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Influence of Texture on the Perception of Saltiness in Wheat Bread

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Supporting Information

ABSTRACT: As a basis for sodium reduction in bread, the influence of crumb texture on the intensity of saltiness and the release of sodium ions during chewing was investigated. A coarse-pored bread crumb was created by extending the proofing time (90/120 min vs 20/40 min as control), whereas the omission of proofing resulted in a fine-pored crumb (0/0 min). A significantly faster sodium release from the coarse-pored bread compared to the fine-pored bread (constant sample weight) was measured in-mouth and in a mastication simulator. This explained the significantly enhanced salty taste of the 90/120 min bread. Corresponding experiments with constant sample volumes revealed a significantly enhanced saltiness despite similar amounts of extracted sodium during the first seconds of chewing. Therefore, saltiness was influenced both by the velocity of sodium release and by crumb texture. Appropriate modification of crumb texture thus leads to enhanced saltiness, suggesting a new strategy for salt reduction in bread.

KEYWORDS: *salt reduction, wheat bread, texture, sodium release, sensory analysis*

INTRODUCTION

Sodium reduction in food has become an important aim due to health reasons. The average daily salt intake in industrialized countries ranges from 8 to 11 g of sodium chloride (salt) per day[1] and is well in excess of the intake recommended by the World Health Organization of only 5 g of salt per day. It is estimated that 30−50% of the population suffers from salt sensitivity.[2] In these persons excess sodium intake leads to hypertension, which is considered to be a main cause of cardiovascular diseases. Bread and cereal products are major sources of sodium in the nutrition of industrialized countries. According to the German National Nutrition Survey II, bread ranks first in the list of sodium sources in Germany and provides about a fourth of the daily sodium intake.[3] In the United Kingdom, bread contributes 35% of the sodium intake.[1] Therefore, a reduction of the salt content in bread could result in a reduced sodium intake of the population from bread consumption. However, reducing salt in bread is not an easy task due to its important technological and sensory properties. Apart from its strengthening effect on gluten, the control of baker's yeast activity and the prolongation of shelf life are important technological functions of sodium chloride in bread.[4] However, preservation of the sensory quality (salty taste, improvement of the entire flavor[5]) is the more serious issue concerning salt reduction in bread.

Several strategies have been proposed for sodium reduction in bread. Apart from the partial replacement of sodium chloride by salt substitutes such as potassium or magnesium salts, the salty taste of sodium can be enhanced by amino acids such as L-lysine or L-arginine.[4,6−8] A partial salt reduction can also be achieved by a stepwise reduction over a longer period of time[9] or by an inhomogeneous salt distribution.[10,11] Nevertheless, all of these methods allow only a partial sodium reduction, and more research into new strategies for salt reduction in bread is required.

Saltiness is mainly evoked by sodium ions, which have to be dissolved in saliva to be detectable via ion channels. Previous studies revealed that a certain chewing time is necessary for a complete sodium extraction from bread crumb during chewing.[12] This retarded sodium release explains why the salt intensity of bread crumb is considerably lower than the salt intensity of an aqueous solution containing the same amount of NaCl.[12] As a consequence, the saltiness of wheat bread could be enhanced by accelerating sodium release during chewing.[12]

It has been reported that salt release from crisps and cheese during mastication was influenced by texture.[13,14] Furthermore, studies have shown that different cheese textures with different ratios of fat/water, fat/protein, or water/protein influenced not only sodium release during cheese consumption but also the perceived salt intensity.[15,16] The influence of texture on salt perception was also shown for xanthan/agar gels with different contents of air bubbles.[17] Studies on viscosity-induced taste suppression revealed that the impaired taste intensity in viscous solutions seems to be caused not only by changes in tastant kinetics but also by perceptual texture−taste interactions.[18,19]

In contrast to taste, there is no single and specific receptor for the perception of texture due to its multiparameter nature. Some textural parameters are already perceived when the food is placed in the mouth, whereas other parameters are detected during the deformation and movement of the food in the oral cavity by chewing and mixing with saliva. Texture-related

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sensations such as touch/pressure, pain, and joint position are perceived by several receptors (somesthetic and kinesthetic) and tissues (e.g., periodontal, mouth mucosa, in the temperomandibular joint).[20]

The aim of this study was to investigate the influence of crumb texture on the velocity of sodium release and on salt intensity. The velocities of sodium release from a very coarse-pored and a very fine-pored bread crumb obtained by altering the proofing times were investigated by two discontinuous sampling techniques with the aid of a mastication simulator and in-mouth as well as by a continuous sampling technique in-mouth. In parallel to these studies, the perceived salt intensities were evaluated by two-alternative forced choice (2-AFC) tests and by compiling time−intensity curves. Because a significantly faster sodium release together with a significantly enhanced salt intensity was found for the softer and more coarse-pored crumb, the same experiments were performed for other bread crumbs with larger pores and a lower firmness obtained by the addition of α-amylase and the surfactant diacetyl tartaric acid esters of mono- and diacylglycerides (DATEM) to determine if the softest bread crumb also showed the fastest sodium release and the highest salt intensity or if there was an optimal crumb firmness concerning sodium release and salt intensity. Furthermore, the salt intensities and the sodium release from bread crumb and dough were determined to have a comparison between two matrices containing the same ingredients, but showing completely different textures. In this way, it was possible to elucidate the influence of specific textural parameters such as the viscoelastic behavior and the free aqueous phase existing in dough, but not in bread crumb.

MATERIALS AND METHODS

Wheat Flour. Commercial wheat flour (Rosenmehl type 550, Rosenmühle, Ergolding, Germany) was obtained and characterized as described previously.[21] Analytical characteristics of the flour were 9.7% moisture, 0.61% ash (dry mass), and 11.8% protein (dry mass).

Chemicals. The quality of all reagents was pro analysi (p.a.) or stated otherwise. α-Amylase from *Aspergillus oryzae* (EC 232-588-1, 35.7 U/mg) was obtained from Sigma-Aldrich (Steinheim, Germany). Sodium chloride, sucrose, and tris(hydroxymethyl)aminomethane (Tris) were obtained from Merck (Darmstadt, Germany). Lametop 300 (80% DATEM and 20% calcium carbonate as anticaking agent) was obtained from BASF Personal Care and Nutrition GmbH (Illertissen, Germany).

Breadmaking. Breads were baked on a scale of 300 g of flour as described by Pflaum et al.[5] Different crumb textures were obtained by using a lower amount of fresh yeast (1.75% instead of 7%) and different proofing times. For the standard bread, dough was fermented for 20 and 40 min. A notably firmer and more fine-pored crumb was obtained by omitting both proofings (0/0 min bread), whereas prolonged proofing times of 90 and 120 min resulted in a significantly more coarse-pored crumb (90/120 min bread). Furthermore, crumb texture was modified by adding α-amylase (5 mg/100 g flour as an aqueous solution) and Lametop 300 (80% DATEM on 20% calcium carbonate as anticaking agent; addition of 0.6% based on flour, added as solid material). In the following, all percentages of NaCl are given on the basis of flour weight, that is, [%] is equivalent to [g NaCl/100 g wheat flour].

Volume Measurement. After the breads had cooled for 2 h, the volume of the breads was measured in a VolScan Profiler (Stable Micro Systems Ltd., Godalming, UK). All measurements were done in triplicate on three breads each.

Texture Measurement. Immediately after the volume measurement, the breads were cut into slices of 1.5 cm thickness, and a cylinder with a diameter of 20 mm was cut out with a cork borer. The texture was measured in triplicate from three different slices from three breads using a TA.XT plus Texture Analyzer (Stable Micro Systems Ltd.) as described by Konitzer et al.[12] Two consecutive measurements were done per sample, and data analysis was performed with the software Texture Exponent (Stable Microsystems Ltd.).

Determination of the Water Content. The determination of the water content of wheat flour and bread was carried out according to ICC Standard 110.

Measurement of the Pore Size. Breads were baked in triplicate and cut into slices of 1.5 cm thickness after cooling for 2 h. Seven images per bread were taken in a specially designed dark chamber under defined, reproducible conditions of illumination, so that in total 21 pictures were available per type of bread. Image analysis was carried out according to the procedure described previously.[22] After the images had been changed to grayscale and a thresholding algorithm applied,[23] the following parameters were extracted from the raw data: total count and total area of pores, average size, area fraction, perimeter, Feret's diameter, circularity, and solidity. After statistical analyses of all parameters by two-way ANOVA (SigmaStat v3.5, Systat, San Jose, CA, USA) and correlation to measurements of bread volume, texture, and amount of extracted sodium after mastication in the mouth for 5 s, the average pore size was selected as the most significant criterion.

Sensory Analyses. *Training of the Sensory Panel.* The panel for all sensory analyses consisted of 15 trained (ISO 8586)[24] subjects (10 women and 5 men, aged 25−31 years) with no history of known taste or smell disorders who had given informed consent to participate in the sensory tests. All sensory analyses were performed in three different sessions in a sensory panel room at 20−22 °C, and panel training was done according to Pflaum et al.[5]

Procedure of the 2-AFC Tests. The saltiness of bread crumb was evaluated by 2-AFC tests as described earlier.[5] Briefly, the panelists were asked to indicate which of two encrypted bread samples tasted saltier. Depending on the number of correct answers, the level of significance α was determined in compliance with ISO 5495[25] according to a table of significance for two-sided paired comparison tests, and α-levels $\leq$0.05 were judged as significant. All 2-AFC tests were done in triplicate.

Time−Intensity (TI). TI measurements were performed using FIZZ Acquisition software v2.46A (Biosystèmes, Couternon, France) for data collection according to the method described by Konitzer et al.[12] In brief, the panelists evaluated the salt intensity over time in three separate sessions on consecutive days by moving their finger along a 14 cm unstructured scale, where the left end represented a salt intensity of 0 and the right end a salt intensity of 10. Analogously to the continuous sampling technique (see below), the total duration of the TI measurements was 60 s, after which data recording was stopped by swallowing the sample and moving the cursor back to the far left end of the unstructured scale, which represented a perceived salt intensity of 0. There was a waiting period of 60 s between two samples, during which the panelists rinsed their mouths with water. A Latin square design was used to rule out effects of sample presentation. Three grams of freshly prepared bread dough with 1.5% NaCl was compared to 3 g of the 1.5% NaCl reference bread. Furthermore, the breads without proofing (0/0 min) and with a very long proofing time (90/120 min) were compared against each other at the same NaCl level of 1.5% at a constant sample weight of 3 g and, in a separate session, at a constant sample volume of 3.375 cm^3, resulting in 3.00 g for the 0/0 min bread and 2.04 g for the 90/120 min bread.

FIZZ Calculations v2.46A (Biosystèmes, Couternon, France) was used for the analysis of the recorded TI curves, and average curves were modeled according to the trapezoid procedure described by Lallemand et al.[26] The curves could be generally divided into three parts: an increasing phase until the maximum intensity (I_{max}) is reached, a plateau, and a decreasing phase with differences in duration, rate, and shape. After extraction of all relevant parameters,[12] the duration D_i, rate R_i, and area A_i of the increasing part of the curve, the duration D_m and area A_m of the 90% I_{max} plateau, and the total area of the trapezoid A were calculated.

Sodium Release during Chewing. The time-resolved release of sodium ions from bread crumb or dough during chewing was investigated by three different methods.[12]

Discontinuous Sampling Techniques. Two different discontinuous sampling techniques were applied. Three grams of bread crumb or dough and 3.375 cm^3 of bread crumb, respectively, were chewed in-mouth and in a mastication simulator (a modified Potter S Homogenizer, Braun, Melsungen AG, Germany), respectively, for 5, 10, 15, 30, and 60 s. For the mastication simulator, human saliva of the same panelists was used as extracting agent, and the added amount of saliva was adapted for each chewing time to the salivary secretion in-mouth. After centrifugation of the resulting chewing pulps (3750*g*, 10 min, 20 °C), the sodium concentrations in the supernatants were quantitated by a sodium-selective electrode. Triplicate determinations were carried out by two panelists (A and B), respectively.

Continuous Sampling Technique. Three grams of bread crumb or dough and 3.375 cm^3 of bread crumb, respectively, was chewed in the mouth for 60 s. The chewing frequency was standardized to 72 chews/min with the aid of a metronome. During mastication, a satin ribbon (width = 3 mm) was pulled through the mouth by a motor (1.1 cm/s). Afterward, the satin ribbon was cut into pieces of 5.5 cm (each corresponding to a chewing period of 5 s), which were extracted with 5 mL of buffer (0.5 mol/L Tris, pH 7.0, with HCl) for 5 min using a vortex mixer. The sodium concentrations in the resulting extracts were measured by a sodium-selective electrode. Triplicate determinations were carried out by two panelists (A and B), respectively.

Ion-Selective Electrode (ISE). Sodium was quantitated by a Metrohm 781 pH-/ion meter (Metrohm, Filderstadt, Germany) and a sodium-selective polymer membrane-based electrode.[5]

Statistical Analysis. Statistical evaluation of all quantitative data was carried out by one-way and two-way analysis of variance (ANOVA) using the software SigmaStat (v3.5, Systat Software Inc.).

RESULTS AND DISCUSSION

In this study, the influence of texture on salt intensity as well as on the velocity of sodium release was investigated. On the one hand, salt intensity and sodium release from bread crumb and dough were compared with each other. On the other hand, the crumb texture was modified and the influence of crumb texture on salt intensity and sodium release was determined. The release of sodium during chewing was determined by using three different methods. Two discontinuous methods were applied: bread crumb and dough, respectively, were chewed in the mouth as well as in a mastication simulator. Although the principles of these two methods were quite similar, both methods were used to cross-check each other, because each method had specific advantages: measuring sodium extraction in the mouth was more realistic, but the mastication simulator allowed the determination of sodium release in a more reproducible manner without any influences of individual mouth or tongue movements of the panelists. In fact, the results obtained with these two methods were quite similar with a marginally faster sodium release in the mastication simulator due to a faster homogenization as described by Konitzer et al.[12] The high degree of consistency between the results obtained by both panelists indicates a high level of comparability to the general population.

Additionally to the discontinuous methods, a continuous method was applied. In contrast to the discontinuous methods, larger differences were observed between the panelists, when this method was used. Besides the influences of individual chewing behavior and individual forms of the oral cavity, these differences were primarily caused by individual rates of saliva secretion leading to different dilutions of the extracted sodium ions.[12] Nevertheless, this method provided consistent results when the extracted amounts of sodium were individually compared for each panelist.[12]

The two discontinuous methods allowed the determination of the extent of extracted sodium (given as absolute amounts or percentages), whereas the continuous method provided the actual sodium concentration in the mouth, which is crucial for the perceived salt intensity. The results of the discontinuous methods tended to be higher than the actual values due to a putative additional extraction during centrifugation, whereas the continuous sampling technique provided lower values and a retarded sodium release, as was the case in reality due to the fact that the chewing point (chewing pulp, in the rear part of the oral cavity) differed from the sampling point (satin ribbon, in the front part of the oral cavity). Therefore, the actual sodium release lay between the results of both the discontinuous and the continuous sampling techniques.[12]

Comparison of Wheat Bread Crumb and Dough. To have a comparison between two completely different textures containing the same ingredients, salt intensity and sodium release from bread crumb and dough were compared with each other. Both bread crumb and dough used for the tests contained 1.5% NaCl. Due to the very good conformity of the measured values of both panelists within each discontinuous sampling method, the results of both panelists were averaged in each case. Significantly lower amounts of extracted sodium were observed for bread crumb compared to dough up to chewing times of 10 s. As can be seen in Figure 1, only 57 ± 6% (in-mouth) and 67 ± 6% (mastication simulator) of the total amount of sodium was extracted from bread crumb after the first 5 s, whereas 72 ± 5% (in-mouth) and 84 ± 5% (mastication simulator) of sodium were extracted from dough. After 10 s, even greater differences were observed between the amounts of extracted sodium from bread crumb (57 ± 7% in-mouth and 67 ± 6% in the mastication simulator) and dough (83 ± 4% in-mouth and 88 ± 5% in the mastication simulator). At longer chewing times of 15 s and above, the extracted

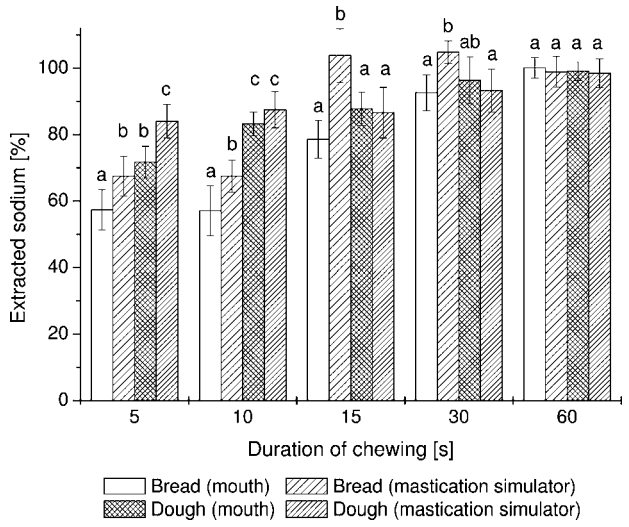

Figure 1. Comparison of the time-resolved sodium release from bread crumb and dough with 1.5% NaCl. Discontinuous sampling techniques included sodium release in-mouth and in the mastication simulator. Error bars represent standard deviations of triplicate determinations by two panelists (A and B). Mean values associated with different letters are significantly different within each chewing time (one-way ANOVA, Tukey test, $p \leq 0.05$).

amounts of sodium in the mouth were comparable in bread crumb and dough. Contrary to the shorter mastication times of 5 and 10 s, sodium was released even more slowly from dough than from bread crumb in the mastication simulator. After 60 s, the entire amount of sodium was extractable from both bread crumb and dough. In total, a slower sodium release was obtained in-mouth than in the mastication simulator, because of the apparently less efficient homogenization in the mouth.

The continuous sampling technique confirmed the faster sodium release from dough compared to bread crumb (Figure 2). However, significant differences ($p \leq 0.05$) between bread crumb and dough occurred only after 20 s (panelist A) and 25 s (panelist B), respectively. This can be explained by the fact that this method provided a retarded sodium release as the sampling point differed from the chewing point. A certain time was necessary for a sufficient distribution of the sodium ions in the mouth. In addition, it should be noted that the results obtained for crumb and dough had to be compared separately for each panelist as the sodium concentrations measured in saliva depended on the saliva secretion of each panelist.[12]

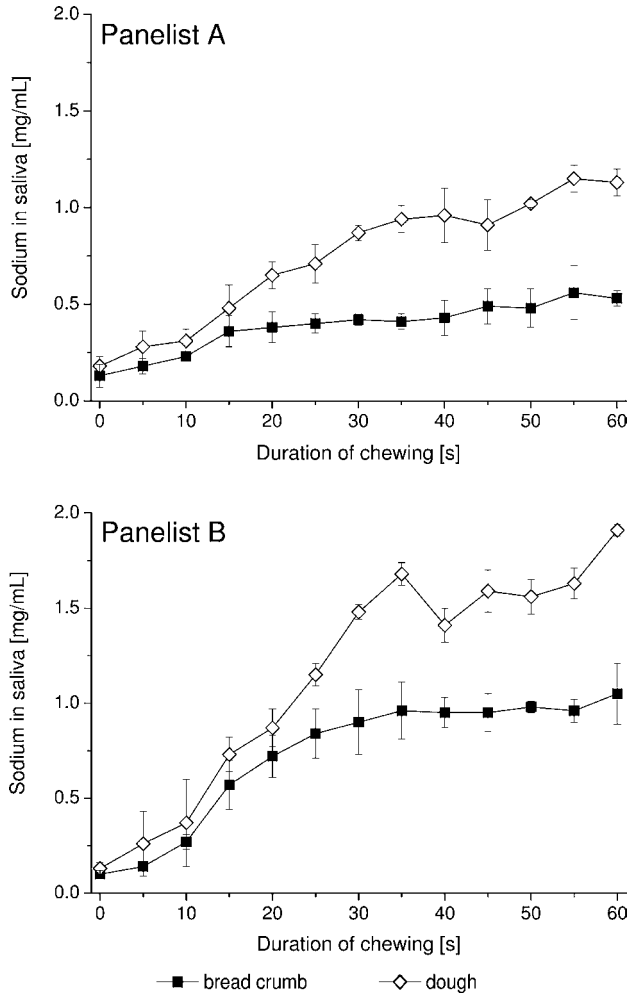

Figure 2. Comparison of the time-resolved sodium release from bread crumb and dough with 1.5% NaCl. A continuous sampling technique was used. Error bars represent standard deviations of triplicate determinations by two panelists (A and B).

The observed differences between bread dough and crumb might be caused by the so-called "dough liquor", the water-soluble phase of bread dough, which contains unbound, free water molecules and can be obtained by ultracentrifugation.[27] Therefore, sodium ions that were already dissolved in this free water phase were initially transferred to saliva more rapidly as opposed to bread, where there is no such free water phase. Later, the release of sodium from dough was slower than from bread crumb, which was due to the glutinous, viscoelastic texture of dough, which made an efficient homogenization more difficult than in bread crumb.

In 2-AFC tests, the sensory panel rated the dough with 1.5% NaCl as significantly ($\alpha = 0.05$) saltier than the same weight (3 g) of bread crumb prepared from the same dough. The water content of bread crumb was 44.9 ± 0.5% and thus a bit lower than in dough (46.3 ± 0.5% water), which means that the sodium concentration based on the water content was slightly higher in bread crumb than in dough. However, the perception of saltiness was still more intense in dough, which can be explained with the faster release of sodium during the first 10 s of mastication.

2-AFC tests were established as the method of choice to compare the intensity of saltiness in bread crumb because of their minimization of carry-over effects, reliability, and good reproducibility in this rather complex food matrix.[5,12] However, they provide information only on the overall perceived salt intensity and not on any changes of taste perception over time. Because time-resolved measurements showed significant differences in sodium release between bread dough and crumb, especially during the first 5−10 s (discontinuous sampling techniques) or after 20−25 s (continuous sampling technique with retarded release) of mastication, the time dependency of salt perception was investigated in more detail by means of TI measurements. On the basis of the results of the instrumental sampling techniques, the increasing phase of the TI curves was deemed to be of particular interest.

The perceived saltiness of freshly prepared dough was compared to the reference bread at the same salt level of 1.5% by the same two panelists (A and B). The overall I_{max} revealed no significant difference between dough (5.5 ± 0.5) and bread crumb (5.1 ± 0.4) for panelist A. In contrast to that, panelist B perceived the dough (I_{max} = 5.6 ± 0.5) as being significantly saltier altogether when compared to bread crumb (I_{max} = 4.4 ±

Table 1. Secondary Parameters[a] Extracted from Time−Intensity Curves of Bread Crumb Compared to Dough at the Same NaCl Level of 1.5%[b]

sample	D_i	R_i	A_i	D_m	A_m	A
bread crumb	32.0 ± 7.2	0.13 ± 0.03	62.5 ± 15.6	18.9 ± 7.8	75.7 ± 31.0	144.8 ± 27.1
dough	18.6 ± 4.0*	0.26 ± 0.06*	38.4 ± 16.9*	23.4 ± 11.9	110.2 ± 57.9	190.8 ± 25.8*

[a] D_i, duration of the increasing phase; R_i, rate of increase; A_i, area under the curve in the increasing phase; D_m, duration of the plateau; A_m, area under the plateau; A, area under the total curve. [b] Mean values ± standard deviation (n = 12). An asterisk (*) indicates statistically significant differences between bread crumb and dough (two-way ANOVA, $p \leq 0.05$).

0.2). The average values of both panelists showed that the rate of increase (R_i) was significantly higher for dough, which was accompanied by a significantly shorter time (D_i) until I_{max} was reached (Table 1). The area under the curve in the increasing phase (A_i) was also significantly smaller for dough, which is mainly attributable to its shorter duration. These significant differences in perceived salt intensity are in accordance with the instrumental measurements of sodium release and most certainly due to the free water phase in dough, leading to a faster transfer of sodium ions into saliva and thus to the ion channels on the tongue. No significant differences were found between dough and bread crumb for the average duration (D_m) and area (A_m) of the 90% I_{max} plateau, because the perceived salt intensity decreased again after 41 s for panelist A. Panelist B did not experience this decrease in perceived salt intensity, which is supposedly due to the lower rate of salivary secretion.[12] Therefore, the sodium concentration in the saliva of panelist B was not diluted as quickly as for panelist A and remained constant until the end of the measurement. The total area A under the curve was significantly larger for dough compared to bread crumb despite the aforementioned differences between the panelists.

In summary, the TI measurements confirmed the results of the 2-AFC tests in which dough was rated as significantly saltier. Significant perceptual differences were found in the increasing phase of the curves at the beginning of mastication. This is in accordance with the instrumental analyses that revealed a faster velocity of sodium release from dough in comparison to bread crumb.

Comparison of Bread Crumbs Baked without Proofing and with Extended Proofing Times. Different crumb textures were obtained by reducing the amount of fresh yeast to one-fourth and by varying proofing times. As different proofing times led to only marginal differences between the crumb textures when 7% fresh yeast was added, the amount of fresh yeast was reduced to 1.75% (based on flour). The omission of both standard proofing times (20 and 40 min) resulted in a notably firmer and fine-pored crumb (0/0 min), whereas a significantly more coarse-pored crumb and a larger bread volume were obtained by prolonged proofing times of 90 and 120 min (Figure 3). Compared to the 0/0 min bread, the volume of the 90/120 min bread was larger by about 75% (0/0 min bread, 771 ± 6 mL; 90/120 min bread, 1353 ± 72 mL) and the average pore size increased by a factor of 2.5 (0/0 min bread, 137 ± 11 pixel^2; 90/120 min bread, 342 ± 57 pixel^2). The salt intensities of both bread crumbs, each containing 1.5% NaCl, were compared by means of 2-AFC tests. The sensory panel rated the more coarse-pored bread crumb of the 90/120 min bread as significantly saltier (α = 0.05) than the same weight (3 g) of the more fine-pored and firmer bread crumb.

The time-dependent sodium releases from both bread crumbs during chewing were determined and compared with each other to find out if the higher salt intensity of the more coarse-pored crumb was due to a faster sodium release during chewing. As expected, the investigations with the mastication simulator as well as both methods for the determination of sodium release in-mouth revealed a significantly faster sodium release from the more coarse-pored crumb of the 90/120 min bread ($p \leq 0.05$, Figures 4A and 5A). According to the discontinuous sampling technique in-mouth, 74 ± 6% of sodium was extracted from the more coarse-pored bread crumb after a chewing time of 5 s, whereas only 55 ± 5% of sodium was extracted from the more fine-pored crumb. Even larger differences were obtained with the mastication simulator (90/120 min bread, 84 ± 4%; 0/0 min bread, 56 ± 7%). The differences decreased with increasing duration of chewing, and significant differences were observed during the first 15 s (in-mouth) and 30 s (mastication simulator), respectively. Therefore, a faster sodium release from the coarse-pored crumb occurred in particular during the first seconds of chewing, which are crucial for salt perception.

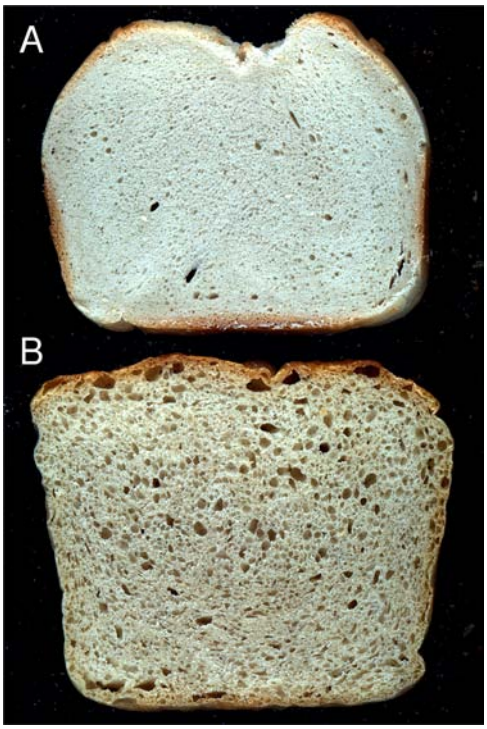

Figure 3. Breads baked with different proofing times: (A) no proofing (0/0 min); (B) extended proofing (90/120 min).

The continuous sampling technique also revealed a faster sodium release from the more coarse-pored bread crumb (Figure 5A). However, the differences in the sodium concentration measured in saliva increased during the first seconds, and the largest differences occurred for both panelists after a chewing time of approximately 30 s. This can be explained again by the fact that the chewing point did not correspond to the sampling point, so that the differences were measured at the sampling point with a certain delay in time.

The faster sodium release from the more coarse-pored bread crumb can explain the increased salt intensity compared to the more fine-pored bread crumb. Interestingly, the coarse-pored bread crumb was rated as significantly saltier by the panelists even when constant sample volumes were used. Due to the different crumb densities, a lower absolute amount of sodium was located in the mouth when the panelists chewed the more coarse-pored bread crumb. Nevertheless, they rated this crumb as significantly saltier (α = 0.05). This effect was examined in closer detail by measuring the sodium release from both bread crumbs while crumb cubes with a constant volume were eaten. A constant crumb volume of 3.375 cm^3 (1.5 cm × 1.5 cm × 1.5 cm) led to sample weights of 3.00 ± 0.32 g (0/0 min bread) and 2.04 ± 0.31 g (90/120 min bread), respectively. In the tests with a constant sample volume, the percentages of extracted sodium obtained by the discontinuous sampling methods corresponded to the extracted percentages obtained with constant sample weights for each crumb texture (data not

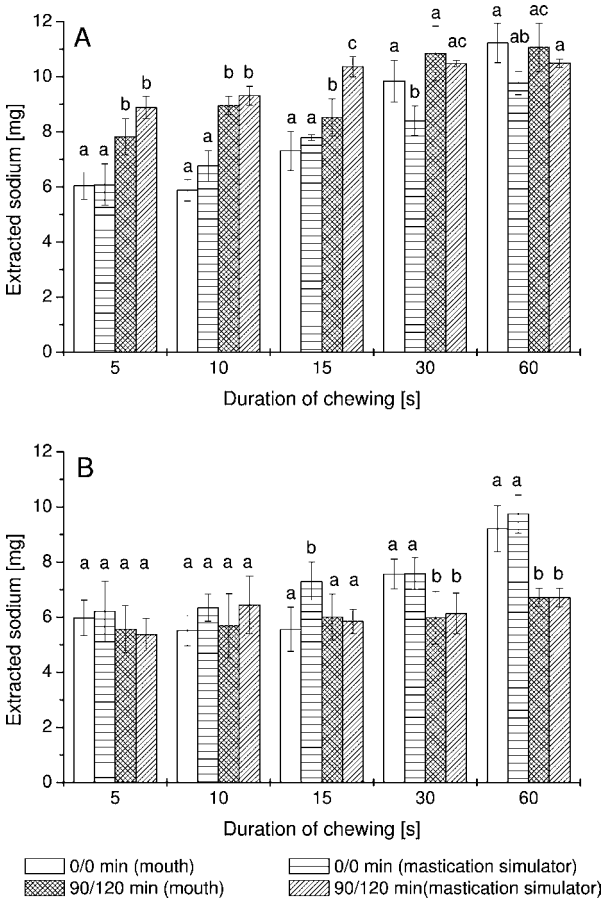

Figure 4. Comparison of the time-resolved sodium release from bread crumb baked without proofing (0/0 min) and with extended proofing times (90/120 min), each containing 1.5% NaCl: (A) constant sample weight (3 g); (B) constant sample volume (3.375 cm^3). Discontinuous sampling techniques included sodium release in-mouth and in the mastication simulator. Error bars represent standard deviations of triplicate determinations by two panelists (A and B). Mean values associated with different letters are significantly different within each chewing time (one-way ANOVA, Tukey test, $p \leq 0.05$).

shown). However, both discontinuous sampling techniques revealed no significant differences between the extracted absolute amounts of sodium from both crumb textures during the first 10 s (Figure 4B). The extracted amount of sodium from the 0/0 min bread exceeded the extracted amount from the 90/120 min bread only after longer chewing times due to the higher sample weight and, thus, the higher absolute amount of sodium in the crumb. The continuous sampling technique even provided identical sodium concentrations in-mouth for both bread crumbs during the entire duration of chewing (Figure 5B) despite the higher absolute amount of sodium in the 3.375 cm^3 cube of the 0/0 min bread. Different crumb textures had no influence on saliva secretion during chewing (data not shown). Therefore, the similar sodium concentrations may be explained by different degrees of distribution of the chewing pulps in the oral cavity. The chewing pulp of the 90/120 min bread was obviously better distributed than the chewing pulp of the 0/0 min bread. This might be due to the higher brittleness of the 90/120 min bread crumb, whereas the compact crumb of the 0/0 min bread led to an inferior distribution of the chewing pulp in the oral cavity.

Just as for dough compared to bread crumb, TI measurements were carried out to compare the perceived salt intensity over time of the 0/0 min bread to the 90/120 min bread at the same NaCl content of 1.5%. Similarly to the 2-AFC tests and the sampling techniques measuring sodium release, the sensory evaluation was carried out both at a constant sample weight (3 g) and at a constant sample volume of 3.375 cm^3, resulting in different sample weights (0/0 min bread, 3.00 g; 90/120 min bread, 2.04 g). At a constant sample weight the I_{max} (panelist A, 6.6 ± 0.3; panelist B, 6.0 ± 0.6) was significantly higher for the 90/120 min bread compared to the 0/0 min bread (panelist A, 5.3 ± 0.3; panelist B, 3.8 ± 0.6). After averaging over all TI curves of both panelists (Table 2), significant differences were found for the duration of the increasing phase (D_i), which was shorter, and the rate of increase (R_i), the area under the curve of the 90% I_{max} plateau (A_m), and the total area (A) under the curve, which were higher for the 90/120 min bread. The mean value for A_m was about 3 times and that for A about twice as large for the 90/120 min bread, because the I_{max} was significantly higher and was also reached much earlier. Only A_i did not reveal any significant differences due to the concurrence of a shorter D_i with a higher I_{max}. The TI curves confirmed the results of the 2-AFC tests and were in good agreement with the instrumental analyses of sodium release, because the higher perceived salt intensity in the 90/120 min bread can be easily explained by the faster velocity of sodium release (Figures 4A and 5A). When the sample volume was kept constant, the perceived I_{max} was also significantly higher for the 90/120 min bread (panelist A, 5.8 ± 0.3; panelist B, 5.1 ± 0.4) than for the 0/0 min bread (panelist A, 5.1 ± 0.3; panelist B, 3.6 ± 0.2). Although the differences for the I_{max} were still significant for both panelists, the relative interval between the maximum intensities was smaller at constant sample volumes than at constant weights. The average values of all TI curves (Table 2) revealed again a significantly shorter D_i and significantly higher values for R_i, D_m, A_m, and A for the 90/120 min bread. Only A_i was significantly smaller for the 90/120 min bread this time, presumably because the shorter D_i coincided with maximum intensities that were not as high as before. The I_{max} began to decrease for panelist A after 48 s in the 90/120 min bread due to a gradual dilution of the salivary sodium concentration with additional secreted saliva. The smaller sample weight also led to a decreased feeling of mouthfulness. Panelist B began to experience a slight decrease in I_{max} only near the end of the measurement, which can be attributed again to the panelist's lower rate of saliva secretion.[12] The results of the TI curves match those of the 2-AFC tests. However, when the sensory results are compared with the sodium release measurements, the more intensely perceived saltiness cannot be explained by a higher availability of sodium ions on the tongue, because the amounts of extracted sodium were similar at constant sample volumes during the first 10 s (discontinuous sampling techniques, Figure 4B) or during the entire time of mastication (continuous sampling technique, Figure 5B). Therefore, other parameters have to be responsible for the sensory difference. As it is known that the perception of texture may influence other sensory sensations, such as the perception of saltiness,[28−30] it can be assumed that the perceived intensity of saltiness has been affected by certain tactile−gustatory interactions. Similarly to these findings, Cook et al.[19] found that the impaired sweetness of sucrose in viscous polysaccharide solutions could not be fully explained by the diffusion/mass transfer, and they suggested that somatosensory tactile stimuli

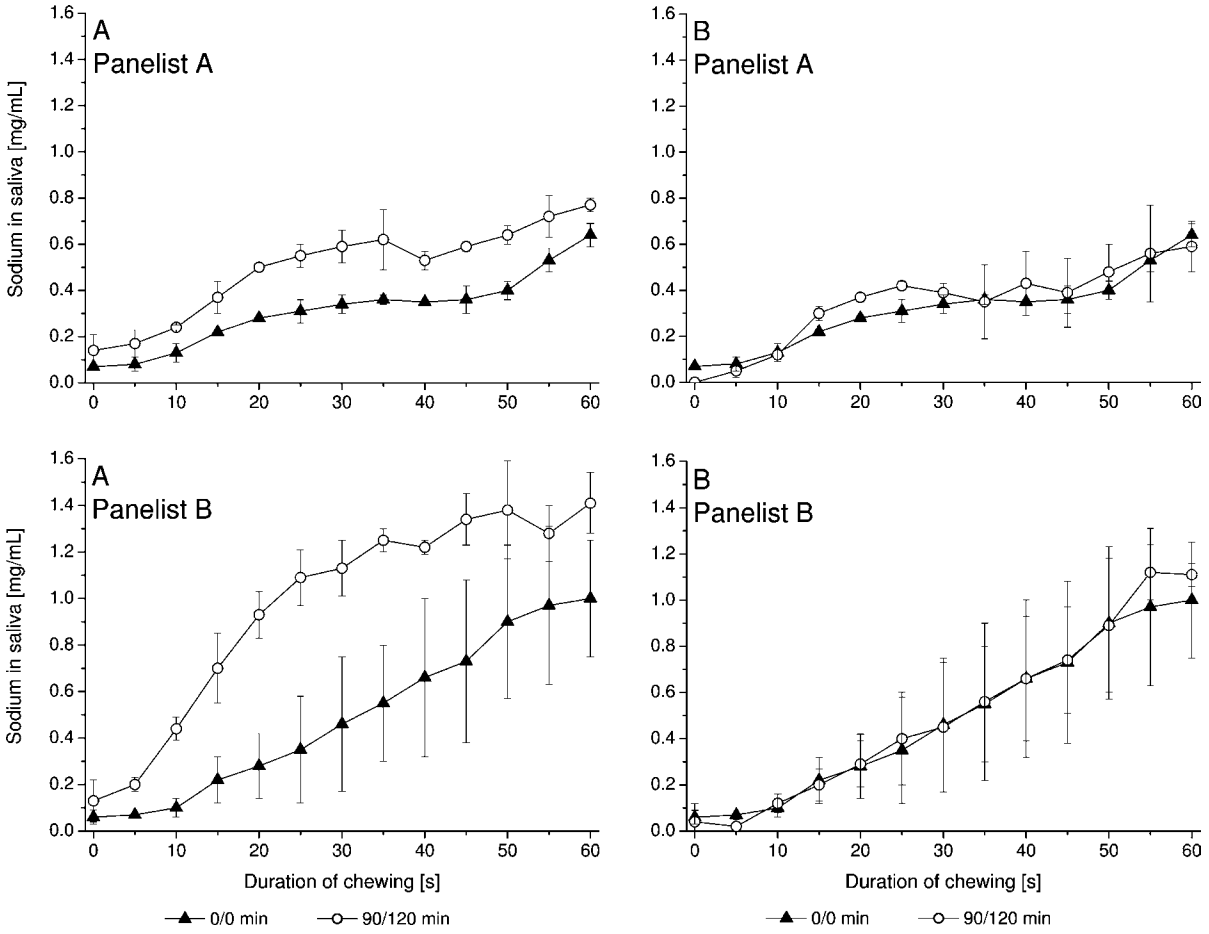

Figure 5. Comparison of the time-resolved sodium release from bread crumb baked without proofing (0/0 min) and with extended proofing times (90/120 min), each containing 1.5% NaCl: (A) constant sample weight (3 g); (B) constant sample volume (3.375 cm^3). A continuous sampling technique was used. Error bars represent standard deviations of triplicate determinations by two panelists (A and B).

Table 2. Secondary Parameters[a] Extracted from Time−Intensity Curves of Breads Baked with Different Proofing Times (0/0 and 90/120 min) at the Same NaCl Level of 1.5%, at a Constant Sample Weight of 3 g and at a Constant Sample Volume of 3.375 cm^3 (0/0 min Bread, 3.00 g; 90/120 min Bread, 2.04 g)[b]

sample	D_i	R_i	A_i	D_m	A_m	A
			Constant Sample Weight (3 g)			
0/0 min	39.4 ± 5.5	0.10 ± 0.03	66.5 ± 13.7	12.3 ± 5.9	50.2 ± 30.0	117.4 ± 31.6
90/120 min	18.1 ± 4.3*	0.31 ± 0.08*	54.1 ± 15.5	31.7 ± 5.7*	168.6 ± 30.5*	238.8 ± 24.5*
			Constant Sample Volume (3.375 cm^3)			
0/0 min	28.2 ± 4.5	0.13 ± 0.03	51.5 ± 10.6	22.2 ± 5.2	80.3 ± 17.0	131.8 ± 15.1
90/120 min	14.3 ± 3.1*	0.32 ± 0.03*	34.0 ± 8.1*	30.6 ± 8.9	138.4 ±30.6*	208.7 ± 12.2*

[a] D_i, duration of the increasing phase; R_i, rate of increase; A_i, area under the curve in the increasing phase; D_m, duration of the plateau; A_m, area under the plateau; A, area under the total curve. [b] Mean values ± standard deviation (n = 12). An asterisk (*) indicates statistically significant differences between the 0/0 min-bread and the 90/120 min-bread (two-way ANOVA, $p \leq 0.05$).

could interact with taste signals to modulate their perception. All sensory analyses were performed with a nose clip to eliminate interfering interactions between taste and orthonasal aroma perception. However, despite this precaution and the reduction of the amount of yeast used, it may be possible that taste- or aroma-active yeast metabolites had an additional modulating effect on saltiness. Amino acids[31] or aroma compounds such as 2-phenylethanol, 3-methylbutanol,[32] 3-methylbutanal, 2,3-butanedione, and phenylacetaldehyde[33] are generated during yeast fermentation. These yeast metabolites might have caused cross-modal odor-induced saltiness enhancement[34] in the long-fermented bread.

Influence of DATEM and α-Amylase. Crumb texture can be influenced not only by variation of the proofing times but also by the addition of surfactants or enzymes. In further experiments, the impact of DATEM and α-amylase on crumb texture, salt intensity, and sodium release during chewing was investigated. The addition of 0.6% DATEM strongly increased bread volume by about 75%, and the average pore size was about twice as large as in the reference bread baked with the standard proofing times (20 and 40 min) and without any additive. When heat-labile α-amylase was added (5 mg/100 g flour), bread volume increased by only about 20% and an increase in the average pore size of about 30% was observed.

Whereas the bread with DATEM showed a quite regular spatial distribution of the different pore sizes, the large pores were accumulated in the middle of the bread with α-amylase. Despite these textural modifications, the sensory panel found no significant difference in saltiness between the breads with additives and the reference bread ($\alpha > 0.2$ for DATEM; $\alpha = 0.2$ for α-amylase). Furthermore, the discontinuous sampling technique in-mouth revealed no significant differences between the time-resolved sodium release from bread crumbs with DATEM or α-amylase and the crumb of the reference bread (data not shown). The results obtained with the mastication simulator revealed that after a chewing time of 10 s, significantly more sodium had been extracted from the DATEM bread (78 ± 3%) and from the α-amylase bread (79 ± 3%) than from the reference bread (67 ± 5%), but a difference as large as between the 0/0 and 90/120 min breads could not be reached. This might be explained by the fact that the pore size ratio between the reference bread and the DATEM bread (factor 2.0) was lower than between the 0/0 and 90/120 min breads (factor 2.5). Furthermore, sodium release from bread crumb might be influenced not only by pore size but also by other texture parameters such as the softness of bread crumb. The crumb firmnesses of the 0/0 and 90/120 min breads differed more from each other (factor 1.9) than the crumb firmnesses of the reference bread and the α-amylase bread (factor 1.1) or the crumb firmnesses of the reference bread and the DATEM bread (factor 1.6). The very soft crumb texture of the bread with DATEM and the resulting stickiness during chewing in the mouth might be the reason that the sodium release in the mouth had not increased despite the significant increase in average pore size (Figure 6A). As no significant increase in salt intensity was obtained by the addition of DATEM or α-amylase, the sodium release from these breads was not examined in closer detail with the continuous sampling technique.

Correlation between Saltiness, Sodium Release, Pore Size, and Texture. To obtain a better understanding of the relationship between saltiness, sodium release, pore size, and texture, the textures of the reference bread, the breads baked with the different proofing times (0/0 and 90/120 min), and the breads with additives (DATEM and α-amylase) were characterized by the determination of the average pore size and by measuring crumb firmness using a texture analyzer. The different breads are listed here in ascending order with regard to pore size: 0/0 min bread, reference bread, α-amylase bread, 90/120 min bread, and DATEM-bread (Figure 6A). An increase in pore size was associated with an increased bread volume and decreased crumb firmness. Moreover, a direct correlation was observed between pore size and the extracted amount of sodium after 5 s (discontinuous sampling technique in-mouth) with the exception of the DATEM bread (Figure 6A). The DATEM bread showed the lowest crumb firmness, which might be caused by the large pore size and by further effects caused by the surfactant. It can be assumed that the crumb of the DATEM bread was compressed more firmly during chewing due to its softness, forming a rather compact chewing pulp in the oral cavity, which resulted in lower sodium extraction efficiency during chewing. With crumb firmness and sodium release from the reference bread, the α-amylase bread, and the 90/120 min bread (Figure 6B) taken into consideration, an inverse correlation between crumb firmness and sodium release can be assumed. However, this inverse correlation seems to exist only in a certain range of crumb

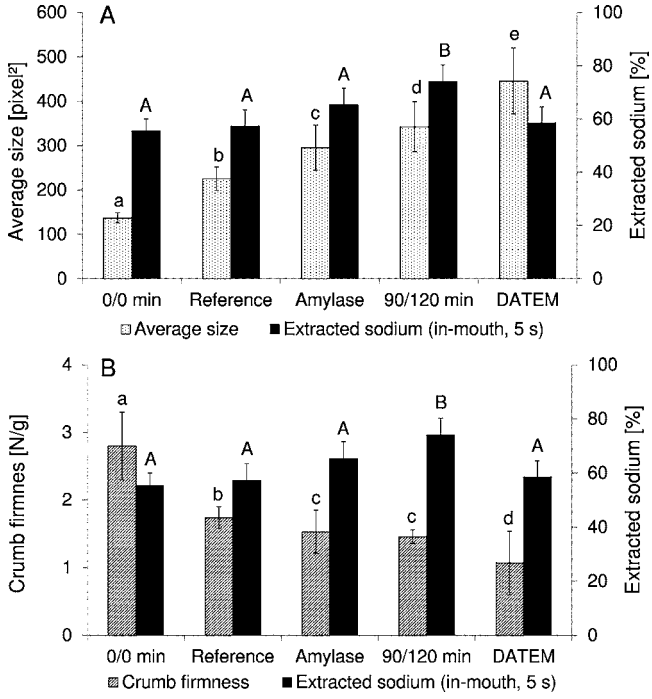

Figure 6. Correlations: (A) average pore size and extracted amounts of sodium after 5 s (discontinuous sampling technique in-mouth); (B) crumb firmness and extracted amounts of sodium after 5 s (discontinuous sampling technique in-mouth). Error bars represent standard deviations ($n = 21$ for average pore size; $n = 6$ for extracted amounts of sodium after 5 s; $n = 27$ for crumb firmness). Mean values associated with different letters (capital letters for extracted amounts of sodium after 5 s in A and B, lower case letters for average pore size in A and for crumb firmness in B) are significantly different (one-way ANOVA, Tukey test, $p \leq 0.05$).

firmness: on the one hand, sodium release after 5 s from the reference bread was as fast as from the 0/0 min bread despite the extreme difference in crumb firmness. On the other hand, sodium release from the DATEM bread was slower compared to the 90/120 min bread despite a further decrease in crumb firmness.

Taken together, these results indicate that sodium release from bread crumb is influenced by texture and, more specifically, by a combination of crumb firmness and pore size. A larger pore size leads to a softer crumb and a faster sodium release, but when the pores are too large and the crumb is too soft, the crumb is converted to a lump by chewing, leading to a diminished rate of sodium release. The crumb firmness must neither be too firm nor too soft to obtain a maximum velocity of sodium release during chewing. With regard to sodium release, the 90/120 min bread seems to have the best crumb texture of all types of bread that were investigated in these studies.

However, the salt intensity of bread crumb is influenced not just by sodium release during chewing. Although no significant difference in sodium release was observed between the 0/0 min bread and the reference bread, the reference bread was rated as significantly saltier ($\alpha = 0.05$). Therefore, the very firm crumb texture of the 0/0 min bread seems to diminish the salt intensity by texture-induced tactile−gustatory interactions in the mouth or brain.

From the results obtained it can be concluded that the salt intensity of bread crumb is determined by texture. On the one

hand, texture has an influence on sodium release from bread crumb during chewing. On the other hand, texture induces tactile−gustatory interactions in the mouth or brain. An enhanced salt intensity of bread crumb could be obtained by an optimized crumb structure with large pores, provided that the resulting crumb firmness is not too low. This suggests a new, effective strategy for salt reduction in bread, and more research would provide further strategies to appropriately modify crumb texture.

■ ASSOCIATED CONTENT

Supporting Information

Additional tables. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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