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Kinetics of Sodium Release from Wheat Bread Crumb As Affected by Sodium Distribution

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Supporting Information

ABSTRACT: As a basis for sodium reduction in bread, the kinetics of sodium release from wheat bread crumb during chewing was investigated by three independent methods using two in-mouth techniques and a model mastication simulator, respectively. Complete sodium extraction in-mouth was achieved after 30 s. Using coarse-grained NaCl in breadmaking significantly accelerated sodium release and led to enhanced salt taste, allowing a sodium reduction in bread by 25% while maintaining taste quality. This salt taste enhancement by accelerated sodium delivery can be explained by the increasing contrast in sodium concentration, which is known to determine salt taste perception. For the first time, the resulting inhomogeneous salt distribution in bread prepared by using coarse-grained NaCl was visualized by means of confocal laser scanning microscopy using a sodium-selective, fluorescent dye.

KEYWORDS: *salt reduction, wheat bread, sodium release, inhomogeneous distribution, sensory contrast, CLSM*

INTRODUCTION

In addition to being an essential nutrient involved in regulating extracellular fluid volume, plasma osmolality, and membrane potential,[1] sodium is essential for the flavor, texture, and preservation of foods. Due to the low endogenous sodium levels in foods (<0.1%), nonprocessed foods contribute only 5−10% to the daily sodium intake, whereas another 10−15% is added to spice foods during cooking and at the table.[2] Accounting for about 75−80% of daily sodium intake, processed, restaurant, canteen, and takeaway foods constitute the main source of sodium in the United Kingdom,[2] other European countries, and North America[3] and have recently been the focus of reduction strategies due to major health risks associated with excess sodium uptake.[4,5] These risks include cardiovascular diseases, stroke, gastric cancer, osteoporosis, kidney stones, and diabetes,[4] which can be ascribed to elevated blood pressure levels caused primarily by excessive dietary sodium consumption. Although other factors such as obesity, physical inactivity, and alcohol consumption also promote hypertension, meta-analyses of scientific studies demonstrated a consistent fall in blood pressure in response to salt reduction.[6] It is estimated that a reduction of 5 g salt/day would cause a 23% decrease in the rate of stroke and a 17% decrease in the rate of cardiovascular disease worldwide.[7] As a result of the U.K. salt reduction campaigns started in 2003−2004, average dietary salt intake had decreased by 0.9 g/day by 2008, which led to about 6000 fewer deaths from cardiovascular disease a year.[2,8] Therefore, a reduction of the current average salt intake of 8−11 g/day to the amount recommended by the World Health Organization of 5 g/day is expected to have a significant beneficial impact on public health.

Bread and cereal products contribute about 24% of the daily sodium intake in Germany,[9] so that new strategies for a reduction of sodium in bread are required. From a technological point of view, a reduction of sodium chloride from 1.2 to 0.6 or 0.3% would be feasible without significantly affecting rheological properties or breadmaking performance, but an improvement of the taste quality in low-salt breads has been determined to be the critical factor.[10] Apart from the partial replacement of NaCl by other inorganic salts[11,12] and the addition of salt-taste-enhancing substances,[13−16] a gradual reduction of the salt content over a longer period of time[17] has been suggested. Due to the limitations of these approaches, the search for new salt reduction strategies is ongoing.

The presentation of prolonged, continuous taste stimuli led to a gradual decrease of perceived taste intensity over time, which was attributed to adaptation of neural taste receptor responses and psychophysical effects.[18] In contrast, alternating pulses of tastant solution and water even enhanced saltiness intensity, possibly through a reduction of adaptation.[18,19] This enhancement of taste intensity was successfully applied in salty[19] and sweet[20] solutions and in sweet model gels.[21] In bread crumb, an inhomogeneous spatial distribution of sodium chloride was achieved with alternating layers of a low-salt and a

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high-salt dough[22] or with the addition of fat-encapsulated salt to a low-salt dough.[23] The observed enhancement of salty taste has been attributed to sensory contrast,[22,23] but a conceivable change in the rate of NaCl crystal dissolution and thus tastant delivery was not studied.

Therefore, the aim of this study was to investigate the impact of the distribution of sodium in the crumb on the velocity of sodium release from wheat bread crumb using a continuous in-mouth sampling method[24] and a mastication simulator,[25] as well as a discontinuous, time-resolved in-mouth mastication setup. Using a homogeneous 1.5% NaCl reference bread, mastication experiments should be performed to study the influence of interindividual variability on sodium extractability, which is known to be affected by salivary flow rate and masticatory performance.[26−28] To investigate the importance of the sensory contrast in salt perception,[22,23] bread showing an inhomogeneous sodium distribution should be prepared using coarse-grained salt, and a possible enhancement of salt taste intensity should be assessed by human sensory analyses using two-alternative forced choice (2-AFC) tests and time−intensity methods.

MATERIALS AND METHODS

Wheat Flour. Rosenmehl type 550 standard wheat flour (Rosenmühle, Ergolding, Germany) was characterized as described by Selmair and Koehler.[29] Analytical characteristics of the flour were 9.7% moisture (w/w), 0.61% ash (dry mass), and 11.8% protein (dry mass).

Chemicals. The following chemicals were commercially available: Patent Blue V calcium salt (Sigma-Aldrich, Steinheim, Germany); sodium chloride, sucrose, and tris(hydroxymethyl)aminomethane (Tris) (Merck, Darmstadt, Germany); 2−3.5 mm coarse-grained sodium chloride (Esco, Hannover, Germany); and SBFI-AM (Life Technologies, Carlsbad, CA, USA).

Breadmaking. The reference bread containing 1.5% NaCl was prepared using 300 g of wheat flour as described recently.[25] An inhomogeneous distribution of NaCl was achieved by preparing doughs with 0.5, 0.25, 0.1, and 0% NaCl, respectively, followed by the addition of 1, 1.25, 1.4, and 1.5% coarse-grained (cg) NaCl, respectively, 30 s prior to the end of dough mixing time. The final doughs always contained 1.5% of total NaCl and were processed into bread as previously described and stored overnight at room temperature.[25] NaCl concentrations in bread are given based on flour weight (= 100%).

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 bread samples each, and the statistical significance of the results comparing the homogeneous breads with 0.25 and 1.5% NaCl to the inhomogeneous bread with 0.25/1.25% cg NaCl was determined by one-way ANOVA at a level of 0.05 with Tukey's test as post hoc procedure (SigmaStat v3.5) (Systat, San Jose, CA, USA).

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 measurement of the crumb cylinder was done in triplicate from three different slices from three breads with a TA.XT plus Texture Analyzer (Stable Micro Systems Ltd.). The crumb cylinder was compressed by 7 mm with a plexiglass cylinder (20 mm diameter), and two consecutive measurements were done per sample as described by Selmair and Koehler.[29] Force−distance and force−time diagrams were recorded, data analysis was performed with the software Texture Exponent (Stable Micro Systems Ltd.), and the statistical significance of the results for the homogeneous breads with 0.25 and 1.5% NaCl, respectively, and the inhomogeneous bread with 0.25/1.25% cg NaCl was determined by one-way ANOVA at a level of 0.05 with Tukey's test as post hoc procedure (SigmaStat v3.5).

Sensory Analyses. *Sensory Panel.* The panel for all sensory analyses consisted of 15 trained (ISO 8586)[30] 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 as reported recently.[25]

Procedure of the 2-AFC Tests. The evaluation of the saltiness of bread crumb was performed by means of a 2-AFC test as described earlier.[25] In brief, the panelists received two encrypted, covered sensory flasks with different bread samples (inhomogeneous 0.5/1.0, 0.25/1.25, 0.4/1.0, or 0/1.5% cg NaCl, respectively, against the homogeneous 1.5% NaCl reference) in an AB or BA presentation design randomized over panelists. The panelists were instructed to rinse their mouths with table water during the 1 min break between samples and give the number of the sample that tasted saltier. The statistical evaluation was done by reference to a table of significance for two-sided paired comparison tests in compliance with ISO 5495.[31] Depending on the number of correct answers, the level of significance p was determined, and p levels ≤ 0.05 were judged as significant. All 2-AFC tests were done in triplicate.

Comparison of Bread and Water Stimuli. The panelists were asked to chew 3 g of bread crumb with 2% NaCl for 30 s, swallow, and rinse their mouths with table water. After a 1 min break, they were asked to take 5 mL of a NaCl solution (7.6 mg NaCl/mL) into their oral cavity and to indicate which sample tasted saltier using the 2-AFC test procedure described above. The amount of NaCl in the bread sample was determined beforehand according to the literature[25] to match the absolute NaCl amount in the bread sample and the aqueous NaCl solution.

Determination of the Salt Recognition Threshold in Bread Crumb. The determination of taste recognition thresholds as reported in the literature[32−35] was adapted for bread samples according to a recent literature protocol.[25] By means of 2-AFC tests, 3 g of bread with 0.1, 0.2, 0.3, 0.4, and 0.5% NaCl, respectively, was tasted against 3 g of bread with 0% NaCl (i.e., no added NaCl) in increasing amounts of salt. The NaCl concentrations were chosen on the basis of previous experience.[25] The panelists were asked to always taste all sample pairs and to indicate the sample that tasted saltier. The salt recognition threshold was determined from the first correct answer on the condition that all successive samples were correctly identified. The determination of the threshold was done in triplicate on consecutive days. The geometric means of the NaCl concentration in the first correctly identified sample and of the NaCl concentration of the previous sample were calculated per panelist. The salt recognition threshold is given as the arithmetic mean (± standard deviation (SD)) of the three geometric means of consecutive determinations.

Time−Intensity (TI). TI measurements were carried out in individual booths using FIZZ Acquisition software v2.46A (Biosystèmes, Couternon, France) for data collection as reported earlier[32] with some modifications. The panelists were asked to touch the screen, take 3 g of bread crumb into their hand, put these 3 g into their mouth after 5 s, and start chewing while simultaneously evaluating the salt intensity over time 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. After 60 s, the panelists were instructed to swallow the bread and stop the measurement by moving the cursor back to the left end of the scale. The waiting period between two samples was 60 s, and the panelists were instructed to rinse their mouths with water during that time. Practice sessions using bread with 1.5% NaCl were done at the beginning to familiarize the panelists with the procedure and train them in using the scale. Sensory evaluations comparing two coded and blinded samples (the homogeneous 1.5% NaCl bread to the inhomogeneous 0.25/1.25% cg NaCl bread) were done in six separate sessions on consecutive days using a Latin square design to rule out effects of sample presentation. Recorded TI curves were modeled according to the trapezoid procedure reported earlier.[36] The maximum intensity (I_{max}), t_{i5}, t_{i25}, t_{i50}, t_{i75}, and t_{i90} (the times corresponding to 5% I_{max}, 25% I_{max}, 50% I_{max}, 75% I_{max}, and 90% I_{max} in the increasing phase) were extracted from the single curves. When the end of the 90% I_{max} plateau was reached before stopping the

measurement at 60 s by swallowing the sample, concurrent t_{d90}, t_{d75}, t_{d50}, t_{d25}, and t_{d5} (the times corresponding to 5% I_{max}, 25% I_{max}, 50% I_{max}, 75% I_{max}, and 90% I_{max} in the decreasing phase) were extracted additionally. 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 and the statistical significance was determined by two-way ANOVA with breads and panelists as factors (SigmaStat v3.5).

Collection of Human Saliva. Human saliva was collected from six volunteers (panelists A−F) who had given informed written consent to the work. Using a literature method[37] with some modifications, the panelists rinsed their oral cavity with table water (8 mL) and spat out. Then, after a waiting time of 60 s and swallowing, the panelists collected saliva in their mouths while simulating chewing movements for 5 min before expectorating. After a waiting time of 1 min, this procedure was repeated until a sufficient amount of saliva (50 mL) had been generated. Saliva samples were collected on the day of use in the mastication simulator, stored at room temperature, and not pretreated in any way. The inherent sodium concentrations were measured directly in the collected saliva by means of a sodium-selective electrode.

Time-Resolved Extraction of Sodium from Bread Crumb during Mastication. The time-resolved sodium release from bread crumb during chewing was determined by using three different methods.

Continuous Sampling Technique. Through adaptation of a literature method,[24] 3 g of bread crumb was chewed in the mouth for 60 s using the frequency controlled by a metronome (72 chews/min). During chewing, a satin ribbon (width = 3 mm) was pulled through the mouth by a motor (1.1 cm/s). Then the satin ribbon was cut into pieces of 5.5 cm corresponding to chewing periods of 5 s. These pieces were extracted with 5 mL of Tris buffer (0.5 mol/L, pH 7) for 5 min using a vortex mixer. The sodium concentrations of these extracts were measured by a sodium-selective polymer membrane-based electrode using a Metrohm 781-type pH-/ion meter (Metrohm, Filderstadt, Germany) calibrated in the range from 5 to 5000 mg Na/L.[25] Triplicate determinations were carried out by six panelists (A−F) for the 1.5% NaCl bread. From these six panelists, two panelists (A with a high and B with a low salivary flow rate) were chosen for further experiments comparing the 1.5% NaCl bread to the 0.25/1.25% cg NaCl bread to take into account interindividual differences in saliva secretion while comparing different bread samples. Statistical significances of differences between panelists A−F during mastication of the 1.5% NaCl bread and between breads (1.5% NaCl vs 0.25/1.25% cg NaCl) assessed by panelists A and B were determined by one-way ANOVA at a level of 0.05 with Tukey's test as post hoc procedure (SigmaStat v3.5).

Discontinuous Sampling Techniques. Two panelists (A with a high and B with a low salivary flow rate) were chosen for the discontinuous sampling experiments.

(a) In-Mouth. Three grams of bread crumb was chewed in the mouth for 5, 10, 15, 30, and 60 s. The resulting chewing pulps were spat out and the saliva secretion was determined gravimetrically. After centrifugation (3750*g*, 10 min, 20 °C), the sodium concentrations in the supernatants were quantitated by the sodium-selective electrode. Independent triplicate determinations were carried out for each panelist.

(b) Mastication Simulator. Mastication was simulated with the aid of a modified Potter S homogenizer (Braun, Melsungen AG, Germany) as described previously.[25] Bread crumb samples (3 g) were extracted for 5, 10, 15, 30, and 60 s using human saliva of panelists A and B as extracting agent. The amount of saliva added was adapted for each chewing time to the respective, gravimetrically determined salivary secretion after mastication in the mouth (1.26, 2.17, 2.97, 3.96, and 5.55 mL for panelist A and 0.43, 0.63, 0.95, 1.45, and 3.34 mL for panelist B for 5, 10, 15, 30, and 60 s, respectively). All determinations were done in triplicate.

For both discontinuous methods, the measured sodium concentrations were converted to extracted sodium (mg/g crumb dry matter), taking into account the volume of saliva secreted, the endogenous water content of the crumb, and the endogenous sodium content in saliva. Statistical significances of differences between panelists A and B, between mouth and mastication simulator, between mastication times, and between different breads (1.5% NaCl vs 0.25/1.25% cg NaCl) were determined by one-way ANOVA at a level of 0.05 with Tukey's test as post hoc procedure (SigmaStat v3.5).

Saliva Secretion. Three grams of bread crumb was chewed in triplicate by six panelists (A−F) for 5−60 s. The chewing pulps were spat out at 5 s intervals, and the saliva secretion was determined gravimetrically. Statistical significances of differences between panelists were determined by one-way ANOVA at a level of 0.05 with Tukey's test as post hoc procedure. Pearson's correlations between saliva secretion, salivary sodium concentration, and salt recognition threshold of the six panelists were calculated (SigmaStat v3.5).

Visualization of the Inhomogeneous Distribution of NaCl in Bread Crumb. The inhomogeneous spatial distribution of NaCl in bread crumb was visualized by two methods. First, NaCl crystals containing the food colorant Patent Blue V served as an indirect, macroscopic indicator of sodium distribution. Second, confocal laser scanning microscopy (CLSM) with the sodium-selective dye SBFI-AM was used to make the distribution of sodium directly visible.

Indirect Visualization of Sodium Distribution Using Stained NaCl. To prepare NaCl crystals stained with Patent Blue V, 54 g of NaCl was dissolved in 150 mL of ultrapure water to obtain a saturated solution. After heating to the boiling point, the hot solution was filtered into a beaker and Patent Blue V calcium salt (0.5 g) was added. A piece of nylon thread was added as crystallization seed, and the beaker was covered with perforated aluminum foil and kept in a vibration-free place at a constant temperature of 22 °C for 2 months. The crystals were then dried at 103 °C for 4 h and passed through a 2 mm sieve, resulting in a coarse-grained fraction with a particle size of 2−3.5 mm. To keep the amount of Patent Blue V constant in all baking experiments, one part of the coarse-grained fraction was carefully ground with a pestle and mortar and again passed through the 2 mm sieve resulting in a fine-grained fraction with a particle size <2 mm. Both fractions were used for the baking experiments.

To quantitate the amount of Patent Blue V in the NaCl crystals, 50−150 mg of the stained NaCl crystals was weighed into 25 mL volumetric flasks and dissolved in distilled water. The extinction of the solutions was measured at 638 nm on a UV-2401 PC UV−vis spectrophotometer (Shimadzu, Kyoto, Japan). Quantitation was done by five-point external standard calibration against water as a blank, and all measurements were done in triplicate. Breads were baked as described above using NaCl crystals containing Patent Blue V instead of nonstained NaCl crystals.

Direct Visualization of Sodium Distribution Using Confocal Laser Scanning Microscopy. Bread pieces (1 × 1 × 0.5 cm) were cut with a razor blade from the middle of the fresh loaf, stained for 5 min with the sodium-selective ion indicator SBFI-AM (3.3% w/v) in acetone, and placed on a cover slide. For analysis, a FluoView 300 confocal laser-scanning system (Olympus, Tokyo, Japan) mounted on an inverted microscope IX 81 (Olympus) with a UPLSAPO 10× objective was used. The excitation wavelength was 405 nm (laser diode), and fluorescence emission (>510 nm) was recorded through a BA510IF emission filter. Fluorescence micrographs were acquired by scanning through the entire sample along the *z*-axis in 5 μm steps from −500 to 500 μm and projecting the 200 layers. Laser intensity, gain, and offset settings were kept rigorously constant in all sample micrographs. Micrographs of the projection of bread pieces were acquired in XYZ layers. A coherent series of five micrographs was taken along the *x*-direction. Then the sample was moved 0.14 cm in the *y*-direction and another row of five micrographs was taken below the first row of micrographs. This procedure was used to scan the entire bread piece. To account for the autofluorescence of SBFI-AM in the absence of sodium and, therefore, to enable a subsequent background correction, bread without addition of NaCl was used as a control. In total, 30 micrographs were taken each of three representative samples of bread crumb with 1.5% NaCl (homogeneous sodium distribution) and without NaCl as well as bread crumb with 0.25/1.25% cg NaCl (inhomogeneous sodium distribution). The

CLSM micrograph areas showing bright, pseudocolored yellow fluorescence indicated a higher concentration of sodium. This was due to the enhancement of fluorescence intensity of SBFI-AM upon binding of sodium within the crown ether structure. Fluorescence spectra of SBFI-AM in solutions of 0−130 mmol NaCl/L (pH 7.0) were recorded on a Cary Eclipse fluorescence spectrophotometer (Agilent Technologies, Waldbronn, Germany) after excitation at 405 nm, and the linearity of fluorescence emission at 510 nm was compared to the literature.[38]

For CLSM micrograph analysis, the micrographs were reduced to grayscale and Scion Image software (Scion Corporation, Frederick, MD, USA) was used to create histograms. Histogram data showing the number of pixels per RGB value from 0 (black) to 255 (white) was exported to Microsoft Excel 2010. Analysis of all micrographs of bread without NaCl revealed that the mean lightest RGB value was RGB 135 (gray). Therefore, the sum of pixels from RGB 0 to 135, which constitutes pores and out-of-focus areas (black) as well as the background fluorescence of SBFI-AM without sodium, was subtracted from the total number of pixels (48000) in all micrographs of bread samples with NaCl. This left only the sum of the pixels with brighter RGB values from 136 to 255, showing the areas rich in SBFI-AM with sodium. For the homogeneous 1.5% NaCl bread and in the inhomogeneous 0.25/1.25% cg NaCl bread, 3 different pieces each were taken, and 12 micrographs covering a representative area in each piece were thus analyzed.

RESULTS AND DISCUSSION

Saltiness Perception in Bread Crumb Compared to Water. After mastication for 30 s, the saltiness of 3 g of bread crumb containing 2% NaCl (37.9 mg NaCl/3 g bread crumb, based on wet weight) was compared to 5 mL of a NaCl solution (7.6 mg NaCl/mL) in bottled water containing the same amount of NaCl. Despite the identical NaCl amounts, the aqueous salt solution was judged by all panelists as being significantly saltier (p = 0.001). In another experiment, the sensory test was performed with 5 mL of a NaCl solution (3.8 mg NaCl/mL) containing only 50% of the NaCl amount. Interestingly, the aqueous salt solution was still rated as tasting significantly saltier (p = 0.001), although the bread contained twice as much salt. As recent studies demonstrated that sodium is completely extractable from bread crumb with water, buffer solution, or human saliva,[25] the binding of sodium ions to bread components such as proteins cannot explain the sensory difference between bread crumb and the aqueous salt solutions. In contrast to the aqueous solution, chewing the bread crumb is expected to entail a time-dependent, gradual increase of sodium levels, resulting in a much less notable salt taste contrast in the oral cavity before and during consumption of the bread. To investigate the kinetics of sodium release from bread crumb, time-resolved mastication experiments were done.

Time-Resolved Release of Sodium from Bread Crumb during Mastication. According to the literature,[25] the total amount of sodium extracted from 3 g of bread crumb containing 1.5% NaCl by using ultrapure water was 6.69 ± 0.07 mg Na^+/g crumb based on dry matter. Taking the endogenous, analyzed water content (45%) of the crumb into account, this corresponded to 3.68 mg Na^+/g crumb (based on wet weight). An addition of 1.5% NaCl (based on flour weight) corresponded to 3.14 mg Na^+/g dough and 3.63 mg Na^+/g crumb (wet weight), considering the determined water loss of 13.6% during baking.

First, the actual sodium concentration in-mouth during the mastication of bread crumb was measured by means of a continuous in-mouth sampling technique based on the method of Davidson et al.,[24] who determined sodium release from cheese and crisps. The in-mouth sodium concentrations during mastication of bread crumb were measured by using a satin ribbon, which was pulled through the mouth during chewing. After the satin ribbon had been cut into pieces corresponding to chewing periods of 5 s, the pieces were extracted with Tris

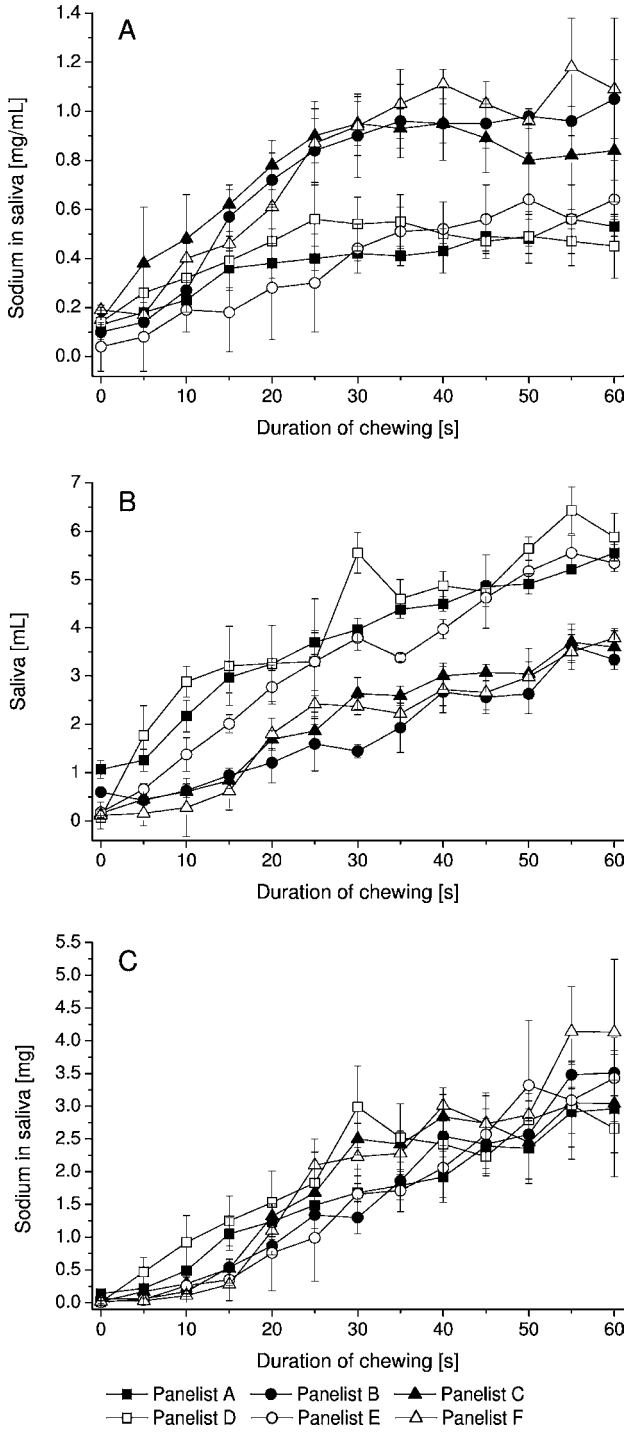

Figure 1. Time-resolved sodium release from bread crumb with 1.5% NaCl: (A) continuous sampling technique, sodium concentration in saliva; (B) saliva secretion during the mastication of 3 g of bread crumb with 1.5% NaCl; (C) continuous sampling technique, multiplication of the sodium concentration in saliva by salivary secretion, shown as absolute amount of sodium in saliva. Error bars represent standard deviations of triplicate determinations (panelists A, B, C, D, E, and F).

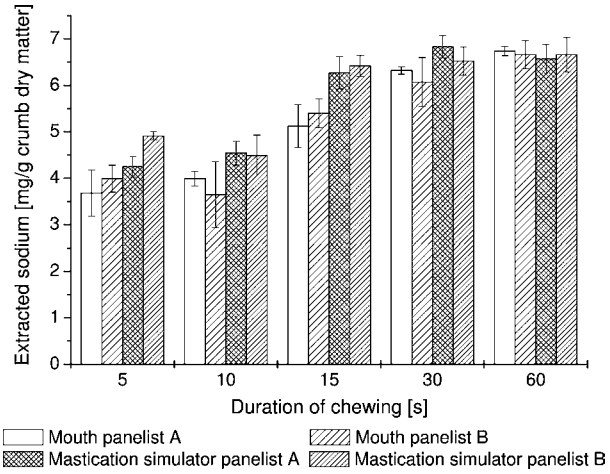

Figure 2. Time-resolved sodium release from bread crumb with 1.5% NaCl. Discontinuous sampling techniques included sodium release in-mouth and in the mastication simulator. Error bars represent standard deviations of triplicate determinations (panelists A and B).

Table 1. Salt Recognition Thresholds for NaCl in Bread Crumb, Sodium Concentrations in Unstimulated Saliva, and Secreted Amounts of Saliva after Bread Mastication for 60 s

panelist	salt recognition threshold[a] (% NaCl in bread based on flour)	Na^+ in saliva[a] (mg/kg)	secreted saliva after 60 s[a] (mL)
A	0.18 ± 0.05	48 ± 3	5.6 ± 0.2
B	0.18 ± 0.05	103 ± 9	3.3 ± 0.2
C	0.28 ± 0.05	216 ± 14	3.6 ± 0.4
D	0.31 ± 0.05	176 ± 7	5.9 ± 0.5
E	0.31 ± 0.10	131 ± 14	5.3 ± 0.2
F	0.18 ± 0.05	260 ± 11	3.8 ± 0.2

[a]Mean values ± standard deviations of triplicate determinations.

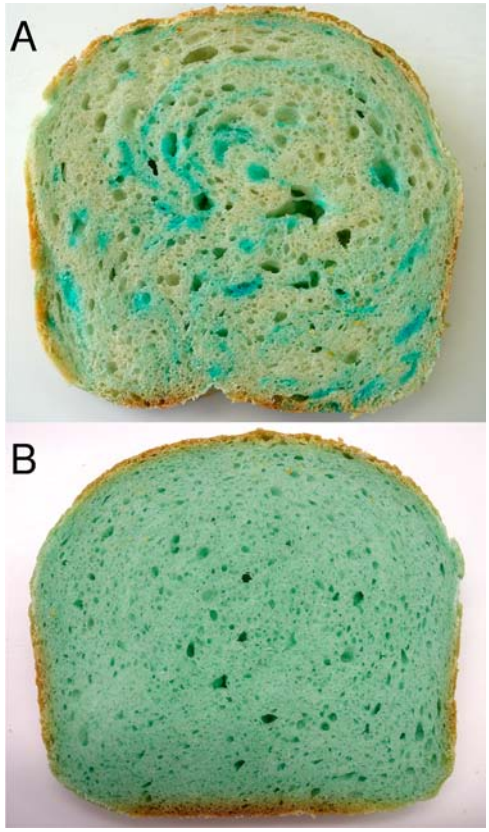

Figure 3. Bread crumb containing 1.5% NaCl: (A) inhomogeneous sodium distribution visualized by adding blue, coarse-grained salt crystals (2−3.5 mm, 1.25%) 30 s before the end of the mixing time to the dough containing 0.25% uncolored salt; (B) homogeneous sodium distribution obtained by adding blue, fine-grained salt crystals (<2 mm, 1.5%) at the beginning of the dough mixing time.

buffer (0.5 mol/L, pH 7) and sodium ions were quantitated by means of a sodium-selective polymer membrane-based electrode. As depicted in Figure 1A, a significant increase in salivary sodium concentration during chewing was measured for six panelists (A−F). However, for chewing times exceeding 20 s, the sodium concentrations in the saliva of panelists B, C, and F were considerably higher ($p \leq 0.028$) than in the saliva of panelists A, D, and E. This might be explained by the different amounts of saliva secreted during chewing. To verify this assumption, the saliva secretion during chewing of 3 g of bread crumb was determined for all panelists. As expected, panelists B, C, and F with higher sodium concentrations showed a lower salivary secretion than panelists A, D, and E with lower sodium concentrations (Figure 1B). The multiplication of the salivary sodium concentrations by the saliva secretions resulted in absolute amounts of sodium that corresponded very well with each other (Figure 1C). These results indicate that the salivary sodium concentrations measured by the continuous sampling technique clearly depend on the saliva secretion of each individual panelist. Furthermore, the slight differences that were still observed between the panelists after the multiplication by saliva secretion could depend to a certain extent on the individual shape of the oral cavity and on individual chewing behavior, because the satin ribbon was not directly pulled through the chewing pulp, which was in the rear part of the oral cavity, but through the front part of the oral cavity. As a consequence, the measurements of sodium release obtained for different breads have to be individually compared for each panelist. Of the six panelists, subject A, showing a high salivary flow rate, and subject B, showing a low salivary flow rate, were chosen for further discontinuous mastication experiments to take interindividual differences in saliva secretion into consideration.

For discontinuous mastication experiments, mastication was simulated by means of a modified Potter S homogenizer as reported recently[25] using collected human saliva of panelists A and B as extracting agent. As shown in Figure 2, only 3.7 ± 0.5 and 4.0 ± 0.3 mg Na^+/g crumb (dry weight) were extracted for panelists A and B, respectively, after a chewing time of 5 s in the mouth. These values corresponded to 55 ± 7% (panelist A) and 60 ± 4% (panelist B) of the total amount of NaCl. This amount remained more or less the same after 10 s (panelist A, p = 0.751; panelist B, p = 0.882), but rose significantly to 5.1 ± 0.5 mg Na^+/g crumb (dry weight) for panelist A (p = 0.010) and to 5.4 ± 0.3 mg Na^+/g crumb (dry weight) for panelist B (p = 0.006) after 15 s. After 30 s, extraction rates were already >91% with 6.3 ± 0.1 and 6.1 ± 0.5 mg Na^+/g crumb (dry weight) for panelists A and B, respectively. The values tended to be higher for both panelists after a mastication time of 60 s, but the respective differences compared to 30 s were not significant anymore (panelist A, p = 0.524; panelist B, p = 0.558). Therefore, almost the entire amount of sodium was extracted in-mouth after a chewing time of 30 s. Using the mastication simulator, the extracted amounts were 4.3 ± 0.3

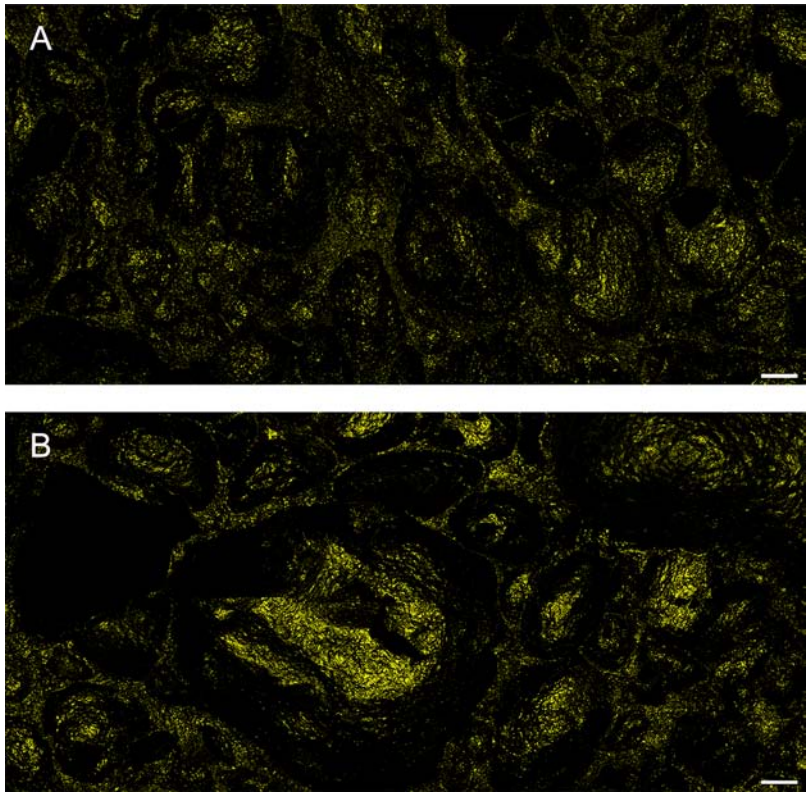

Figure 4. CLSM micrographs of bread crumb containing 1.5% NaCl: (A) homogeneous sodium distribution (1.5% NaCl); (B) inhomogeneous sodium distribution (0.25/1.25% cg NaCl).The figure consists of six individual, reassembled micrographs showing a representative area in each of the breads. The white scale bar is equivalent to 200 μm.

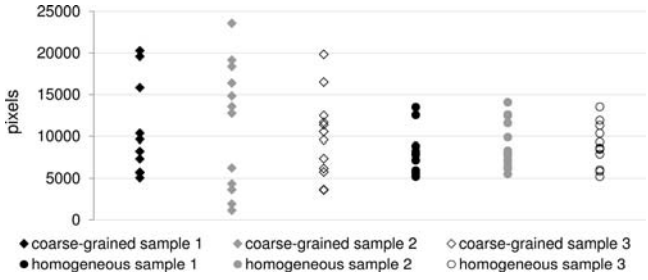

Figure 5. Frequency of pixels (sum of RGB values 136−255) per micrograph in samples of breads with a homogeneous sodium distribution (1.5% NaCl) compared to breads with coarse-grained NaCl (inhomogeneous sodium distribution of 0.25/1.25% cg NaCl). Three different samples were taken for each of the homogeneous and the inhomogeneous breads. One data point represents 1 of 12 micrographs per sample.

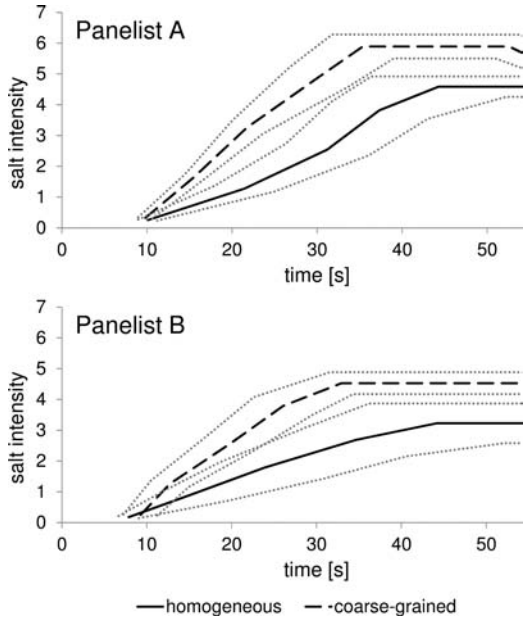

Figure 6. Time−intensity curves of the bread with coarse-grained (cg) NaCl (inhomogeneous distribution of 0.25/1.25% cg NaCl) compared to the homogeneous reference bread (1.5% NaCl). All determinations were done six times by two panelists (A and B). Small dotted, gray lines represent the respective limits of the confidence interval of 95%.

and 4.9 ± 0.1 mg Na^+/g crumb (dry weight) for panelists A and B, respectively, after 5 s corresponding to 63 ± 3 and 73 ± 1% of total sodium, respectively. No significant differences were observed between 5 and 10 s of simulated mastication, but there was a significant increase in sodium extraction rates after 15 s to 6.3 ± 0.4 mg Na^+/g crumb (dw) for saliva of panelist A ($p < 0.001$) and to 6.4 ± 0.2 mg Na^+/g crumb (dw) for saliva of panelist B ($p < 0.001$), which are equivalent to >94% of total sodium. When simulated to in-mouth mastications were compared for each chewing time, the sodium amounts after 5 s were significantly higher in the simulator than those found after extraction in the mouth for panelist B ($p = 0.020$), but not for panelist A ($p = 0.075$). Looking at the chewing time of 10 s, higher sodium amounts were extracted in the simulator only for panelist A ($p = 0.049$). However, there was a significant difference after 15 s for both panelists (A, $p = 0.003$; B, $p = 0.002$), because almost the entire amount of sodium had already been extracted in the mastication simulator, whereas

Table 2. Secondary Parameters Extracted from Time–Intensity Curves of the Bread with Coarse-Grained (cg) NaCl (Inhomogeneous Distribution of 0.25/1.25% cg NaCl) Compared to the Homogeneous Reference Bread (1.5% NaCl)[a]

parameter[b]	homogeneous	coarse-grained
I_{max}	4.3 ± 0.9	5.8 ± 0.9*
t_{max} (s)	52.0 ± 8.0	41.0 ± 5.0*
D_i (s)	35.2 ± 7.7	24.6 ± 2.9*
R_i	0.11 ± 0.03	0.20 ± 0.04*
A_i	65.2 ± 21.8	66.3 ± 12.1
D_m (s)	15.8 ± 7.9	22.1 ± 5.9
A_m	56.5 ± 30.4	106.5 ± 22.6*
A	128.6 ± 34.7	196.2 ± 24.8*

[a]Mean values ± standard deviation (two panelists, $n = 6$ each). An asterisk (*) indicates statistically significant differences between breads (two-way ANOVA, Tukey test, $p \leq 0.05$). [b]I_{max}, maximum intensity; t_{max}, time until maximum intensity is reached; 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.

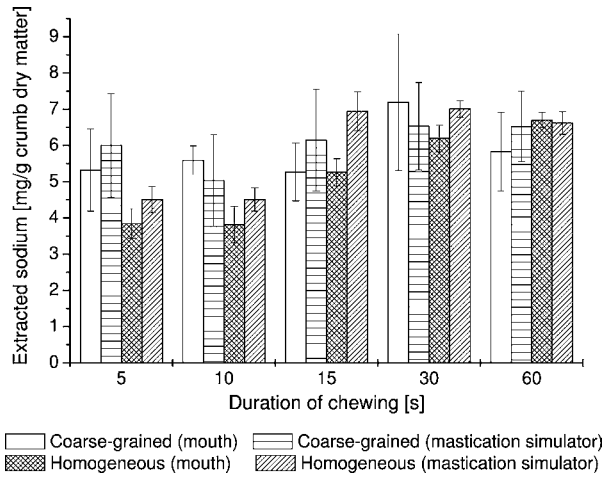

Figure 7. Comparison of the time-resolved sodium release from the reference bread (homogeneous distribution of 1.5% NaCl) and from bread baked with coarse-grained NaCl (inhomogeneous distribution of 0.25/1.25% cg NaCl). Discontinuous sampling techniques included sodium release in-mouth and the mastication simulator. Error bars represent standard deviations of triplicate determinations averaged over two panelists (A and B).

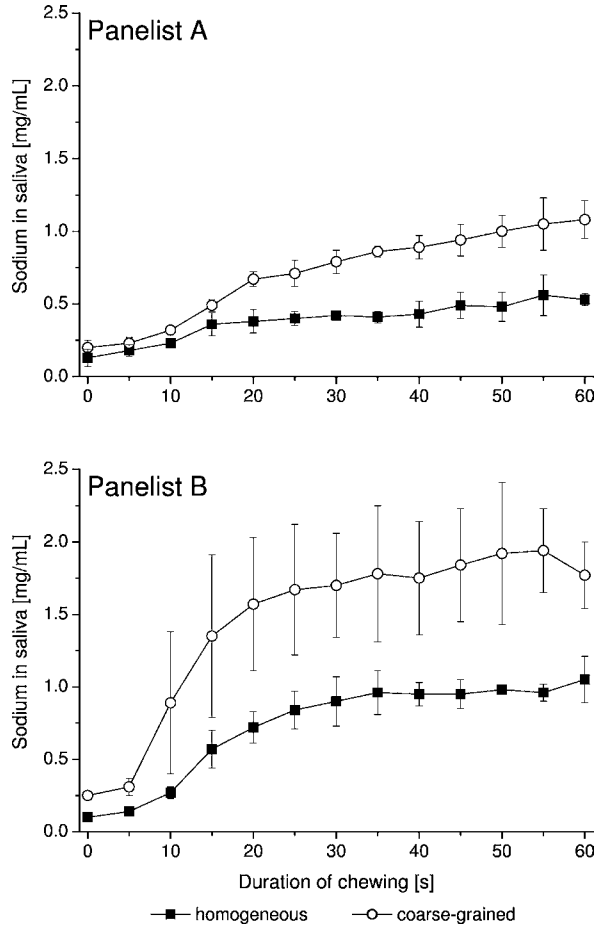

Figure 8. Comparison of the time-resolved sodium release from the reference bread (homogeneous distribution of 1.5% NaCl) and from bread baked with coarse-grained (cg) NaCl (inhomogeneous distribution of 0.25/1.25% cg NaCl). A continuous sampling technique was used. Error bars represent standard deviations of triplicate determinations (panelists A and B).

only about 80% had been released after chewing in the mouth. The amounts of extracted sodium obtained with the two panelists showed a very good agreement for both methods ($p \geq 0.26$ for mastication in-mouth; $p \geq 0.06$ for the mastication simulator). Therefore, the values obtained with the two panelists in the discontinuous sampling methods could be averaged when the release of sodium from different breads was compared. In conclusion, these results showed that sodium release from bread crumb is dependent on the time and extent of homogenization, so that a complete extraction of sodium was only achieved after 30 s (in-mouth) and 15 s (mastication simulator).

Comparison of Sampling Techniques. Whereas the discontinuous sampling techniques allow the determination of the amount of extracted sodium after a given time, the continuous sampling technique is suitable for measuring the actual salivary sodium concentration in the mouth during chewing. After multiplication of this salivary sodium concentration by the saliva secretion of the panelists, absolute amounts of sodium can also be obtained by the continuous sampling technique. Nevertheless, the sodium release obtained by this method was slower than the sodium release measured by the discontinuous sampling techniques. For example, after a chewing time of 5 s, only marginal amounts of extracted sodium were obtained by the continuous sampling technique (Figure 1C), but >50% of the total amount of sodium was extracted according to the discontinuous sampling technique in-mouth (Figure 2). This can be explained by the fact that the sampling point (oral area of the satin ribbon, front part of the oral cavity) did not correspond to the chewing point (chewing pulp in the rear part of the oral cavity). Given that a certain amount of time is necessary for a sufficient distribution of sodium during chewing, the sodium concentration gradient is less steep compared to the actual sodium concentration gradient in the chewing pulp. Furthermore, the amounts of extracted sodium were lower for continuous than for discontinuous sampling. The continuous sampling technique revealed that about 3.2 mg Na^+/3 g crumb (wet weight) (1.1 mg Na^+/g crumb) was extracted after 60 s (Figure 1C), whereas about 6.7 mg Na^+/g crumb (dry weight) was extracted

according to the discontinuous sampling techniques (Figure 2). This corresponds to 100% of the sodium content in 3 g of bread crumb with 1.5% NaCl (3.7 mg Na^+/g crumb, wet weight). This effect can also be explained by the spatial divergence of chewing point and sampling point. Additionally, a permanent dilution caused by saliva secretion in the front part of the oral cavity by the sublingual salivary glands may lead to lower sodium concentrations in the sampling area. Nevertheless, this method could describe the sodium concentration gradient in the front part of the mouth. On the other hand, the values obtained by the discontinuous sampling techniques may have been higher due to a putative additional sodium extraction during centrifugation. Both discontinuous methods were used for verification purposes and vice versa. Measuring sodium release after chewing in the mouth was the most realistic method, but it showed more variability of results due to interindividual differences in chewing behavior. The mastication simulator provided results in a more reproducible way, but revealed a faster sodium release after 15 s due to a more efficient homogenization. Due to the respective limitations of each method, all three methods were used to compare different breads, and the actual sodium release may be limited by the discontinuous and the continuous sampling techniques.

All sampling techniques revealed that a certain time of chewing is necessary for a maximal sodium extraction from bread crumb. At the same salt level (7.6 mg/mL), this slower release of sodium from bread crumb may explain why a salt solution was judged as significantly saltier in the 2-AFC test. At the lower salt level (3.8 mg/mL), the actual sodium concentration in the mouth was higher after 30 s during the chewing of bread crumb. Therefore, other factors such as texture-induced tactile gustatory interactions[26,27,39] may play an important role in inhibiting salty taste in bread crumb. As salt perception depends on the contrast of sodium concentration and as the increase in sodium concentration during the first seconds is crucial for the perceived salt intensity,[19] an accelerated sodium release from bread crumb during chewing may be a possibility to enhance the saltiness of bread crumb.

Correlation between Saliva Secretion, Salivary Sodium Concentration, and Salt Recognition Threshold in Bread Crumb. As the sodium concentration of a NaCl solution directly correlates with the perceived salt intensity in a certain range of concentrations,[14] it may be assumed that the salt recognition threshold for NaCl in bread crumb is lower for panelists with a lower saliva secretion due to the resulting higher sodium concentration than for panelists with a higher saliva secretion. Saliva secretion, unstimulated salivary sodium concentration, and the salt recognition threshold for NaCl in bread crumb were determined for the same six panelists who had participated in the tests using the continuous sampling technique to study the release of sodium from bread crumb. As can be seen in Table 1, the threshold values for all six panelists did not differ much from each other. Significant differences (p = 0.047) were observed only for panelists A and D, B and D, and D and F. No correlations could be derived either between the salivary sodium concentrations and the salt recognition thresholds (r = 0.212, p = 0.686) or between saliva secretion and the salt recognition thresholds (r = 0.450, p = 0.371). As a consequence, the individual salt recognition thresholds could be explained by further factors, for example, by the sensory experience of the panelists or the individual daily salt consumption.[40]

Properties of Bread with an Inhomogeneous Spatial Distribution of Salt. A base dough containing only a small quantity of sodium chloride was prepared, and an appropriate portion of cg NaCl was added 30 s prior to the end of dough mixing time, so that the total amount of NaCl was always 1.5%. The late addition of coarse-grained salt with a particle size of 2−3.5 mm during mixing led to an inhomogeneous spatial distribution of salt in bread crumb with spots of a high sodium concentration in contrast to the surrounding crumb. These salty spots were barely visible to an untrained eye and were not remarked by the sensory panel. After thorough visual inspection small, soggy, and salty spots could be detected, which are believed to be caused by water migration, as has been noted before.[23]

The volume of the inhomogeneous bread was significantly increased (1395 ± 43 mL, p = 0.008) compared to the homogeneous 1.5% NaCl reference bread (1147 ± 61 mL) and was similar to the volume of homogeneous bread with 0.25% NaCl (1341 ± 47 mL, p = 0.439). This can be attributed to the fact that the coarse-grained salt crystals dissolved in the dough only to a small extent and thus did not inhibit yeast activity, leading to a higher fermentation rate and thus to a larger bread volume.[41]

Texture analyses 2 h after baking revealed that the crumb firmness of the inhomogeneous bread (1.19 ± 0.20 N/g) was comparable to that of the homogeneous 0.25% NaCl bread (1.33 ± 0.11 N/g, p = 0.09) and lower than that of the homogeneous 1.5% NaCl bread (1.74 ± 0.16 N/g, p = 0.29). The additionally modified structural characteristics of the inhomogeneous bread as well as the diffusion behavior of salt and water were not the focus of the present work, but may be the subject of further studies on technological aspects.

To visualize the sodium distribution in the inhomogeneous bread crumb, coarse-grained, Patent Blue V colored NaCl crystals (1.25%, particle size of 2−3.5 mm) were added to bread dough containing 0.25% uncolored NaCl 30 s before the end of the dough mixing time, so that the resulting bread crumb was colored blue at the spots where a colored crystal had been incorporated. As can be seen in Figure 3A, the coarse-grained crystals were partially dissolved during the last 30 s of dough mixing, but the major part of the crystals remained intact and was integrated as salty spots in the surrounding crumb. The reference bread was prepared by adding the fine-grained, stained NaCl crystals (1.5%, particle size of <2 mm) at the beginning of the dough mixing time, so that the overall contents of NaCl (1.5%) and colorant (1.3 mg Patent Blue V/100 g wheat flour) were the same in both breads. As displayed in Figure 3B, the blue color was evenly distributed across the entire bread crumb, pointing to a completely homogeneous sodium distribution. Patent Blue V was incorporated into the crystalline structure of sodium chloride so that the blue color is an indicator for the distribution of sodium in bread crumb. This was also verified by two unbiased panelists, who tasted the blue regions and reported that they tasted intensely salty compared to the uncolored areas, which tasted rather bland. However, there are most likely differences in diffusion characteristics between sodium and Patent Blue V due to their completely different chemical properties and structures, so that the blue color can only be regarded as an indirect indicator of sodium distribution. Therefore, an advanced staining method using CLSM with a fluorescent sodium-selective dye was established to directly visualize the distribution of sodium in bread crumb in the following.

To enable a more direct visualization of sodium distribution by means of CLSM, the sodium-selective, fluorescent dye SBFI-AM[38] was used for CLSM. Figure 4A shows a representative crumb area in homogeneous bread with 1.5% NaCl. The fluorescence intensity was evenly distributed across the entire sponge-like structure in bread crumb. Because the fluorescence of SBFI-AM could be seen everywhere within the structure of homogeneous bread, there was no evidence of an enrichment of sodium in particular chemical structures, for example, in proteins. This supports the previous observation using Patent Blue V that NaCl was completely and homogeneously dissolved in bread crumb. In contrast, a cumulated region of intense fluorescence emission was visible in the middle of the inhomogeneous 0.25/1.25% cg NaCl bread crumb sample (Figure 4B), whereas the surrounding area appeared much darker. The crumb around these salty spots also contained 0.25% NaCl, which is why there was still slightly more fluorescence emission than in the bread sample without addition of NaCl (not shown). Therefore, the existence of sodium-rich spots, which was made visible indirectly with the help of Patent Blue V, could be confirmed by directly and selectively staining sodium.

To substantiate this visual observation with objective data, 36 (3×12) micrographs each were analyzed for the homogeneous 0 and 1.5% NaCl breads and the inhomogeneous 0.25/1.25% cg NaCl bread. Figure 5 shows the distribution of SBFI-AM with sodium after background correction within one sample comprising 12 separate coherent micrographs. One data point is equivalent to the sum of pixels with bright RGB values from 136 to 255 in 1 of 12 coherent micrographs per sample, so that there are 12 data points per sample and 3 samples each for the homogeneous and inhomogeneous breads.

In the homogeneous 1.5% NaCl bread, the smallest sum of bright pixels per micrograph was 5196 (micrograph 8 in homogeneous sample 1) and the highest was 14070 (micrograph 9 in homogeneous sample 2). All other values lay in the comparatively narrow margin in between, so that the brightness was quite homogeneously distributed across the 12 micrographs per sample. Because the brightness is equivalent to the fluorescence of SBFI-AM with sodium, it can be concluded that the distribution of sodium across the sample was even.

However, in the inhomogeneous bread with coarse-grained NaCl, the smallest sum of bright pixels per micrograph was only 1146 (micrograph 1 in coarse-grained sample 2) and the highest was 23578 (micrograph 11 in coarse-grained sample 2). This distribution of brightness across the 12 micrographs revealed that there were bright micrographs with intense fluorescence as compared to other rather dark micrographs with low fluorescence. The difference of over 22400 pixels between the smallest and the highest sum was much larger than in the homogeneous samples, where the difference was only 8874 pixels. Contrary to the homogeneous samples, the data points within one coarse-grained sample were also scattered over this much broader range. The same was true for all three different bread samples with coarse-grained NaCl, so that the brightness was unevenly distributed across the samples. This shows that there were areas with low concentrations of sodium as well as areas rich in sodium (salty spots) within one coarse-grained bread sample. In conclusion, the distribution of sodium in bread crumb could be visualized directly by CLSM with the help of the sodium-selective dye SBFI-AM. Furthermore, it could be confirmed that the late addition of coarse-grained NaCl to dough led to an inhomogeneous spatial distribution of sodium in bread crumb. The salt crystals dissolved into the dough only to a small extent, so that a large part of the sodium ions remained in accumulated sodium-rich regions.

Influence of an Inhomogeneous Spatial Distribution of Salt on Saltiness Perception and Sodium Release. By means of 2-AFC tests, the saltiness of the four different inhomogeneous breads was always compared to the reference bread with a homogeneous distribution of NaCl at the same overall salt level of 1.5%. The 0.5/1.0% cg bread could not be distinguished from the reference ($p = 0.2$), presumably because the sensory contrast between the salty spots and the surrounding crumb was still too weak. All other breads with an inhomogeneous salt distribution were judged as being significantly saltier ($p = 0.01$ for 0.25/1.25% cg and $p = 0.05$ for 0.1/1.4 and 0/1.5% cg) than the reference by the panel. The combination of a base dough containing 0.25% NaCl with the late addition of 1.25% coarse-grained NaCl proved to be most suitable in terms of dough handling, so that this formulation was used for all following experiments. To evaluate the potential for salt reduction by inhomogeneous spatial distribution of NaCl, the inhomogeneous 0.25/1.25% cg bread with a total NaCl content of 1.5% was compared to bread with homogeneously distributed 1.9% NaCl in a 2-AFC test. Although the homogeneous bread contained 0.4% more salt, the two breads could not be distinguished by the panel in terms of saltiness ($p = 0.2$). In a corresponding 2-AFC test, inhomogeneous 0.17/0.83% cg bread with a total NaCl content of 1% could not be distinguished from homogeneous bread with 1.35% NaCl ($p = 0.2$). As has been shown before,[25] this difference of 0.35% NaCl would have been readily detected by the panel. Therefore, the late addition of coarse-grained sodium chloride allowed a NaCl reduction of up to 25% without using additives and without negative effects on the sensory quality of bread.

Because the perception of taste in food is a dynamic process due to mastication, breathing, salivation, tongue movements, and food breakdown, the perceived intensity of a certain taste changes over time.[42] TI curves offer a more detailed insight into the perceived maximum intensity (I_{max}), the time until I_{max} is reached, the rate and shape of the increasing or decreasing phase, and the duration of a possible plateau.[42] Inspired by the continuous sampling technique to quantitate sodium release during chewing of bread crumb in the mouth, TI measurements were made in which the salt intensity of bread crumb during mastication was scaled over time. The sensory experiment was set up to come as close as possible to the instrumental measurements of sodium release during mastication. The longest chewing time for the discontinuous sampling techniques was 60 s. To ensure comparability, the TI measurements were also done over this chosen time period. Because bread is simply swallowed after a sufficient mastication time, causing an abrupt decrease in salt intensity, the TI measurement was simply stopped after 60 s by swallowing and moving the cursor back to the left end of the scale. The average trapezoidal curves obtained from both panelists (panelists A and B, $n = 6$, respectively) comparing the saltiness of the homogeneous 1.5% NaCl reference to the inhomogeneous 0.25/1.25% cg NaCl bread are shown in Figure 6. For both panelists the overall perceived I_{max} was significantly higher for the bread with coarse-grained NaCl compared to the I_{max} of the reference ($p < 0.001$). Note that the plateau of the trapezoids depicted in the diagrams represents 90% I_{max}. Two-way ANOVA with breads and panelists as factors revealed no

subject effects ($F \leq 3.392$; $p \geq 0.08$) for the chosen parameters, so that the secondary parameters were averaged over both panelists. The slope was steeper, that is, the rate of increase (R_i) was significantly higher ($p < 0.001$), and thus the time (D_i) until I_{max} is reached was significantly shorter ($p < 0.001$) for the coarse-grained sample (Table 2). No significant differences ($p = 0.883$) could be seen between the areas under the curve in the increasing phase (A_i) due to the concurrence of a higher I_{max} but a shorter duration. However, significant differences were found for the area under the plateau (A_m), which was significantly larger ($p < 0.001$) in the coarse-grained sample. The same holds true for the overall area under the curve (A, $p < 0.001$) which can be attributed to the faster increase and most notably to the higher perceived maximum of salt intensity. Interestingly, the salt intensity began to diminish after 53 s for panelist A for the coarse-grained sample, which may be due to adaptation or a gradual dilution of the sodium concentration in the mouth with saliva. This effect was not observed in the reference, so that the reduction of salt intensity due to salivary dilution was more notable in the sample with an overall higher perceived intensity. Panelist B did not experience this decline, which can be explained by the lower rate of saliva secretion (Figure 1B) leading to a delayed dilution. It should also be noted that, despite training, each panelist is known to make an individual signature curve[36,42,43] with a different usage of the scale. This is one reason the recorded maximum intensities differ for the same bread sample. Other reasons include individual differences due to mastication, saliva secretion, tongue movements,[42,43] and eating habits, in this case especially the preference for high- or low-salt foods. With all of these data taken into account, TI measurements supported the sensory analyses by 2-AFC tests, where the coarse-grained bread was rated as being significantly saltier.

To find out if the enhanced salt intensity of bread crumb with an inhomogeneous salt distribution was due to only sensory contrast or also due to a faster sodium release during chewing, the time-resolved sodium release from the inhomogeneous bread was determined and compared to that of the homogeneous bread. Analogously to the sodium release studies described above, the velocity of sodium release was determined by two discontinuous sampling techniques as well as by a continuous sampling technique. For the discontinuous sampling techniques, the inhomogeneous 0.25/1.25% cg bread and the homogeneous reference bread were chewed for 5, 10, 15, 30, and 60 s, respectively, in the mouth and in the mastication simulator. The measured values of both panelists were in good accordance and, thus, the results of the discontinuous sampling methods were averaged. According to expectations, the standard deviations were rather high for the inhomogeneous bread due to the uneven distribution of NaCl, which led to varying absolute sodium contents in the amount of bread crumb (3 g) applied in the tests (Figure 7). Nevertheless, significantly more sodium was extracted from the inhomogeneous bread than from the reference bread after chewing times of up to 10 s (mouth, 5 s, $p = 0.002$; 10 s, $p = 0.002$) and 5 s (mastication simulator, $p = 0.029$), respectively. After 5 s, 5.3 ± 1.1 mg Na^+/g crumb (dry weight) were extracted in-mouth from the inhomogeneous bread (79 ± 17% of the mean sodium content), whereas only 3.8 ± 0.4 mg Na^+/g crumb (dry weight) were extracted from the reference bread in-mouth after the same chewing time (57 ± 6%). In the mastication simulator, more sodium was extracted after 5 s from both breads. Nevertheless, the difference between the extracted amounts of sodium from the inhomogeneous bread (6.0 ± 1.4 mg Na^+/g crumb, dry weight, 90 ± 21% of the mean sodium content) and the homogeneous bread (4.5 ± 0.4 mg Na^+/g crumb, dry weight, 67 ± 6%) was as large as in the mouth. The continuous sampling technique confirmed these results. For both panelists, significantly higher salivary sodium concentrations ($p \leq 0.047$) were observed for the inhomogeneous bread compared to the homogeneous bread, which indicates a significantly faster sodium release from the bread crumb with coarse-grained NaCl (Figure 8). The higher standard deviations measured for panelist B in the inhomogeneous bread were probably due to the uneven distribution of NaCl in this bread.

In summary, the kinetics of sodium release from bread crumb during mastication were studied using one continuous method in the mouth and two discontinuous sampling methods in the mouth and by means of a mastication simulator. All three methods demonstrated that sodium release was time-dependent with a complete extraction in-mouth only after 30 s. The use of coarse-grained NaCl in breadmaking led to an inhomogeneous spatial distribution of sodium in the crumb, which was visualized by CLSM. Sensory analyses revealed an enhanced salty taste in the inhomogeneous samples, allowing a sodium reduction in bread by 25% while maintaining taste quality. This enhanced saltiness could be attributed not only to sensory contrast but also to a faster sodium release. Therefore, the acceleration of sodium release during mastication may be used to reduce the salt content of foods while maintaining taste quality.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

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

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Notes

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