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

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Abstract

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

Keywords

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

Introduction

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

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

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

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

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

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

Material and Methods

Characterizing bread and sample types

Nine types of French baguettes were used in this study; all had the same composition (white flour, water, yeast, and salt). For these breads, pictures and texture measurements were preliminary performed at different times of purchase before the study to control the stability of production. One came from a supermarket (S), and five came from three local bakeries (b1, b2, and b3). These baguettes fell into two categories: (i) ordinary breads (O), produced using short fermentation times and possibly containing additives, and (ii) traditional breads (T), produced using long fermentation times and containing no additives.[19] Three other baguettes were produced in the laboratory run by Lesaffre International (B1, B2, and B3; Marcq-en-Baroeul, France), as described in previous studies[20,21]; they were par baked and frozen until usage. Two sample types were studied: crumb only (CO) and crumb with crust (CC). The supermarket/bakery baguettes were used within 4 hours of purchase (which occurred in the morning), and the laboratory-produced baguettes were used no more than 2 hours after the cooling phase had ended.

The nine baguette types were characterized based on their density, crumb water content, and crumb elasticity as performed in Jourdren *et al.* (2016).[20] Bread density was measured using the rapeseed displacement method. Crumb water content was determined by weighing samples before and after oven drying. Crumb elasticity (i.e., Young's modulus) was estimated using compression tests performed on crumb cylinders (h: 2.5 cm; d: 3.0 cm) with a TA.XT Plus Texture Analyzer (Stable Micro System, UK). All measurements were performed in triplicate (Table 1).

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

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

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

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

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

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

Helium was the carrier gas (constant flow rate of 1.2 mL/min). Oven temperature increased from 40°C to 200°C at a rate of 4°C/min and then remained at 200°C for 9 min.

In the **PTR-MS analyses**, 5 g of crumb (frozen at -20°C for 30 min) were stored at 20°C for one hour in 100-mL Schott flasks that were equipped with valved caps (GL 45, Duran Group, Wertheim, Germany). A highly sensitive PTR-MS apparatus (Ionicon Analytik, Innsbruck, Austria) was used in SCAN mode over a mass range of *m/z* 20 to 200; dwell time was 100 ms per peak. Five cycles (90 s) were dedicated to measuring the ambient air (i.e., the background signal), and 15 cycles (290 s) were dedicated to measuring the sample headspace. The gas above the samples was introduced into the system through a capillary line heated to 110°C at a flow rate of 100 mL/min. The PTR-MS settings were as follows: H_2O flow rate of 7.0 mL/min; drift tube pressure of 200 Pa; drift tube temperature of 60°C; and drift voltage of 600 V (E/N = 153 Td).

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

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

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

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

In each series, levels of *m/z* 21, 37, and 59 were quantified to verify measurement conditions. The *m/z* 21 ion, which corresponds to $H_3{}^{18}O^+$, is the isotopolog of the *m/z* 19 reagent ion. It is measured instead of *m/z* 19 to protect the PTR-MS apparatus from excessively high levels of the latter (natural isotopic ratio of $^{18}O/^{16}O \approx 0.2$).[7] Humidity levels were checked by monitoring the abundance of the *m/z* 37 ion, which must represent less than 2% of reagent ion signal intensity to limit the background signal. The relative amounts of the *m/z* 30 (NO^+) and 32 ($O_2{}^+$) ions were considered to be low enough that they did not contribute to ionization. The *m/z* 59 ion (resulting from propan-2-one protonation) was monitored to trace participant breath.[22]

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

were as follows: H_2O flow rate of 6.0 mL/min; drift tube pressure of 200 Pa; drift tube temperature of 60°C; drift voltage of 600 V (E/N = 152 Td).

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

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

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

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

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

Chewing behavior quantified by electromyography

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

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

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

Data analyses

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Results

Bread crumb properties

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

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

Relative VOC contributions to target ions

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

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

In vivo release

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

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

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

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

Chewing behavior dynamics

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

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

Perception dynamics for the three par-baked breads

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

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

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

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

Discussion

VOC contribution to ion fragments

To our knowledge, this study is the first to statistically relate ion fragment data obtained using PTR-MS to VOC data obtained using GC-MS with the purpose of quantitating the contributions of VOCs to ion fragments. An important caveat is that the relationships described here are only applicable to the study food: French baguette crumb. For *m/z* 71, 95, and 97, the linear regressions were not significant. There could be different explanations for this finding: (i) all the VOCs capable of generating these ions were not included in the statistical models; (ii) background noise (i.e., excessive variability) could be obscuring the relationship given that both the ions and the VOCs occurred at low levels; and/or (iii) some of the VOCs could display a lesser affinity for the purge-and-trap sorbent (i.e., Tenax®), skewing their abundance and ratios relative to their actual occurrence in bread.[36] However, for five of the target ions, the coefficients of determination were high enough that a percentage contribution could be calculated. The results are consistent with those found with data on baker’s yeast starters obtained via PTR-MS utilizing a time-of-flight mass analyzer[34]; however, the latter study focused exclusively on VOCs produced during fermentation. In this study, the VOCs produced during both fermentation and baking were examined. This statistical analyses have helped clarify the dynamics behind the ion fragments generated by complex food

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

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

As expected, the release of crumb markers (i.e., *m/z* 45, 47, 57, and 73) during crumb consumption was influenced by the crumb's initial VOC profile. In breads B1, B2, B3, and b1_T, both the initial content and intensity of crumb markers were high. Furthermore, the release of crumb markers was also impacted by crumb firmness. Indeed, breads with high Young's modulus values, such as b3_T or b1_O1, also displayed high intensity values for crumb markers, even though these markers were present at intermediate or low levels. The greater release of crumb markers in breads with firm crumb could be explained by the fact that firm breads induced more intense chewing activity. This result concurs with results found for model cheeses varying in hardness.[16] So, the high degree of muscular activity associated with firm crumbs[37] could lead to a more thorough breakdown of the food product and thus result in greater ion release. When crust was present, there was a large release of crust markers (*m/z* 73 and 87). Indeed, crust markers had a greater rate of release than did crumb markers. Structural differences between the crumb and the crust could be at the origin of this observation. The soft and elastic crumb is surrounded by the rigid and brittle crust.[38,39] During consumption, the crust is probably broken down more rapidly than the crumb due to its placement on the bread's surface and its brittle structure, thus leading to a faster release of crust markers.

To conclude, in addition to initial VOC profile, crumb firmness and, in all likelihood, crust brittleness are important factors that can impact the *in vivo* release of VOCs. They should therefore be accounted for when designing the aromatic properties of breads.

Impact of chewing behavior on *in vivo* aroma release

It is well known that individuals vary in their oral processing and thus release aromas differently.[17,40] Mastication, saliva volume, and saliva composition all have a major impact.[41] To delve further into aroma release dynamics, we studied the chewing behavior of three of the participants in the *in vivo* study. The results revealed that greater muscular activity appears to release greater amounts of VOCs. However, although a previous *in vitro* study[10] had found that the volume of saliva added to the bolus plays a key role in limiting aroma release, the links were not obvious here. In fact, contrary to expectations, participant #3, who had a more hydrated bolus than did participants #1 and #8, also showed more intense chewing activity and greater levels of ion release (bolus data come from a previous study involving the same three subjects[20]). Thus, under *in vivo* conditions, mastication appears to have a greater effect on aroma release than does salivation. However, this result must be confirmed with a greater number of subjects. In summary, participant physiology affects VOC release. More specifically, intense chewing activity releases greater amounts of VOCs in the oronasal cavity. However, the differences in VOC release were more dramatic among bread types than among participants. A study involving a larger number of subjects would be required to validate these results.

Impact of *in vivo* aroma release on perception dynamics

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

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

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

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

Abbreviations

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

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Figure captions:

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

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

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

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

Figure 5: Temporal patterns of sensation dominance for crumb-only (CO) samples (on the left) and crumb-with-crust (CC) samples (on the right) during consumption. Time was standardized (% of time available for perception). SL = significance line; CL = chance 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 *a*	51.5 ± 0.2 *a*	15.0 ± 4.1 *b*
B2	Laboratory	Par-baked	0.164 ± 0.001 *g*	49.3 ± 0.1 *b*	3.8 ± 0.9 *e*
B3	Laboratory	Par-baked	0.220 ± 0.003 *c*	50.6 ± 0.1 *a*	12.5 ± 0.6 *bc*
S_O	Supermarket	Ordinary	0.164 ± 0.001 *g*	45.4 ± 0.3 *e*	7.8 ± 0.9 *cde*
b1_O1	Local bakery 1	Ordinary	0.180 ± 0.002 *e*	46.6 ± 1.8 *d*	11.0 ± 4.2 *bcd*
b1_O2	Local bakery 1	Ordinary	0.171 ± 0.001 *f*	47.1 ± 0.3 *cd*	7.1 ± 4.6 *de*
b1_T	Local bakery 1	Traditional	0.216 ± 0.002 *c*	48.2 ± 0.5 *bc*	11.5 ± 3.5 *bcd*
b2_T	Local bakery 2	Traditional	0.192 ± 0.001 *d*	48.5 ± 0.3 *b*	5.7 ± 1.1 *e*
b3_T	Local bakery 3	Traditional	0.265 ± 0.006 *b*	46.6 ± 0.5 *d*	25.3 ± 2.1 *a*

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

m/z ion	Chemical formula	Dwell time (ms)	Series 1	Series 2
21	H_2O, H^+	50	x	x
37	$(H_2O)_2$, H^+	50	x	x
45	-	100	x	
47	C_2H_5OH, H^+	100	x	
57	-	100	x	
59	C_3H_6O, H^+	50	x	x
71	-	100	x	
73	-	100		x
87	-	100		x
95	-	100		x
97	$C_5H_4O_2$, 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 R^2 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 R^2.

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 [9]			<1
	2-heptanol	116	2.34E-05	*100*			<1
47	ethanol	46	5.67E-06	70 [9]	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 [25]			10 ± 13
	nonanal	142	4.93E-04	9 [9]			6 ± 19
	1-butanol	74	9.99E-06	90 [9]			<5
	1-pentanol	88	1.33E-05	89 [9]			<5
	1-hexanol	102	1.76E-05	16 [9]			<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 [9]			<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 [9]			
	2-pentanol	88	1.33E-05	39 [25]			
73	2-butanone	72	6.58E-05	100 [9]	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 [9]			9 ± 11
	2-pentanone	86	8.73E-05	85 [9]			<5
	2,3-butanedione	86	1.97E-07	89 [9]			<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 *b*	41 ± 2 *c*	25 ± 3 *e*	61 ± 7 *a*	61 ± 6 *a*	34 ± 4 *d*
Chewing frequency	s^{-1}	1.46 ± 0.06 *ab*	1.34 ± 0.08 *c*	1.40 ± 0.07 *b*	1.47 ± 0.04 *a*	1.41 ± 0.09 *ab*	1.46 ± 0.07 *a*
Mean burst duration	ms	284 ± 16 *d*	339 ± 19 *b*	381 ± 31 *a*	274 ± 17 *d*	310 ± 9 *c*	326 ± 15 *b*
Mean EMG activity	µV.s	26 ± 7 *c*	29 ± 6 *c*	17 ± 2 *d*	44 ± 4 *b*	59 ± 7 *a*	39 ± 1 *b*
Total EMG activity	mV.s	1.30 ± 0.41 *c*	1.22 ± 0.26 *c*	0.44 ± 0.09 *d*	2.66 ± 0.38 *b*	3.60 ± 0.70 *a*	1.33 ± 0.41 *c*

Figure 1.a

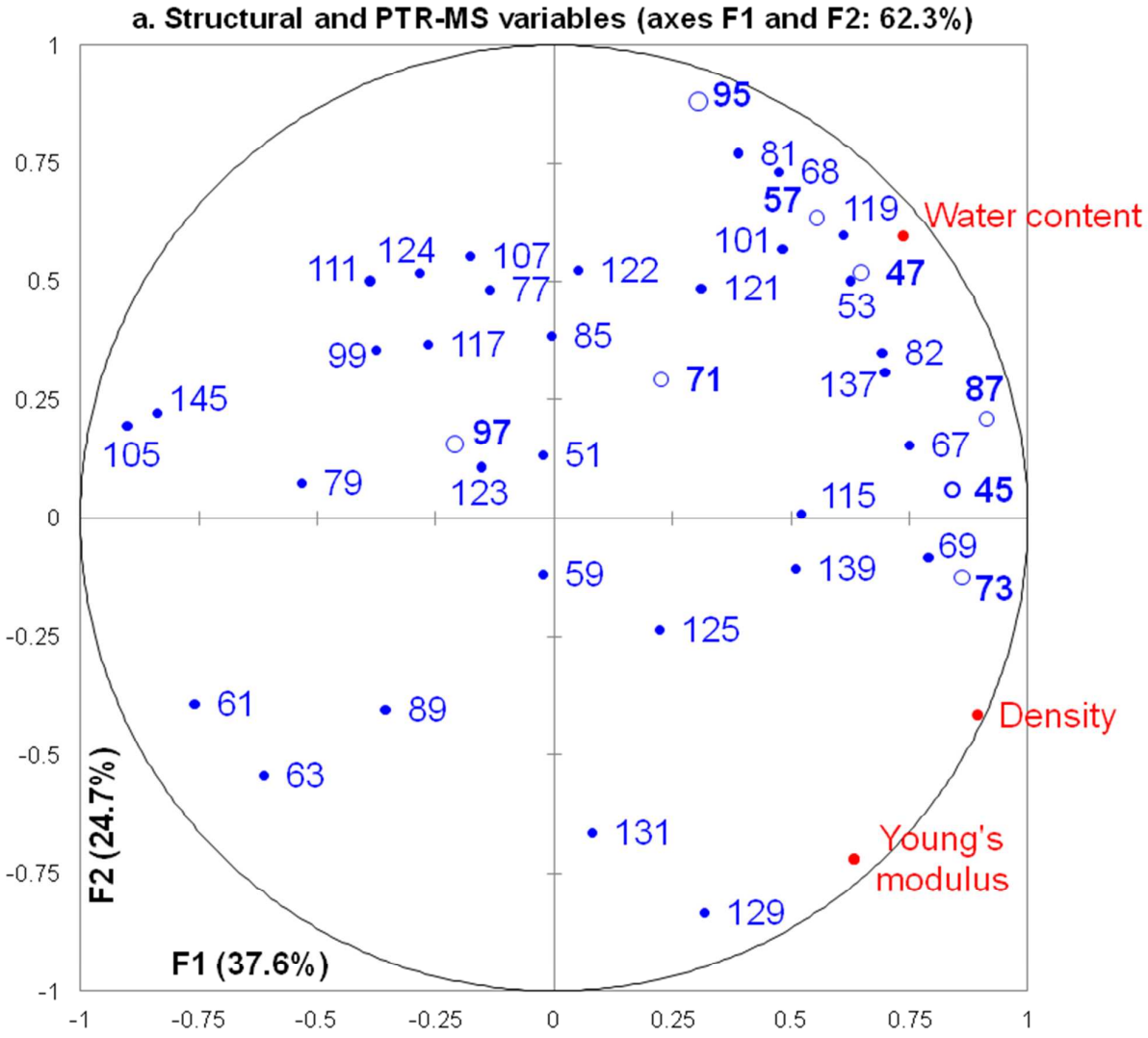

a. Structural and PTR-MS variables (axes F1 and F2: 62.3%)
F1 (37.6%)
F2 (24.7%)
95
81
68
57
119
Water content
101
124
111
107
77
122
121
47
53
99
117
85
71
82
137
87
145
105
97
51
67
79
123
115
45
69
59
139
73
125
61
89
Density
63
131
Young's modulus
129

Figure 1.b

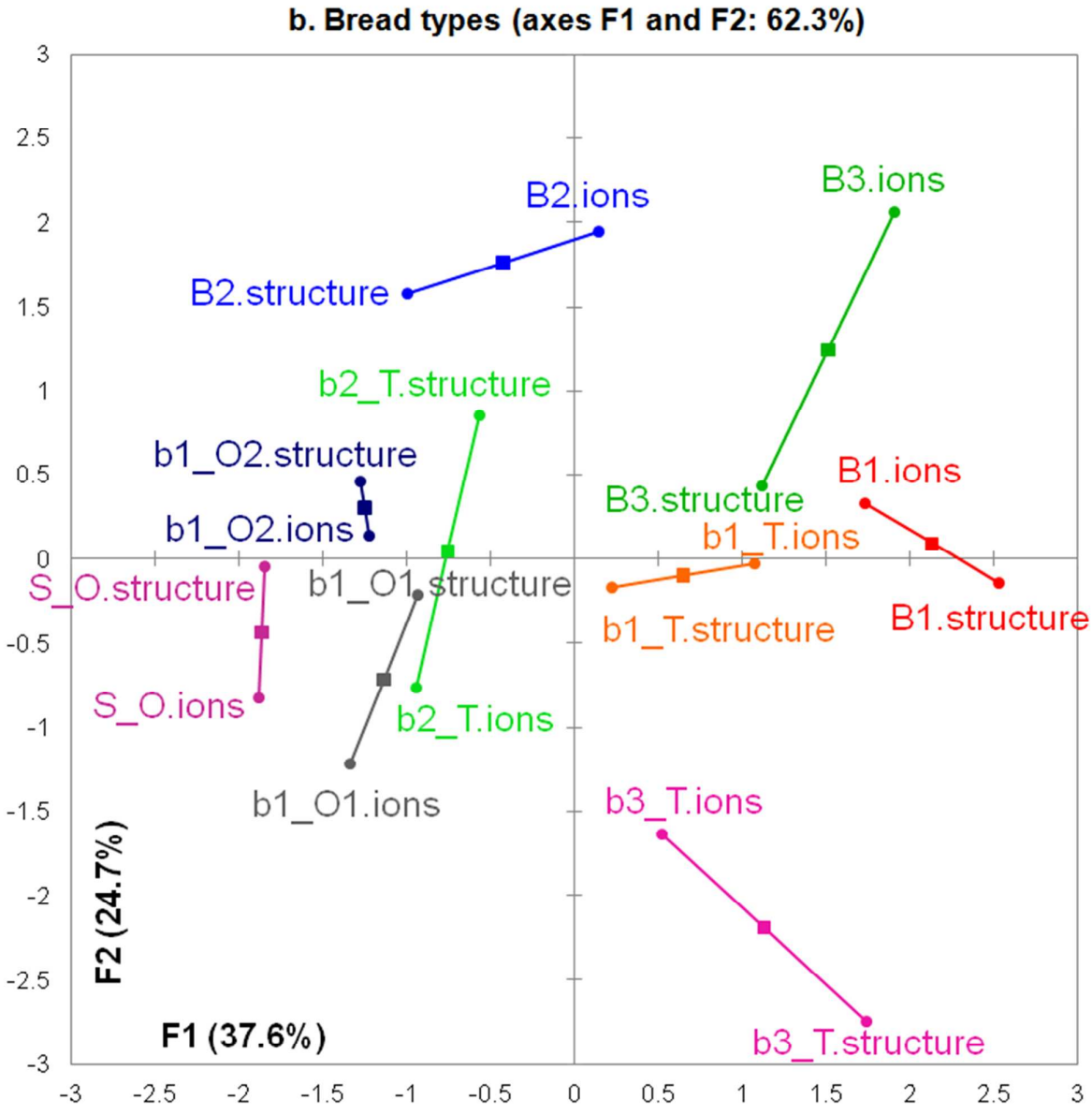

b. Bread types (axes F1 and F2: 62.3%)
B2.ions
B3.ions
B2.structure
b2_T.structure
b1_O2.structure
B1.ions
B3.structure
b1_T.ions
b1_O2.ions
S_O.structure
b1_O1.structure
b1_T.structure
B1.structure
S_O.ions
b2_T.ions
b1_O1.ions
b3_T.ions
b3_T.structure
F2 (24.7%)
F1 (37.6%)
3
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-2.5
-3
-3
-2.5
-2
-1.5
-1
-0.5
0
0.5
1
1.5
2
2.5
3

Figure 2

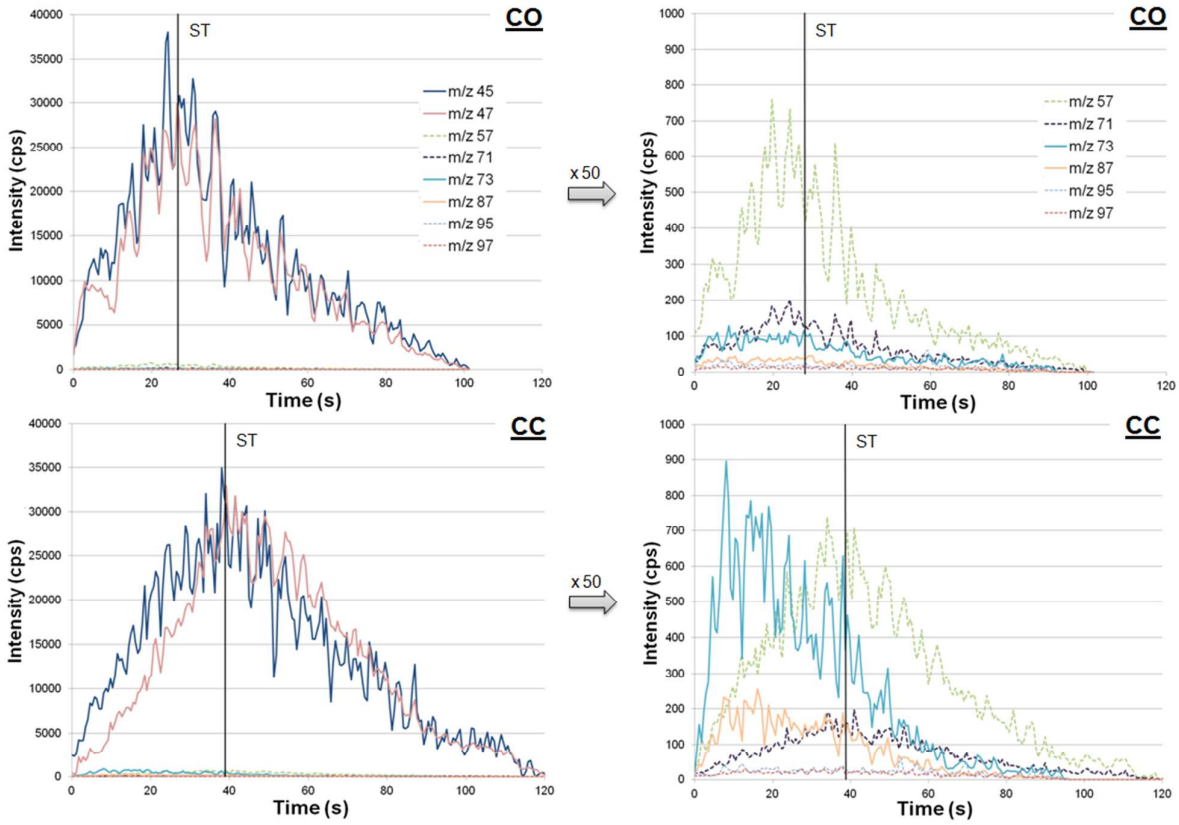

CO
ST
m/z 45
m/z 47
m/z 57
m/z 71
m/z 73
m/z 87
m/z 95
m/z 97
Intensity (cps)
Time (s)
x 50
CC

Figure 3

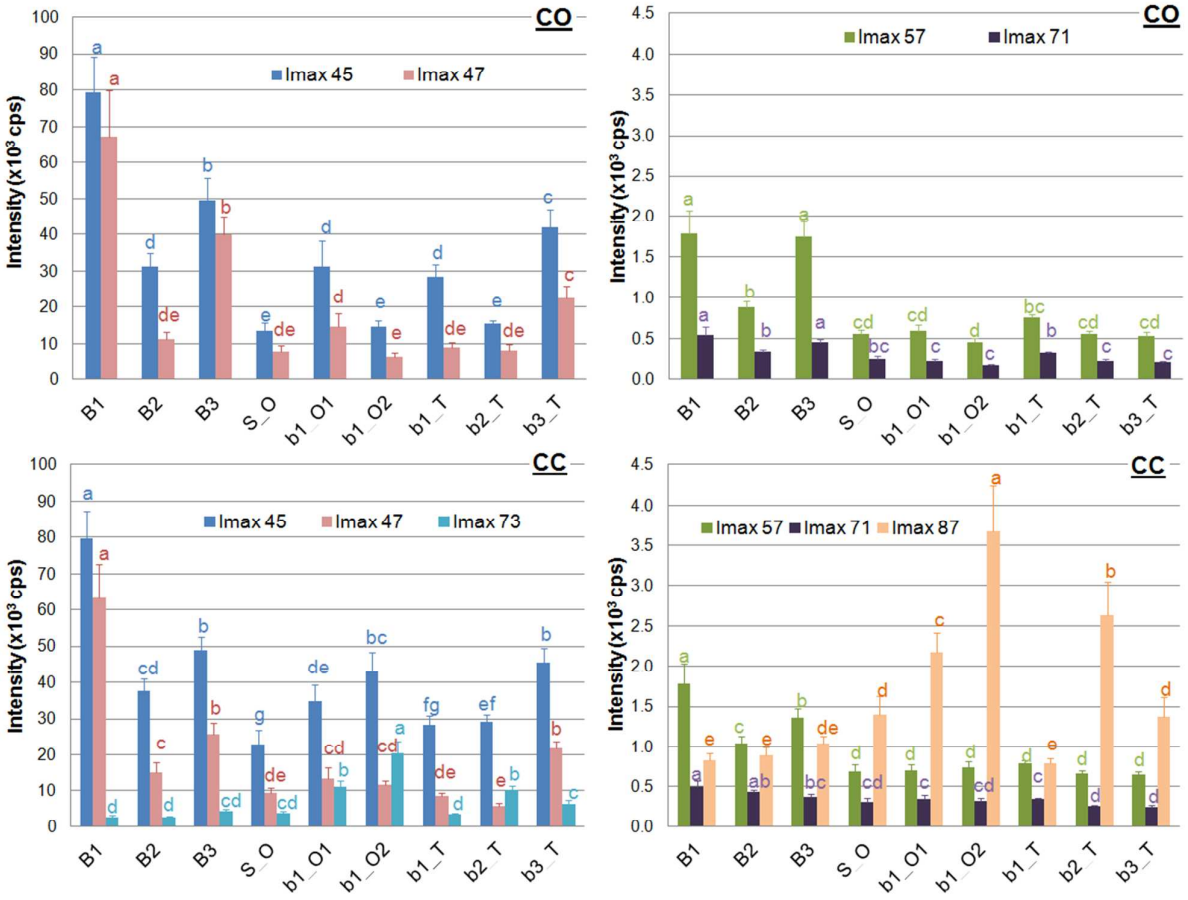

CO
Imax 45
Imax 47
Intensity (x10³ cps)
B1
B2
B3
S_O
b1_O1
b1_O2
b1_T
b2_T
b3_T
CO
Imax 57
Imax 71
Intensity (x10³ cps)
CC
Imax 45
Imax 47
Imax 73
Intensity (x10³ cps)
CC
Imax 57
Imax 71
Imax 87
Intensity (x10³ cps)

Figure 4

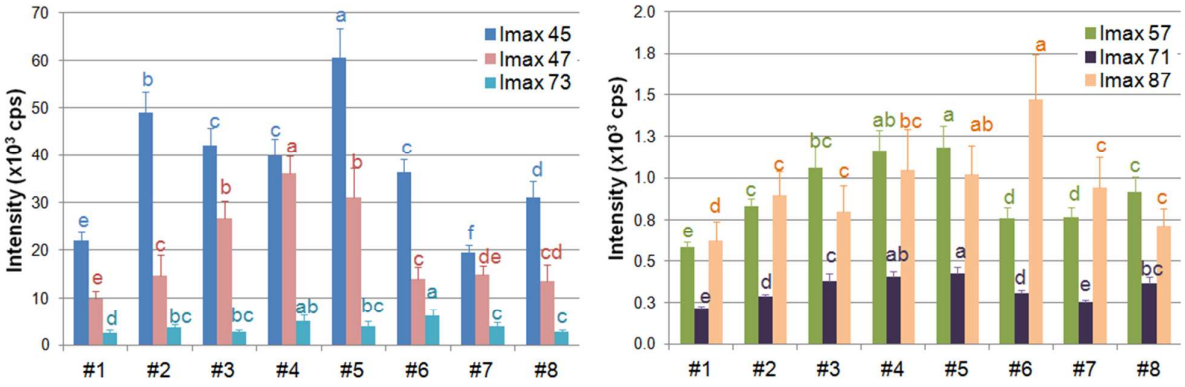
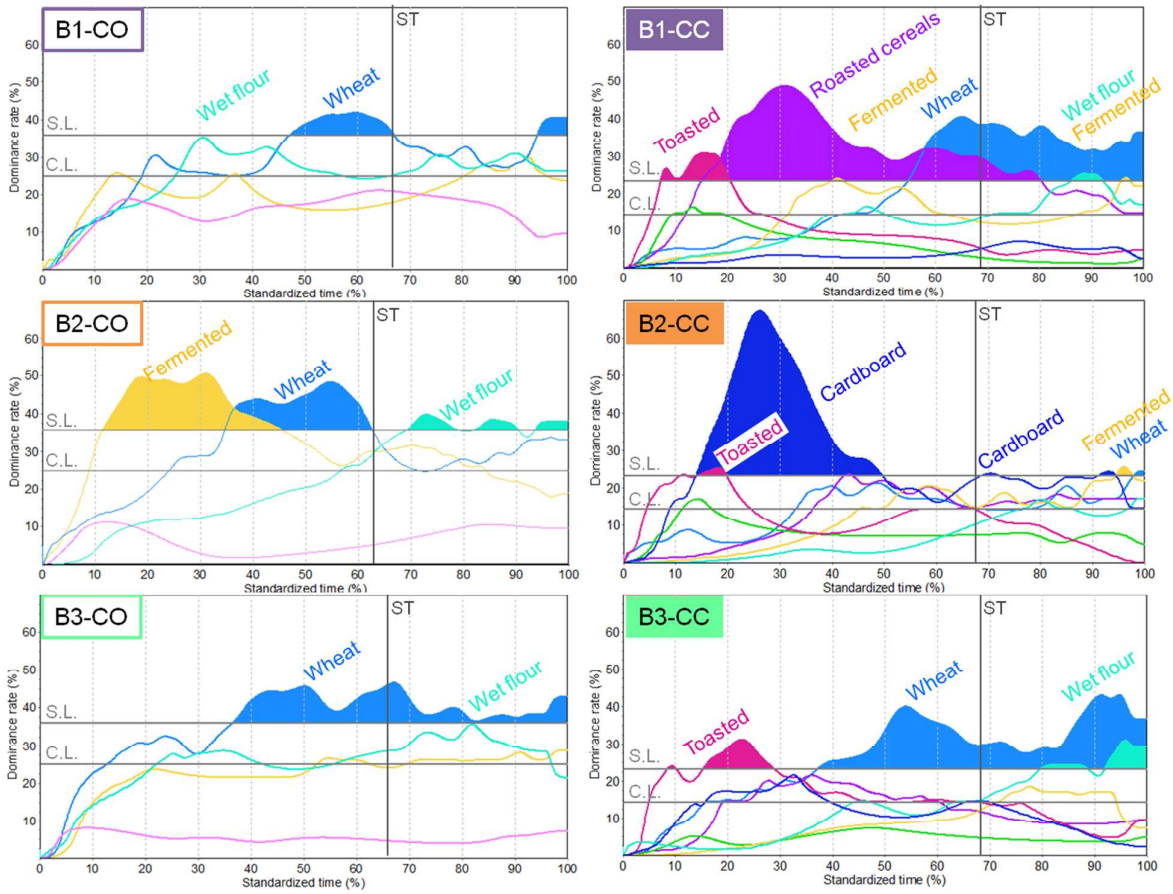
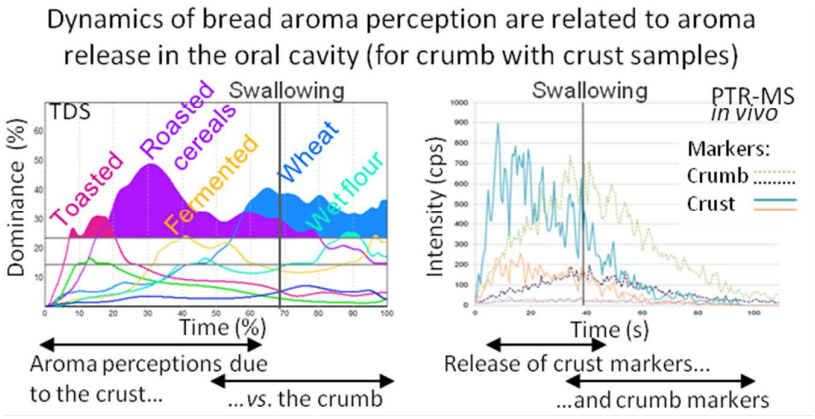

Intensity (x10³ cps)
lmax 45
lmax 47
lmax 73
#1
#2
#3
#4
#5
#6
#7
#8
Intensity (x10³ cps)
lmax 57
lmax 71
lmax 87
#1
#2
#3
#4
#5
#6
#7
#8

Figure 5


B1-CO
Wet flour
Wheat
S.L.
C.L.
ST
B1-CC
Toasted
Roasted cereals
Fermented
Wheat
Wet flour
Fermented
B2-CO
Fermented
Wheat
Wet flour
B2-CC
Cardboard
Toasted
Cardboard
Fermented
Wheat
B3-CO
Wheat
Wet flour
B3-CC
Toasted
Wheat
Wet flour
Dominance rate (%)
Standardized time (%)

TOC graphic


Dynamics of bread aroma perception are related to aroma release in the oral cavity (for crumb with crust samples)
TDS
Swallowing
Toasted
Roasted cereals
Fermented
Wheat
Wet flour
Dominance (%)
Time (%)
Aroma perceptions due to the crust...
...vs. the crumb
Swallowing
PTR-MS in vivo
Markers:
Crumb
Crust
Intensity (cps)
Time (s)
Release of crust markers...
...and crumb markers