>	48.5 ± 0.3 <i>b</i>	5.7 ± 1.1 <i>e</i>
b3_T	Local bakery 3	Traditional	0.265 ± 0.006 <i>b</i>	46.6 ± 0.5 <i>d</i>	25.3 ± 2.1 <i>a</i>

Table 2: Ions that were monitored during *in vivo* PTR-MS measurements.

<i>m/z</i> ion	Chemical formula	Dwell time (ms)	Series 1	Series 2
21	H ₂ O, H ⁺	50	x	x
37	(H ₂ O) ₂ , H ⁺	50	x	x
45	-	100	x	
47	C ₂ H ₅ OH, H ⁺	100	x	
57	-	100	x	
59	C ₃ H ₆ O, H ⁺	50	x	x
71	-	100	x	
73	-	100		x
87	-	100		x
95	-	100		x
97	C ₅ H ₄ O ₂ , H ⁺	100		x

Table 3: Percentage of contribution of VOCs (with a molar mass M) to the corresponding fragments, calculated by taking the volatility (V), the proportion of fragmentation into the corresponding ion (F) of each VOC and the mean R² of the linear regression between X (sum of the quantities of VOCs measured by purge and trap) and Y (quantity of the ion measured *in vitro* by PTR-MS) into account. Compounds were identified based on their linear retention indices and mass spectra. We used a mass standard MS database: the Wiley Registry of Mass Spectral Data. Identification was not confirmed by standard injection. The volatility (V) was characterized by Henry's law constant (partition coefficient of VOCs between air and water at 25°C), calculated using the EPI Suite's experimental database. The proportion of fragmentation (F) was founded in literature. If not the maximal coefficient was chosen (100, in italic). SD = standard deviation of R².

m/z	VOC (tentatively identified by GC-MS)	M (g.mol ⁻¹)	V (atm.m ³ /mol)	F (%)	R ²	SD	% contribution
45	acetaldehyde	44	6.78E-05	100 ²⁵	0.520	0.077	43 ± 5
	2-methylbutanal	86	1.59E-04	32 *			8 ± 5
	2-pentanone	86	8.73E-05	82 ^g			<1
	2-heptanol	116	2.34E-05	100			<1
47	ethanol	46	5.67E-06	70 ^g	0.780	0.049	78
57	2-methyl-1-propanol	74	9.99E-06	100 ²⁸	0.823	0.079	58 ± 24
	2-propenal	56	3.58E-05	100 ^{2b}			10 ± 13
	nonanal	142	4.93E-04	9 ^y			6 ± 19
	1-butanol	74	9.99E-06	90 ^y			<5
	1-pentanol	88	1.33E-05	89 ^g			<5
	1-hexanol	102	1.76E-05	16 ^g			<5
	1-hydroxypropan-2-one	74	1.73E-06	20 *			<1
	1-heptanol	116	2.34E-05	100			<1
	2-heptanol	116	2.34E-05	100			<1
1-octen-3-ol	128	2.31E-05	3 ^y	<1			
71	2-butenal	70	5.61E-05	100 ²⁸	0.318	0.096	
	3-hydroxybutan-2-one	88	1.03E-05	100			
	2-methylbutanol	88	1.33E-05	100			
	3-methylbutanol	88	1.33E-05	39 ^y			
	2-pentanol	88	1.33E-05	39 ^{2b}			
73	2-butanone	72	6.58E-05	100 ^g	0.609	0.126	38 ± 8
	2-methylpropanal	72	1.20E-04	100 ²⁸			18 ± 10
	tetrahydrofuran	72	8.43E-05	77 ²⁶			<5
87	2-methylbutanal	86	1.59E-04	49 *	0.622	0.116	34 ± 9
	3-methylbutanal	86	1.59E-04	10 *			15 ± 6
	pentanal	86	1.59E-04	5 ^g			9 ± 11
	2-pentanone	86	8.73E-05	85 ^y			<5
	2,3-butanedione	86	1.97E-07	89 ^y			<1
	3-methylbut-3-en-1-ol	86	1.55E-05	1 *			<1
	1-penten-3-ol	86	9.88E-06	100			<1
	2-penten-1-ol	86	1.17E-05	100			<1
95	methylpyrazine	94	3.22E-06	100 *	0.185	0.120	
	phenol	94	5.61E-07	98 *			
	2,3-pentanedione	100	2.62E-07	3 *			
97	furfural	96	3.77E-06	96 *	0.429	0.131	

*IONICON Analytik GmbH data (2008)

Table 4: Chewing activity parameters measured by electromyography for crumb only (CO) and crumb with crust (CC) samples of B1, B2 and B3 breads and for 3 panelists. Mean and standard deviation of number of bites, chewing frequency, mean burst duration, mean EMG activity and total EMG activity. Letters a to e indicate means that significantly differ between panelists and samples at $p < 0.05$ (Fisher's LSD test).

		CO samples			CC samples		
		Panelist #1	Panelist #3	Panelist #8	Panelist #1	Panelist #3	Panelist #8
Bite number	-	49 ± 6 <i>b</i>	41 ± 2 <i>c</i>	25 ± 3 <i>e</i>	61 ± 7 <i>a</i>	61 ± 6 <i>a</i>	34 ± 4 <i>d</i>
Chewing frequency	s ⁻¹	1.46 ± 0.06 <i>ab</i>	1.34 ± 0.08 <i>c</i>	1.40 ± 0.07 <i>b</i>	1.47 ± 0.04 <i>a</i>	1.41 ± 0.09 <i>ab</i>	1.46 ± 0.07 <i>a</i>
Mean burst duration	ms	284 ± 16 <i>d</i>	339 ± 19 <i>b</i>	381 ± 31 <i>a</i>	274 ± 17 <i>d</i>	310 ± 9 <i>c</i>	326 ± 15 <i>b</i>
Mean EMG activity	μV.s	26 ± 7 <i>c</i>	29 ± 6 <i>c</i>	17 ± 2 <i>d</i>	44 ± 4 <i>b</i>	59 ± 7 <i>a</i>	39 ± 1 <i>b</i>
Total EMG activity	mV.s	1.30 ± 0.41 <i>c</i>	1.22 ± 0.26 <i>c</i>	0.44 ± 0.09 <i>d</i>	2.66 ± 0.38 <i>b</i>	3.60 ± 0.70 <i>a</i>	1.33 ± 0.41 <i>c</i>

Figure 1.a

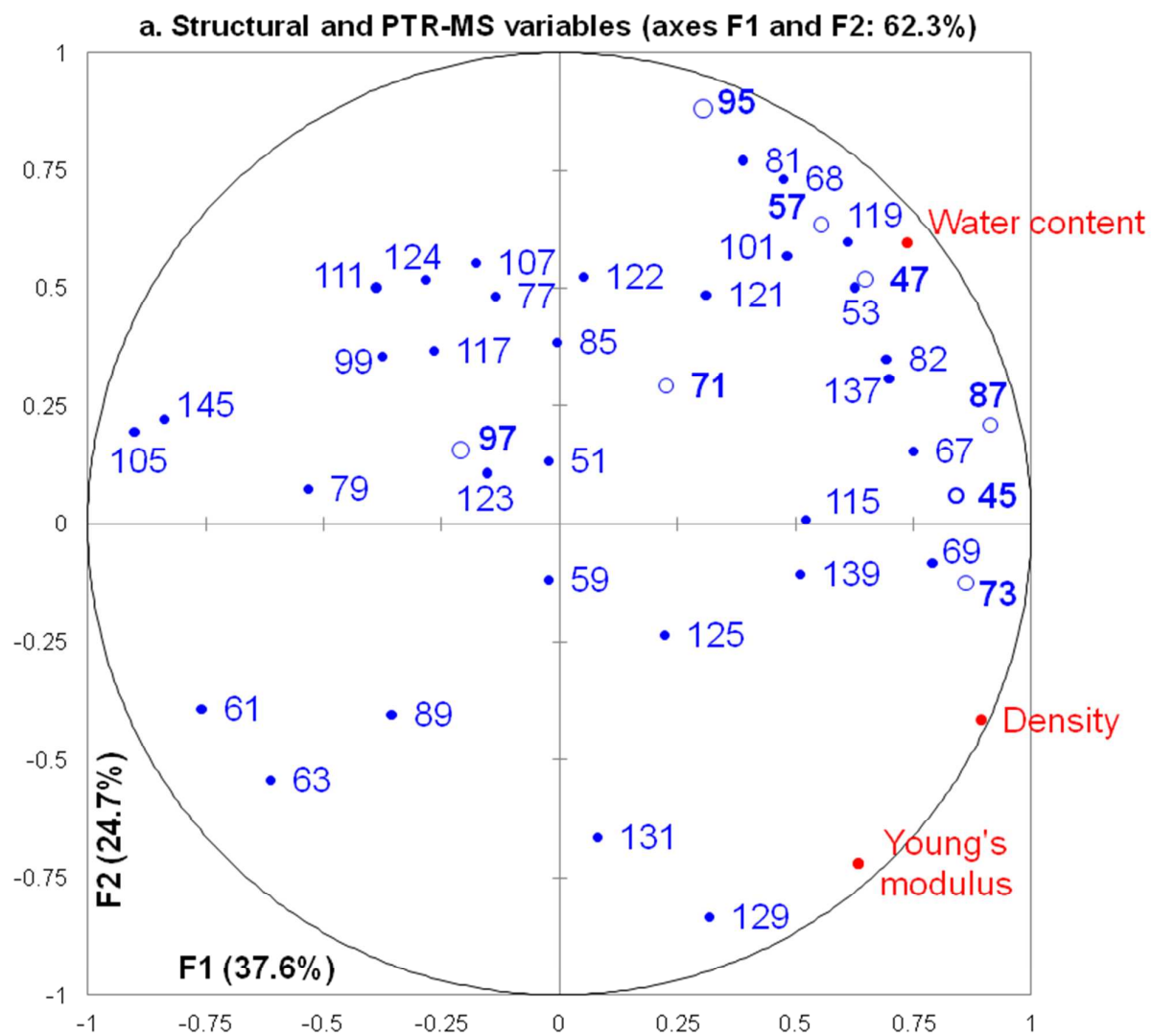


Figure 1.b

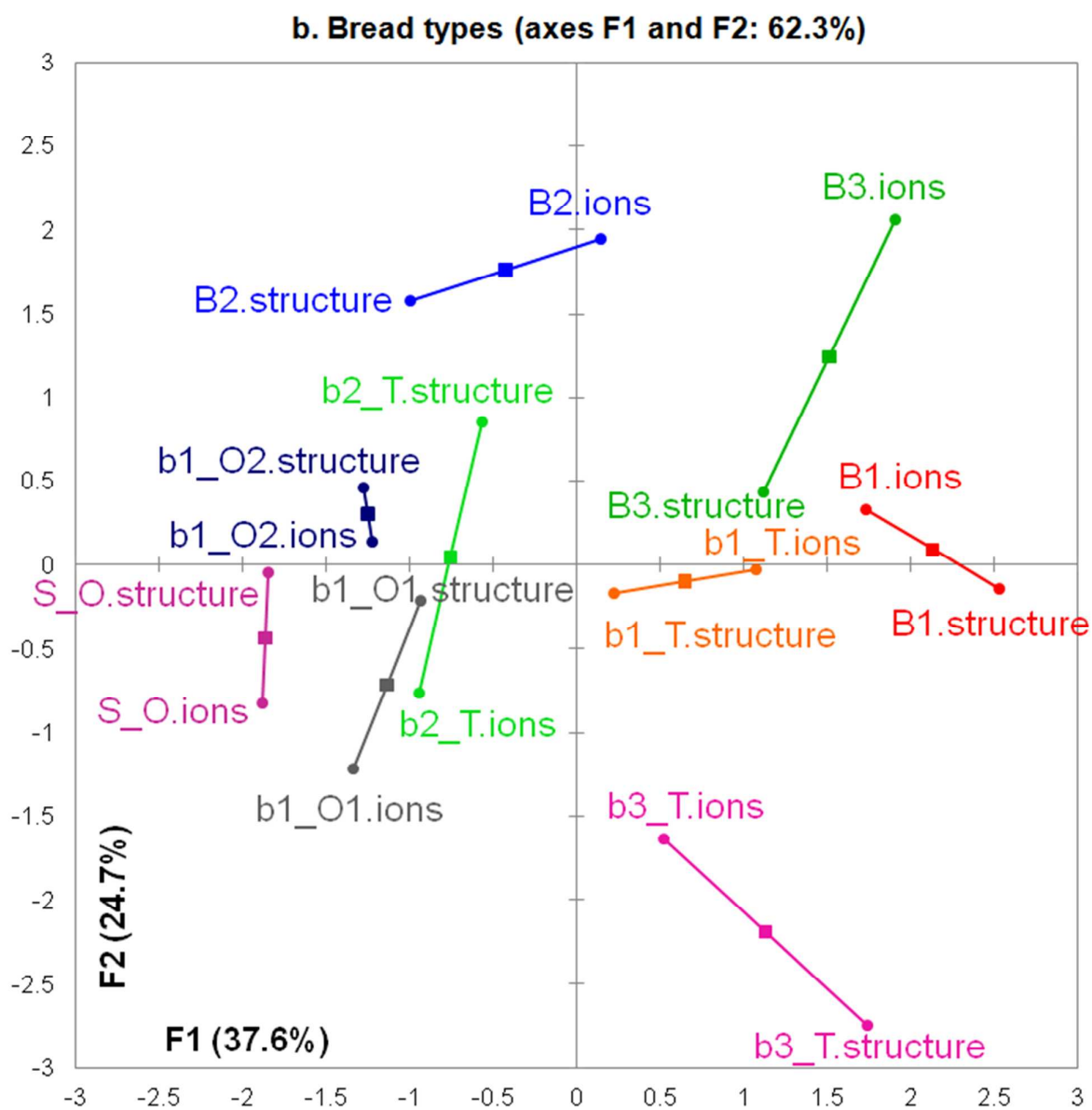


Figure 2

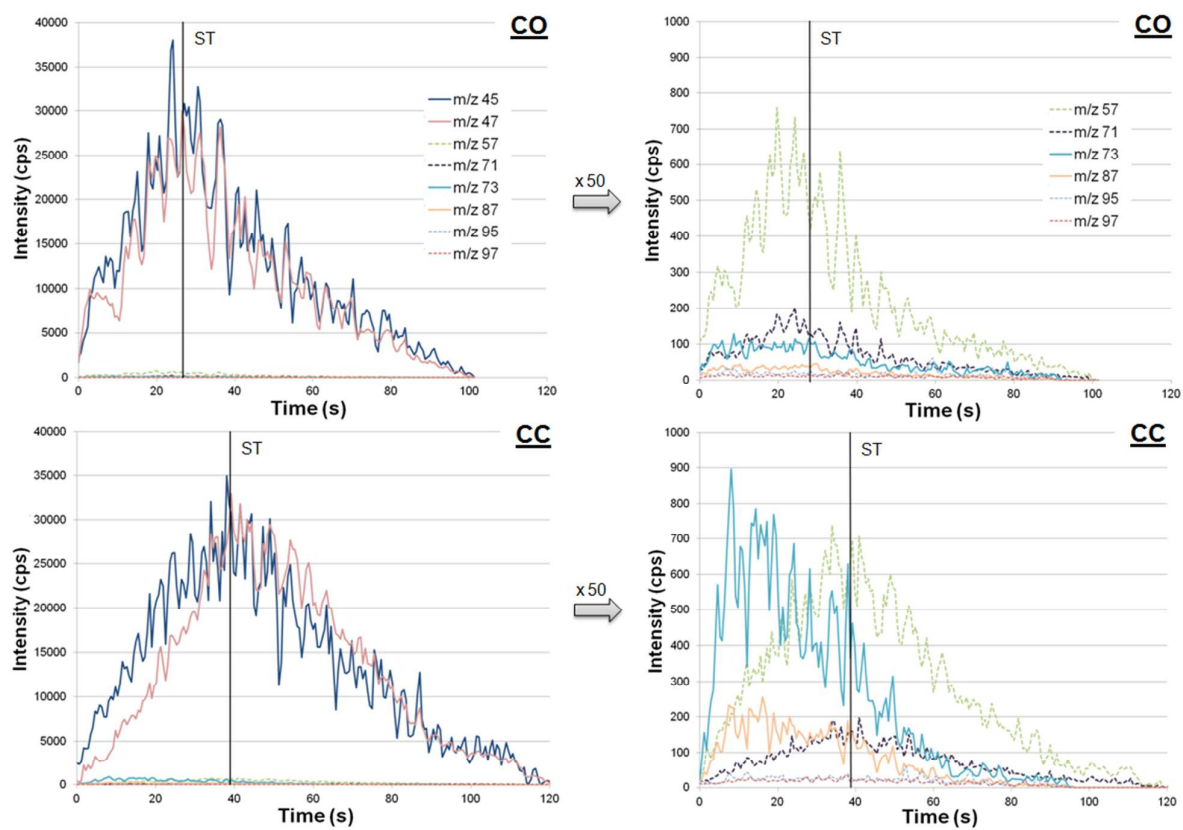


Figure 3

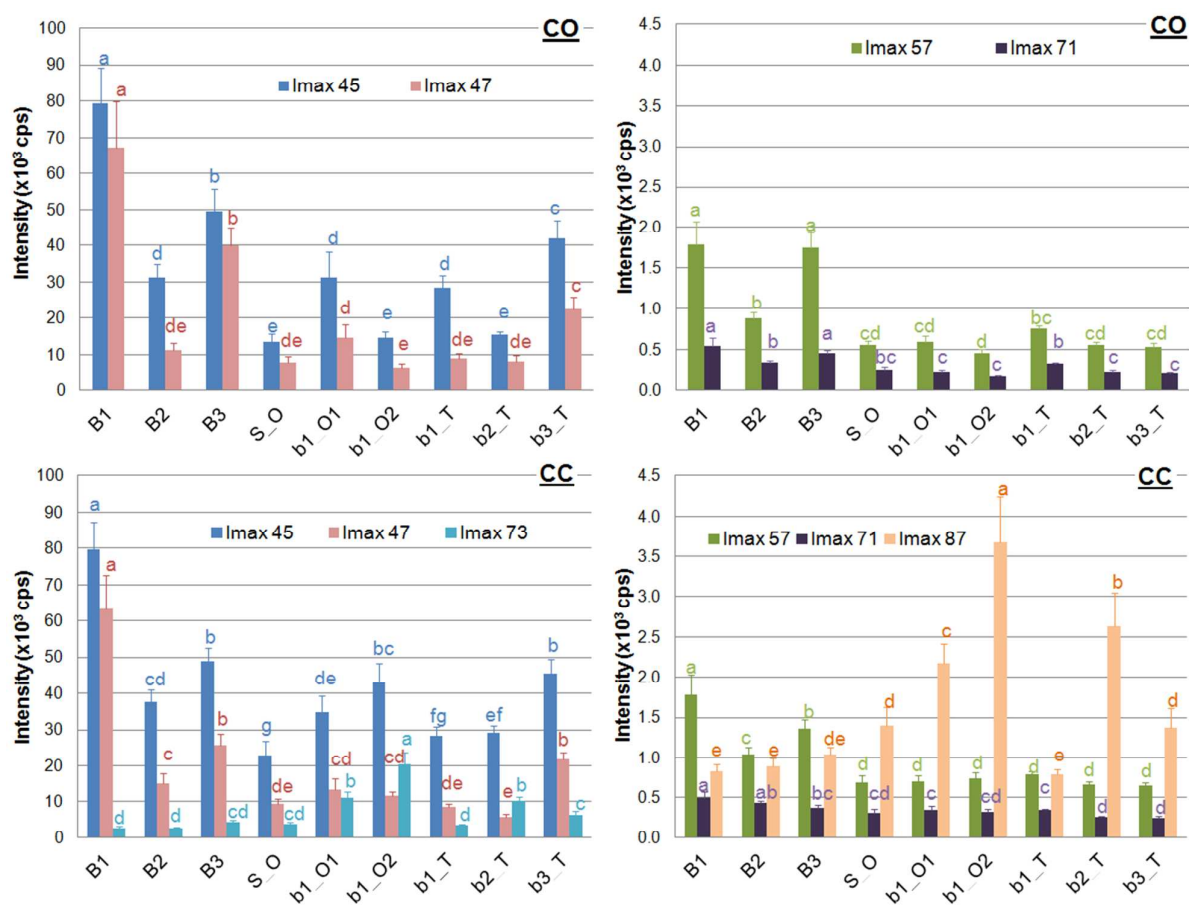


Figure 4

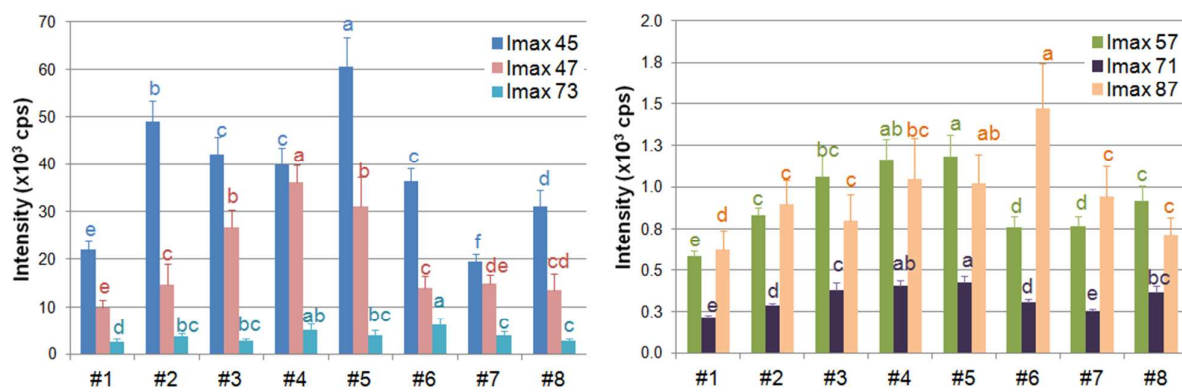
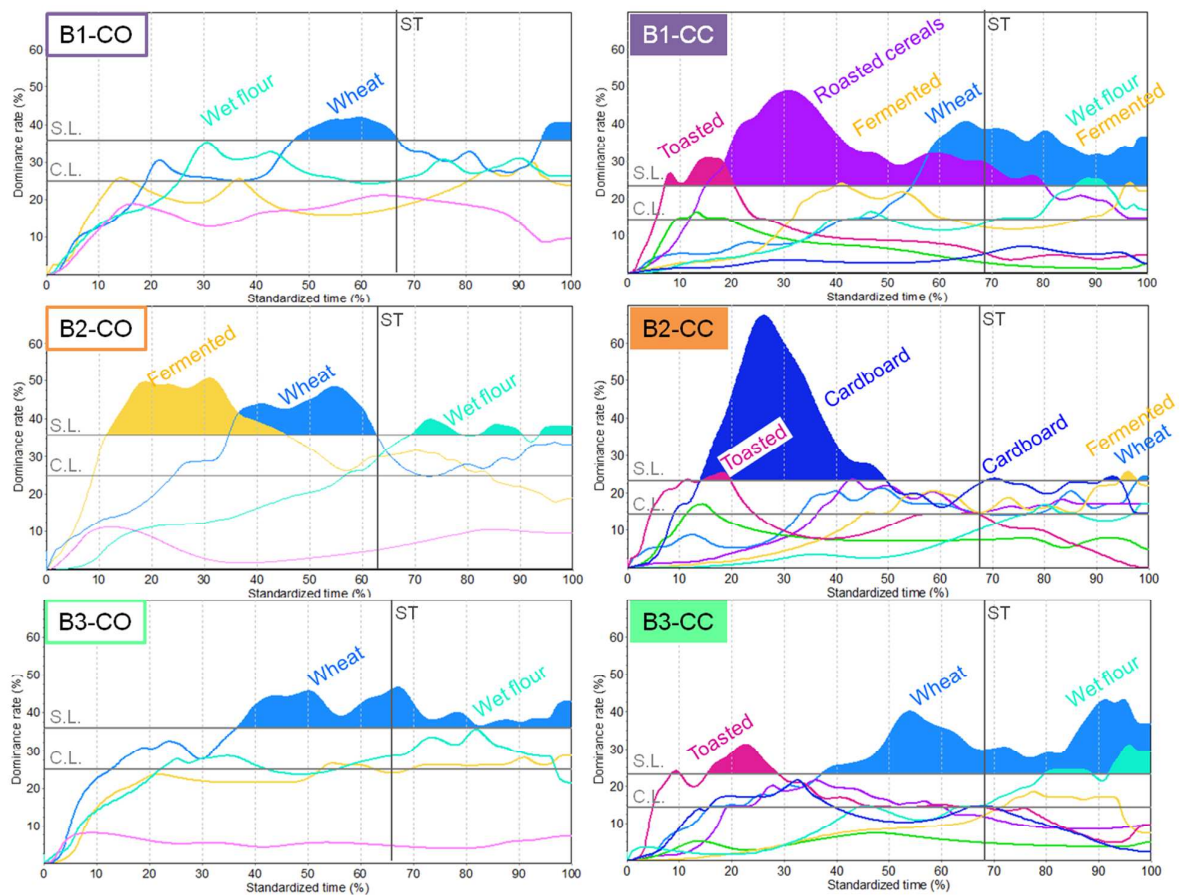


Figure 5



TOC graphic

Dynamics of bread aroma perception are related to aroma release in the oral cavity (for crumb with crust samples)

