

# Mechanistic Model To Understand in Vivo Salt Release and Perception during the Consumption of Dairy Gels

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**ABSTRACT:** The objective of this study was to develop a model to simulate salt release during eating. Salt release kinetics during eating was measured for four model dairy products with different dynamic salty perceptions. A simple in vivo model of salt release was developed to differentiate between the contribution of the individual and of the product to salt release. The most difficult model parameter to determine or predict is the evolution of the contact area between the product and the saliva. Fitting the model to the experimental data showed that the subject's masticatory performance and fracture initiation energy of the product determined the contact area between the product and the saliva generated by mastication. Finally, the role of release dynamics on sensory time-intensity profiles is discussed.

**KEYWORDS:** Mechanistic, cheese, temporal, sensory, NaCl

## 1. INTRODUCTION

Reducing salt content in food is a major concern for public health authorities worldwide and a major challenge because it often induces a reduction in sensory quality and technological properties of food products with regard to their functionality and safety. Understanding the mechanisms involved in perception could help to formulate products that satisfy both nutritional and sensory criteria.

During food consumption, salt must be released from the product and diluted in the saliva to reach the taste receptors located on the tongue and, thus, induce salty perception. Understanding and modeling taste compound release during the mastication of "solid" foods is a challenging task due to the complexity of the phenomena that occur in the mouth: dilution by saliva, mastication, temperature modification, etc.

Few studies have attempted to measure in vivo salt release kinetics during mastication. Jack et al.,<sup>1</sup> Davidson et al.,<sup>2</sup> and Neyraud et al.<sup>3</sup> monitored conductivity during eating with electrodes placed in the interdental space between the incisors. However, conductivity probes placed in the mouth can disturb normal mastication behavior. A noninvasive technique consisted of sampling saliva from the tongue at different moments during the eating process to analyze the components present in the saliva.<sup>3–5</sup>

Most of the descriptors (initial slope, maximal concentration, etc.) extracted from these kinetics can be statistically related to subject's physiological parameters, such as saliva flow rates or masticatory performance, as well as to product composition.<sup>4,5</sup> However, these statistical approaches are global and do not offer the possibility of understanding the contribution of the product and the individual to stimuli release.

Modeling was shown to be an effective tool for understanding, quantifying, and predicting the influence of each parameter on release kinetics.

The first model of flavor release during the consumption of solid products was developed by Harrison et al.<sup>6</sup> The transport of aroma compounds across the food–saliva interface was described by the interfacial theory of mass transfer. Saliva flow, mastication, and swallowing were incorporated into the model. Mastication was modeled by selection and breakage functions to generate particle size distributions but was independent of physiological parameters.<sup>6</sup> Wright and Hills<sup>7,8</sup> calculated these functions from the individual's mastication pattern, which was acquired with electromyography and spit-out experiments. Detailed knowledge of the mastication process is important to predict particule size distribution and, consequently, the contact area between the product and the saliva and between the product and the air.<sup>6–8</sup> Food fragmentation can be modeled from empirical laws fitted to experimental data obtained from spit-out experiments<sup>9,10</sup> or by statistical models that consider mastication as a selection and breakdown process.<sup>6,7,11,12</sup> Another approach was developed by de Loubens et al.<sup>13</sup> In their study, salt release kinetics was measured after compression of dairy gels. The salt release kinetics from fragmented products was modeled. To model salt release during mastication (<40 s), they show that knowledge of the size distribution of the particles is unnecessary. The main parameter that has to be known is the evolution of the contact area between the particles and the saliva. These considerations considerably simplify the physical formulation of the model and reduce the number of variables that are often difficult to experimentally determine. The main difficulty in predicting the contact area is due to the fact that it depends a

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**Table 1. Composition and Codes of Model Dairy Products**

|                                 | 250/40 | 250/0 | 150/40 | 150/0 |
|---------------------------------|--------|-------|--------|-------|
| milk retentate powder (g/kg)    | 250    | 250   | 150    | 150   |
| anhydrous milk fat (g/100 g DM) | 40     | 0     | 40     | 0     |
| water (g/kg)                    | 573    | 740   | 740    | 850   |

priori on the subject's masticatory behavior and on the fracture mechanics and reagglomeration of food, which depends on food structure and composition.

The objective of the present study was to develop a model of salt release during the consumption of gel food products and to determine the influence of the product and physiological parameters on the evolution of the product/saliva contact area on the basis of experimental *in vivo* salt release kinetics.

First, four model dairy products with different compositions were chosen because they had already been extensively characterized in terms of rheology and texture,<sup>14</sup> as well as in terms of bolus formation<sup>15</sup> and breakdown properties.<sup>13</sup> In this study, their sensory dynamic characteristics were determined. An experimental method was used to measure salt release kinetics during eating. The panelists were characterized on a physiological basis. A simple model of salt release was then developed on the basis of the main assumption that generation of the contact area between the product and the saliva governs salt release. This assumption has already been validated by de Loubens et al.<sup>13</sup>

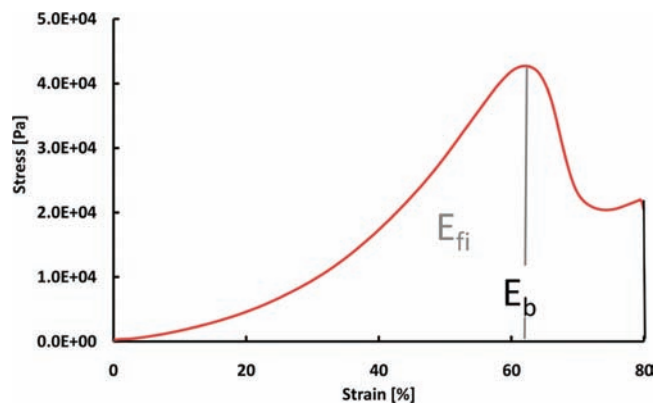
On the basis of the experimental data and the model, the specific contributions of the product and of the panelist to this parameter were studied and related to experimentally measurable parameters. Finally, the role of release dynamics on sensory time-intensity profiles is discussed.

## 2. MATERIALS AND METHODS

**2.1. Sample Preparation.** Dairy gels were prepared as described by Sain-Eve et al.:<sup>16</sup> ultrafiltrated skim milk retentate powder PL60 (Triballat, Noyal-sur-Vilaine, France), anhydrous milk fat (Corman, Goe, Belgium), and sodium chloride (Prolabo, France) were mixed and gelled by the addition of rennet. Four model cheeses (Table 1) were studied in the present work, varying only in their fat content (0 or 40%, dry basis) and their retentate powder concentration (150 or 250 g/kg). The other composition parameters remained constant: salt content (1% w/w) and pH value (6.2). Throughout the paper, products will be referred to according to the following code: PL 60 concentration (150 or 250 g/kg)/fat content (0 or 40%).

These products had already been extensively characterized in terms of mechanical properties in ref 14. Figure 1 shows a typical stress–strain curve obtained during product compression (experiments carried out in ref<sup>14</sup>). For all of the products, this curve presented a maximum that corresponds to the fracture initiation. In this study, we calculated two parameters: (i) breakdown energy  $E_b$  (in  $\text{kJ}/\text{m}^3$ ), defined as the energy necessary to break up the product at 80% of strain, and (ii) fracture initiation energy  $E_{fi}$  (in  $\text{kJ}/\text{m}^3$ ), defined as the energy necessary to initiate the fracture in the product.

**2.2. Sensory Analysis.** Sixteen volunteer panelists were recruited and trained for the evaluation of salty perception and for time-intensity (TI) methodology. The four training sessions were focused on salty perception in water and in model cheeses (identification, ranking, intensity evaluation, and temporal perception), and on software training (using Fizz software). During the product evaluation, the four products were tested in a session, and two replicates for the session were performed. The samples were presented monadically. The presentation orders of the products were defined using an orthogonal Latin square.



**Figure 1.** Typical stress–strain curve during product compression. The maximum of the curve represents fracture initiation. Breakdown energy  $E_b$  is defined as the energy necessary to break up the product at 80% of strain (i.e., total area under the curve). Fracture initiation energy  $E_{fi}$  is defined as the energy necessary to initiate the fracture in the product. These data were calculated from the experiments carried out in ref 14.

The samples were coded with three-digit random numbers, and 5 g of sample was served at 15 °C. Panelists were asked to rinse their mouth with water and to eat a piece of apple between each sample. The tests were run under white light conditions. For each evaluation, the panelists clicked on the left extremity of a horizontal unstructured scale (corresponding to no sensation) when they put the product into their mouth. Panelists were then asked to move the cursor along the scale as the sensation evolved until the end of the perception.

For each sample, average TI curves were drawn by averaging the data at each time across the 16 subjects and the two replicates. No specific averaging method was used.

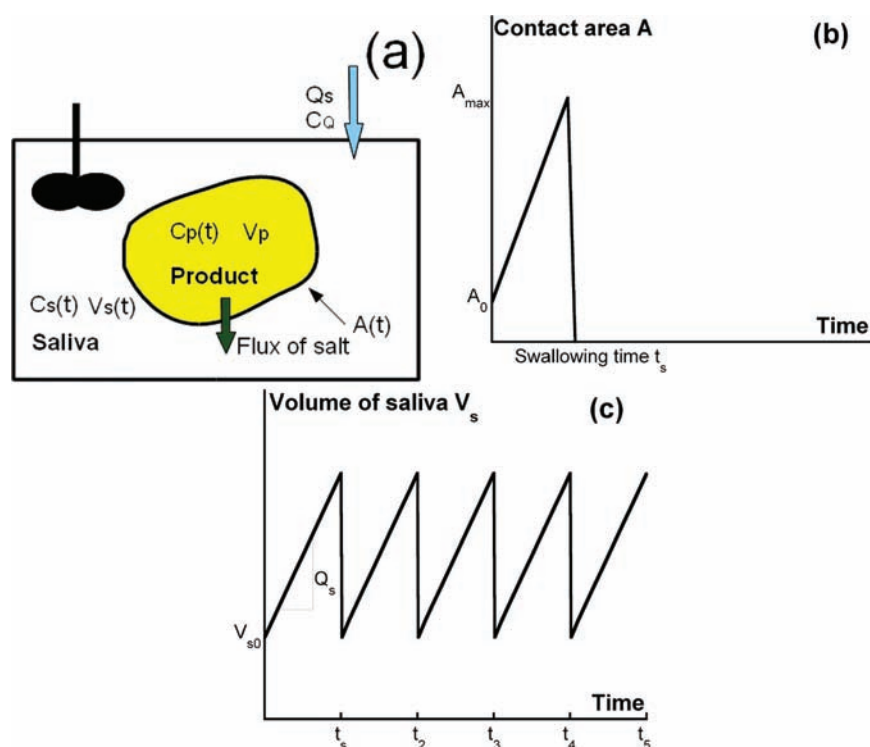
**2.3. Physiological Measurements.** **2.3.1. Masticatory Performance.** The masticatory performance (MsP) of each subject was measured. Each subject was instructed to chew standardized cylinders (weight, 3 g; height, 1.8 cm; diameter, 1.4 cm) of Optosil silicone dental (Perrigot et Cie, Dijon, France) during 20 masticatory cycles.

They were then asked to spit the sample onto a filter paper. The pieces of the chewed sample were spread on paper and dried in an oven for 1 h at 75 °C. The particles were then separated using a sieve with a 4 mm mesh. The MsP of each subject over 20 s was defined as the amount of sample that passed through the sieve over the amount of chewed sample. This procedure was repeated three times.

**2.3.2. Stimulated Saliva Flow Rate.** Stimulated saliva flow rates  $Q_s$  were measured for each panelist. Panelists chewed 0.5 g of parafilm (American National Can, Menasha, WI) without swallowing and spit out their saliva at 30 and 60 s. The saliva flow rate is obtained by dividing the mass of saliva collected by the time of the experiment.

**2.4. Experimental Measurement of Salt Release Kinetics during Eating.** **2.4.1. Saliva Sampling.** Eight panelists were recruited for this study. Before the product was consumed, the saliva was stimulated with parafilm. Saliva sampling during the consumption of 5 g of product consisted of the application of a Whatman filter paper for 1–2 s on the tongue surface. A first sampling was done at time  $t = 0$  before product introduction into the mouth. A second sampling took place just after the time of the first swallowing event  $t_s$ , and the four other samplings took place at  $t_s + 10$  s, 40, 60, and 80 s (from  $t_2$  to  $t_5$ ). Before each sampling, panelists were instructed to swallow and to clear their tongue with their teeth. Each piece of filter paper was weighed before and after sampling to determine the quantity of saliva sampled. Four replicates per product and per panelist were performed.

**2.4.2. Measurement of Salt Concentration in Saliva.** Salt concentration in saliva was measured with a conductivity probe (Heito, France)



**Figure 2.** Model assumptions: (a) principles and notations of the in-mouth salt release model during eating; (b) hypothesis of the change of the contact area between the product and the saliva during eating; (c) hypothesis of evolution of saliva volume in the mouth during eating.

that was calibrated at 20 °C with aqueous NaCl solutions prepared with deionized water. In the present work, the concentrations are given in equivalent grams per liter of NaCl.

In addition to NaCl, model cheeses contained other solutes such as potassium, calcium, phosphates, citrates, and lactates.<sup>17</sup> Moreover, saliva contains naturally different solutes<sup>3</sup> the concentrations of which differ depending on whether the saliva is stimulated or not. This is why saliva was stimulated with parafilm before product consumption and its conductivity was measured just before eating to take this value into account. In the present paper, we defined “salt” as all of the species that contribute to the conductivity signal.

The piece of filter paper used for saliva sampling was placed in 15 mL of deionized water and shaken during 15 min at 20 °C. The conductivity of the solution was measured.

Given the dilution rate, the conductivity of the saliva sample was determined. The filter paper contribution to the conductivity signal was measured beforehand and subtracted from the global signal.

**2.5. Mathematical Model.** *2.5.1. Hypothesis of the Developed Model.* *2.5.1.1. Mass Transfer Theory.* During mastication, the product is fractionated into little pieces and its contact area with saliva increases. To model flavor release from a chewed bolus, Harrison et al. and Wright and Hills<sup>6–8</sup> calculated the particle size distribution generated by mastication. Modeling the particle size distribution is of minor interest except to calculate the contact area. In fact, considering that the mastication time ( $\sim 10$  s in the experiments) is relatively short compared to the characteristic time of release, the size distribution is expected to have a low impact on the kinetics. For example, considering that salt release is due to a Fickian diffusion ( $D \sim 10^{-9}–10^{-10}$  m<sup>2</sup>/s<sup>14,18</sup>), the product thickness where the concentration significantly decreases is given by  $(4Dt)^{1/2}$  (see ref 18) and, consequently, is approximately 0.06–0.2 mm. Considering a melting surface phenomenon, the thickness is given by  $t\nu$ , where  $\nu$  is the velocity of the surface layer melting ( $\nu \sim 3 \times 10^{-5}$  m/s (see ref 8)), that is approximately 0.3 mm. This thickness range is relatively small compared to the median particle size

measured just before swallowing, which is approximately 2.8 mm for carrots and 1 mm for peanuts.<sup>19,20</sup> Therefore, the salt concentration in the bulk product has not yet the time to significantly decrease and remains homogeneous. Hence, the time of mastication is sufficiently short to not take the particle size distribution into account. Knowledge of the saliva/product contact area is sufficient to satisfactorily represent the mass transfer phenomena and to consider that the concentration in solutes inside the product is homogeneous. These considerations considerably simplify the mastication model formulation and reduce the number of variables required for the model that are often difficult to determine experimentally.

Finally, as shown in Figure 2, we can assume that the release of solutes can be described by a mass transfer coefficient  $k_p$  (m/s) and that salt concentration in the bulk product  $C_p$  can be considered uniform during the short residence time of the product in the mouth.

For short times (<40 s), this type of model has already been successfully validated on salt release kinetics from the same dairy gels reduced into small particles by standardized compression.<sup>13</sup>

*2.5.1.2. Evolution of the Contact Area between Product and Saliva and of the Volume of Saliva.* Bolus volume increases with the saliva flow rate. At each swallowing event, part of the product and of the saliva transit to the pharynx. Hence, product and saliva volumes and contact area instantly decrease. Between two swallows, saliva volume increases linearly with the saliva flow rate. The variations of contact area  $A(t)$  and of saliva volume  $V_s(t)$  are shown in Figure 2b,c. The initial area  $A_0$  is given by the initial geometry of the sample. We consider that before the first swallowing event, the evolution of the contact area between product and saliva is linear. First, the products being fragmented during the course of the mastication, its contact area with saliva increases. Second, assuming a linear increase is the simplest assumption that does not overparametrize the model according to the experimental data. It is assumed that the contact area at the swallowing time  $A_{max}$  depends on the product and on the panelist. Formally, this can be written as the product of two functions:

$$A_{max} = F(\text{product}) \times G(\text{panelist}) \quad (1)$$



**Table 2. Model Parameters: Mean Values and Standard Deviation (SD)**

| model parameter                              | unit                 | symbol   | mean $\pm$ SD   | ref   |
|--|----------------------|----------|-----------------|---|
| saliva flow rate                             | mL/min               | $Q_s$    | $2.2 \pm 0.9$   | $1.7 \pm 0.6^3$<br>$1.2 \pm 0.82^1$<br>$1.5 \pm 0.52^2$ |
| volume of saliva                             | mL                   | $V_{s0}$ | $1.1 \pm 0.6$   | $0.7 \pm 0.3^{23}$                                      |
| salt concentration<br>in stimulated saliva   | g/L equiv<br>NaCl    | $C_Q$    | $1.3 \pm 0.6$   | $2.5 \pm 1.2^3$   |
| salt partition coefficient<br>product/saliva |                      | $K$      | $0.85 \pm 0.15$ | $0.75 \pm 0.15^{27}$                                    |
| mass transfer coefficient                    | $\times 10^{-6}$ m/s | $k_p$    | $2.2 \pm 0.3$   |   |

$F$  and  $G$  are two functions that depend on the product and on the panelist, respectively.

We observed experimentally that very few products remained in the mouth after the first swallowing event  $t_s$ . Thus, the contact area and the product volume in the mouth are considered to be null.

**2.5.2. Mathematical Model Formulation.** The evolution of salt concentration in saliva  $C_s$  over time  $t$  is given by a mass balance between product and saliva and takes the dilution by the saliva flow rate  $Q_s$  into account (Figure 2a). The salt concentration in the inlet saliva is referred to as  $C_Q$

$$V_s(t) \frac{dC_s}{dt} = k_p A(t) (KC_p(t) - C_s(t)) + Q_s (C_Q(t) - C_s(t)) \quad (2)$$

$K = C_s^{eq} / C_p^{eq}$  is the saliva/product partition coefficient and  $k_p$  the mass transfer coefficient (m/s). The remaining salt concentration in the product is

$$V_p \frac{dC_p}{dt} = -k_p A(t) (KC_p(t) - C_s(t)) \quad (3)$$

where  $V_p$  is the volume of product ( $m^3$ ). As previously explained, the contact area is given by (Figure 2)

$$A(t) = \begin{cases} A_0 + (A_{\max} - A_0) \frac{t}{t_s} & \text{if } t < t_s \\ 0 & \text{else} \end{cases} \quad (4)$$

where  $A_{\max}$  is defined by eq 1.

Considering that saliva volume  $V_s$  decreases to its initial value at each swallowing (Figure 2), we have

$$V_s(t) = \begin{cases} V_{s0} + Q_s t & \text{if } t < t_s \\ V_{s0} + Q_s (t - t_s) & \text{if } t_s < t < t_i \\ \dots & \dots \end{cases} \quad (5)$$

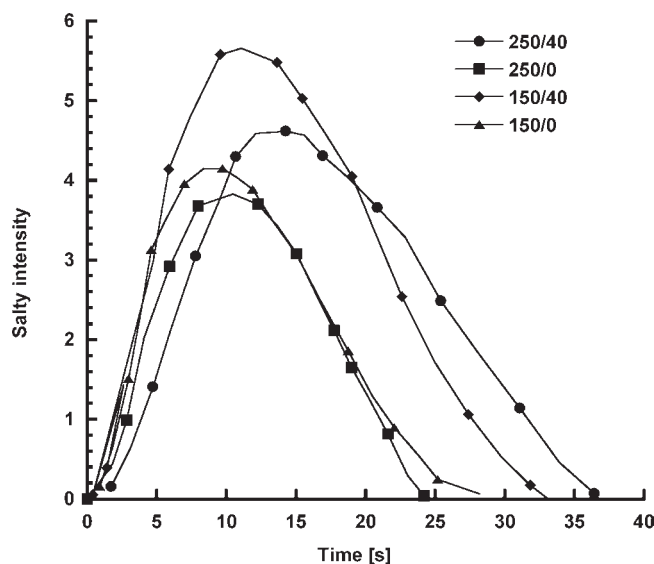
where  $t_i$  ( $i \in \overline{2..5}$ ) are the times of the different swallowing events (Figure 2). The initial conditions are

$$C_s(t = 0) = C_Q \quad (6)$$

$$C_p(t = 0) = C_{p0} \quad (7)$$

**2.5.3. Inverse Method for Determining Unknown Model Parameters.** This equation system was solved with Matlab 7 (The Mathworks, Natick, MA). Model parameters are summed in Table 2. The values of  $K$ ,  $k_p$  and  $C_{p0}$  were fixed and were those obtained by de Loubens et al.<sup>13</sup> for the same products. Partition coefficients  $K$  were between 0.7 and 1, mass transfer coefficients  $k_p$  ranged between  $2 \times 10^{-6}$  and  $3 \times 10^{-6}$  m/s, and initial salt concentration in the products  $C_{p0}$  was between 13 and 17 g/L. Saliva flow rate  $Q_s$  was measured experimentally for each panelist.

For each data set, salt concentration in inlet saliva  $C_Q$  was given by the concentration at the first point of the kinetics ( $t = 0$ ), which is the salt concentration in stimulated saliva. Three model parameters were

**Figure 3.** Mean of the time-intensity profiles for the four dairy gels.

unknown beforehand: the two functions  $F$  and  $G$  and the initial saliva volume  $V_{s0}$ , which was assumed to be dependent on the panelist alone.

In a first step, fitting the model to all experimental data from the swallowing events (from  $t = t_s$  to  $t = t_5$ ) made it possible to determine the initial volume of saliva  $V_{s0}$  for each panelist. In fact, the concentration measured at the first swallow ( $t = t_s$ ) is considered to be an initial condition; the equation system is reduced to eqs 2 and 5, where  $A = 0$  and is therefore independent of  $F$  and  $G$ .

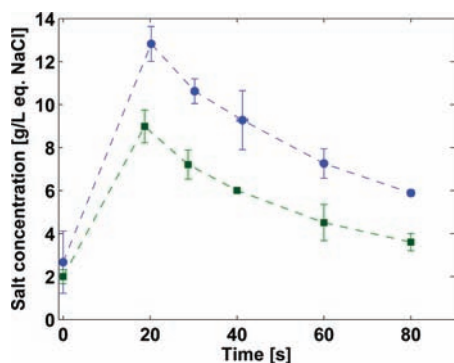
In a second step, given  $V_{s0}$ , functions  $F$ (product) and  $G$ (panelist) were determined by fitting the model to the two first experimental points (from  $t = 0$  to  $t = t_s$ ) for all data. Because  $F$  and  $G$  are defined up to a common multiplicative factor, it is necessary to fix the value of one of these functions for a panelist or a product. We initially set  $F(250/40) = 1$ .

### 3. RESULTS AND DISCUSSION

**3.1. Impact of Product on Salty Perception.** The differences in perception of the studied dairy gels were previously highlighted by a profile method<sup>14</sup> and a temporal dominance of sensations method,<sup>13</sup> which revealed, in particular, a strong effect of fat content, a weaker effect of dry matter content on salty perception, and a high product effect on the dynamics of dominance of salty and texture perception. Subsequent to this result, it seems important to study the dynamics of salty perception to identify and quantify the salt release mechanisms at the origin of perception using an experimental and modeling approach.

Figure 3 represents the time-intensity profile averaged for each product and all panelists. A one-way ANOVA with product as factor was performed to analyze which time-intensity parameters showed significant differences. The area under the curve (AUC) and the maximum intensity ( $I_{\max}$ ) were statistically influenced by the structure and composition of the products ( $p < 10^{-4}$ ). The maximum perceived intensity of the salty taste is significantly increased by fat addition for products with low dry matter. Moreover, when fat was present in the product, the maximum perceived intensity occurred later, contrary to low-fat products.

**3.2. Physiological Parameters.** The values of the physiological parameters used in the model are given Table 2. The experimental stimulated salivary flow rates  $Q_s$  were between 0.6 and 3.6 mL/min. The saliva volume  $V_{s0}$  obtained by fitting the



**Figure 4.** Examples of experimental release kinetics of salt in saliva for a panelist and two products: 250/40 (blue circles); 250/0 (green squares).

model to the experimental data was between 0.3 and 2.5 mL. The salt concentration in stimulated saliva  $C_Q$  was approximately 1.3 g/L equiv NaCl. These data were in agreement with those in the literature.<sup>3,21–23</sup>

**3.3. Product and Subject Influences on Salt Release Kinetics.** Figure 4 shows typical evolutions of salt concentration in the mouth during the consumption of model dairy products for one panelist. For each piece of data, the associated error bars show the minimum and maximum of the four replicates and are relatively small.

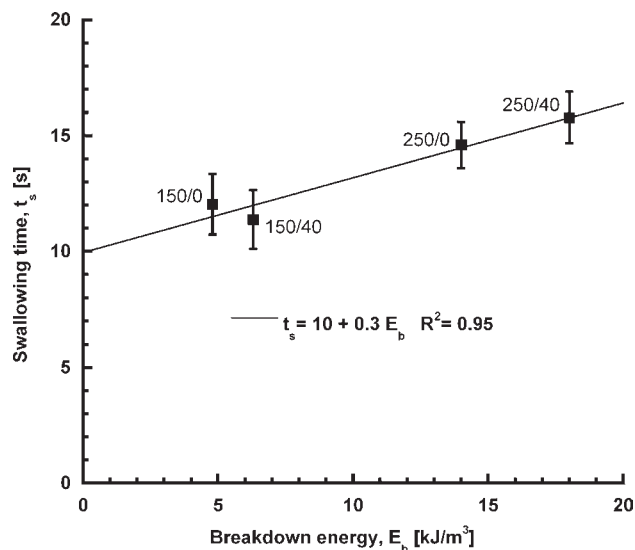
Regardless of the product or the panelist, the kinetics had similar profiles. Between the time at which the product was put in the mouth and the first swallowing event, salt concentration increased due to the salt release from product to saliva. After the first swallowing event, the concentration decreased because the saliva remaining in the mouth was diluted by the saliva flow rate from the salivary glands; moreover, the product was swallowed. The maximal concentrations of salt measured at the first swallowing event ranged from 8 to 13 g/L. This value can be compared to the initial salt concentration in the products that are between 15 and 17 g/L. Thus, at the first swallowing event, the solute distribution between the product and the saliva was relatively close to the equilibrium, implying that the maximal salt concentration could be reached at the first swallowing event.

Product and subject effects on salt release kinetics were tested statistically. Tests were performed on the following descriptors extracted from the release kinetics: (i) first swallowing time  $t_s$ , (ii) concentration at the first swallow  $C_s(t_s)$  normalized by the initial concentration of solutes in the product  $C_{p0}$  and the partition coefficient  $K$  (i.e.,  $C_s(t_s)/KC_{p0}$ ), and (iii) characteristic time of the decreasing part of the kinetics  $\tau$  (determined by fitting an exponential law on this part of the kinetics).

The first swallowing time ranged from 8 to 22 s. This parameter was influenced by product and subject, and a product–subject interaction was observed ( $p < 10^{-4}$ ). The swallowing time was correlated with the breakdown energy  $E_b$  that describes mechanical properties of the product under large deformation ( $R^2 = 0.95$ ; Figure 5).

The normalized concentration at the first swallowing time was influenced by product and subject, and a product–subject interaction was observed ( $p < 10^{-4}$ ). The product 250/40 was significantly different from 150/40 (Table 3).

The characteristic time of the decreasing part of the kinetics  $\tau$  provided information about the “salt persistence”. The longer  $\tau$  is, the longer salt remains in the mouth and the more persistent



**Figure 5.** Mean swallowing time  $t_s$  as a function of the breakdown energy  $E_b$  for each product. Breakdown energy  $E_b$  is defined as the energy necessary to break up the product at 80% of strain during a compression test (data calculated from the experiments carried out in ref 14). Error bars represent confidence intervals.

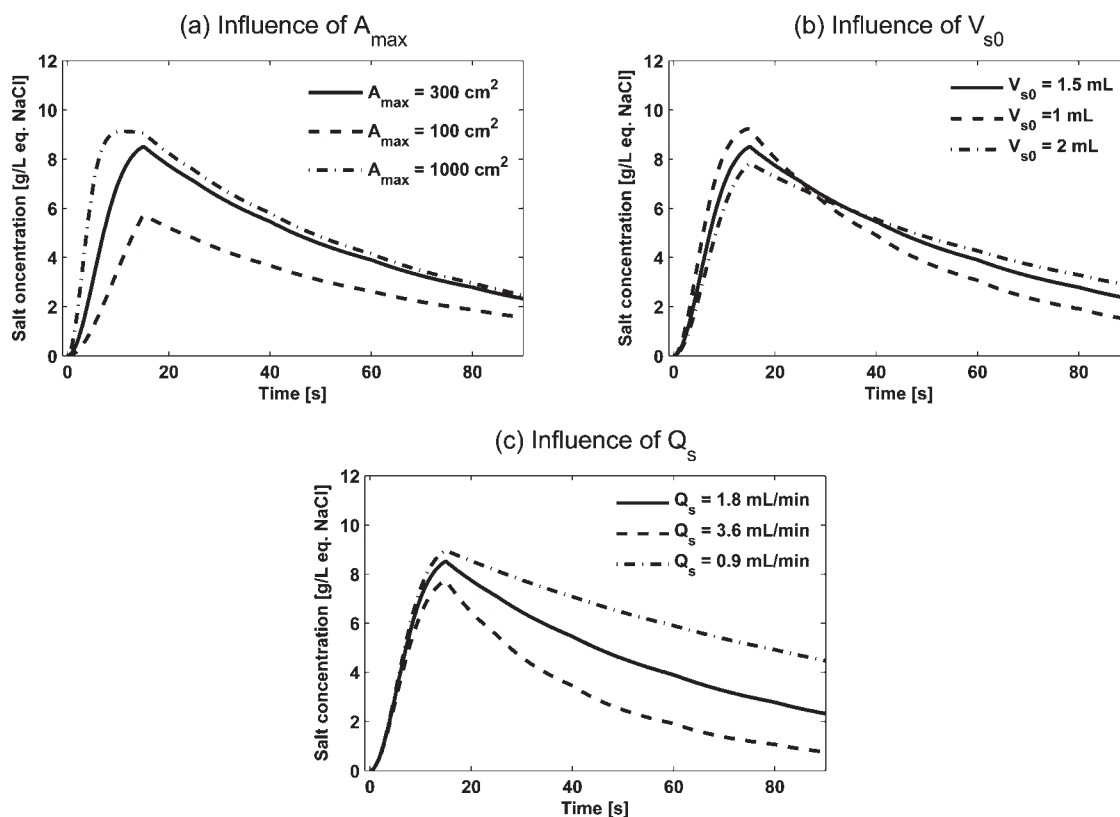
**Table 3.** Normalized Concentration  $C_s(t_s)/KC_{m0}$  at the First Swallowing and Mean Characteristic Time  $\tau$  of the Decreasing Part of Release Kinetics for the Different Products with Confidence Intervals<sup>a</sup>

|        | normalized concn<br>$C_s(t_s)/KC_{m0}$ | characteristic time of<br>decay $\tau$ (s) |
|--------|--|--|
| 250/40 | $0.77 \pm 0.04$ (a)                    | $56 \pm 14$ (a)                            |
| 250/0  | $0.71 \pm 0.04$ (a,b)                  | $45 \pm 11$ (b)                            |
| 150/40 | $0.63 \pm 0.03$ (b)                    | $43 \pm 8$ (b)                             |
| 150/0  | $0.70 \pm 0.05$ (a,b)                  | $45 \pm 12$ (b)                            |

<sup>a</sup> The products are statistically classified in two groups referred to as a and b (SNK test).

the product is. As in the case of the other parameters,  $\tau$  was influenced by the subject, the product, and the product–subject interaction. Subject influence could be mainly explained by the saliva volume and the salivary flow rate. Products were statistically classified into two groups (Table 3). The first group was composed of 250/40 only and the second one of 250/0, 150/0, and 150/40. Two hypotheses can be formulated to explain this phenomenon: either the saliva flow rate depended on the product or some little pieces of product adhered to the tongue surface and acted as a reservoir for solute release. Further studies are needed to improve our understanding of these phenomena.

In conclusion, product and subject influenced release kinetics and a product–subject interaction was observed. In the model, product influence was represented by the salt physicochemical properties in the product (mass transfer coefficient  $k_p$  and partition coefficient  $K$ ), and subject influence was represented by its physiological parameters (the initial saliva volume and the salivary flow rate). The product–subject interaction was introduced by considering that the maximal contact surface during mastication  $A_{max}$  was a function of both the product and the panelist. We assumed that the initial volume of saliva  $V_{s0}$  and the salivary flow rate  $Q_s$  were product- and time-independent.



**Figure 6.** Parameters influence on model predictions: influence of maximal contact area,  $A_{\max}$  (a); influence of initial volume of saliva in the mouth,  $V_{s0}$  (b); influence of saliva flow rate  $Q_s$  (c). The swallowing events take place at 15, 25, 40, 60, and 80 s. Unless otherwise specified, parameter values are  $A_{\max} = 100 \text{ cm}^2$ ,  $V_{s0} = 1 \text{ mL}$ ,  $Q_s = 1.8 \text{ mL/min}$ ,  $K = 0.8$ ,  $k_p = 2 \times 10^{-6} \text{ m/s}$ ,  $V_p = 5 \text{ mL}$ .

### 3.4. Modeling Salt Release during Food Consumption

**3.4.1. Model Sensitivity Analysis.** In this section, the effects of some of the parameters on model predictions are analyzed. The values used for the sensitivity analysis were chosen according to the physiological values measured in this study and the physicochemical parameters measured by de Loubens et al.<sup>13</sup> As shown in Figure 6a, the product/saliva contact area  $A_{\max}$  mainly has an influence on release kinetics before the first swallowing event. The greater the contact area is, the faster the solute release is. When the area is sufficiently large, the salt equilibrium between the saliva and the product is reached before the first swallowing event.

The initial volume of saliva  $V_{s0}$  has an influence on the salt concentration at the first swallowing event and on the decay of salt concentration after this event (Figure 6b). A small saliva volume induces a high salt concentration in saliva, but the decay of the concentration after the first swallowing event is faster. Because the characteristic time of the decay is given by the ratio  $V_{s0}/Q_s$ , a large saliva volume and a weak saliva flow rate increase the salt persistence of the product.

A high saliva flow rate  $Q_s$  reduces the salt concentration at the first swallowing event and accelerates the decay by diluting salt in saliva (Figure 6c).

**3.4.2. Relationship between Maximal Contact Area  $A_{\max}$ , Product, and Subject.** In section 2.5, the maximal product/saliva contact area  $A_{\max}$  was assumed to be the product of two functions  $F$  and  $G$  that depend only on the product and the panelist, respectively:  $A_{\max} = F(\text{product}) \times G(\text{panelist})$ . This equation allowed us to determine the respective contributions of the product and of the panelist to the dynamics of contact area generation.

After the model had been fitted to the salt release kinetics as explained in section 2.5, a good regression between the function  $G$  and the masticatory performance MsP was found ( $R^2 = 0.90$ ; Figure 7):

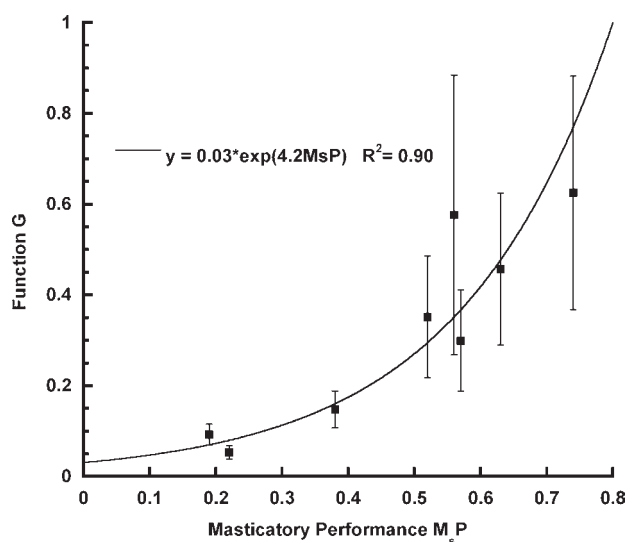
$$G = \alpha \exp(\beta \times \text{MsP}) \quad (8)$$

$\alpha$  and  $\beta$  are two constants. Finally,  $A_{\max}$  is reduced to a function of  $F$  ( $F \leftarrow \alpha F$ ) and MsP:

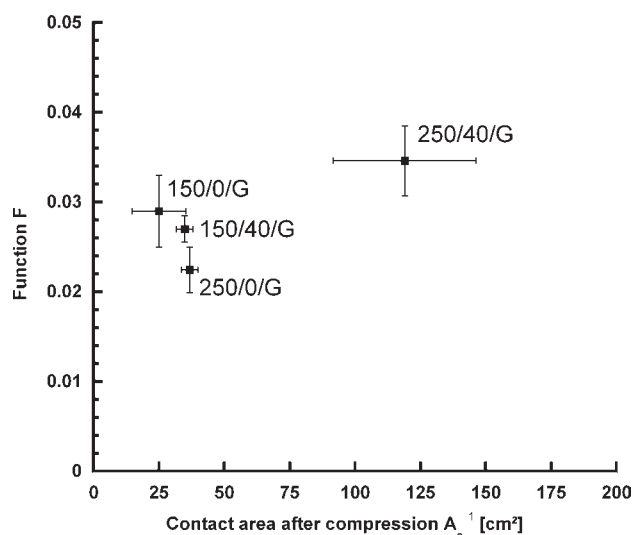
$$A_{\max} = F(\text{product}) \exp(\beta \times \text{MsP}) \quad (9)$$

Considering that  $A_{\max}$  is given by eq 2, the values of  $F$ ,  $\beta$ , and  $V_{s0}$  previously obtained were used as an initial attempt to fit the model to the complete kinetics (from  $t = 0$  to  $t = t_5$ ; Figure 2) to refine the values of  $F$ ,  $\beta$ , and  $V_{s0}$ . The determination coefficient  $R^2$  was 0.91. We determined that  $\beta = 3.9 \pm 0.2$  and  $V_{s0}$  was between 0.3 and 2.5 mL.

In Figure 8, we can observe that the values of  $F$  between each product were close compared to the interindividual variability (function  $G$ ). We compared the value of  $F$  with the area generated by a standardized compression under in vitro conditions  $A_c$ , which was determined by de Loubens et al.<sup>13</sup> (Figure 8). This parameter ( $A_c$ ) is a representation of the breakdown product properties translated in terms of contact area of the product with the surrounding aqueous phase. The product 250/40 had a value of  $F$  that was slightly higher than those of the other products. This difference was more evident after the in vitro compression. In fact, panelists adapt their mastication behavior according to the product structure (Figure 5): the firmer the product is, the longer the subject chews it before swallowing and



**Figure 7.**  $G$  as a function of the masticatory performance. Each point represents a panelist. Error bars represent confidence intervals.

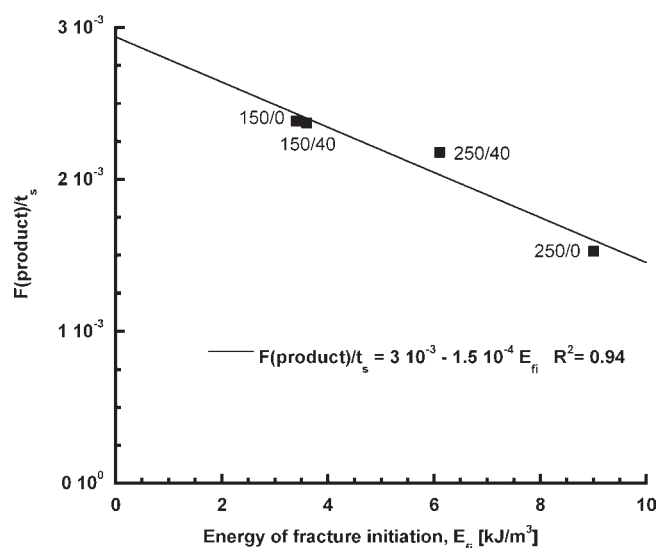


**Figure 8.**  $F$  as a function of the contact area obtained after a standardized compression  $A_c$  (data from ref 13). Error bars represent confidence intervals.

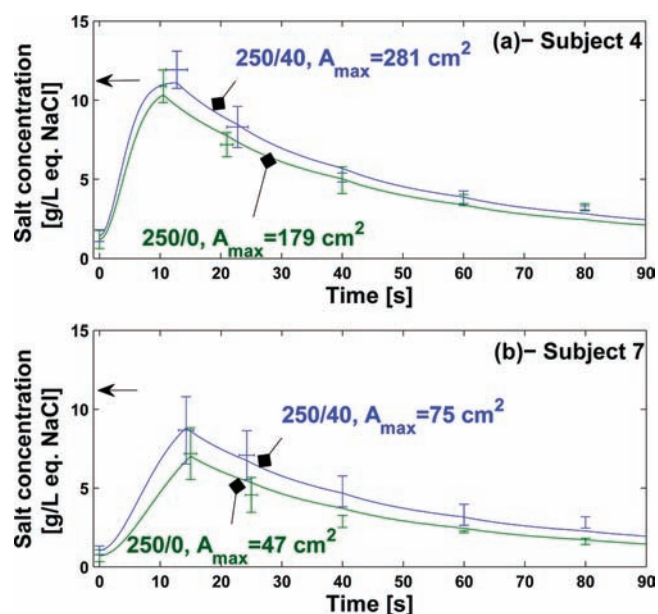
the greater the quantity of salt released is. Similar results have already been observed.<sup>24–27</sup> Depending on their texture perception, subjects adapted their chewing time to reach the same bolus state. The analysis of the ready-to-swallow bolus by image analysis revealed similar results:<sup>19</sup> mean particle width and particle size distribution do not depend on the specific food (e.g., carrot and radish) but, instead, on the food type (e.g., dried nuts and raw vegetables).

Finally,  $A_{\max}$  is clearly related to the subject's masticatory performance and is slightly dependent on the product.

However, as shown in Figure 9, the dynamics of contact area evolution (represented by the ratio  $F(\text{product})/t_s$ ) is very different between the products.  $F(\text{product})/t_s$  is very well correlated with the fracture initiation energy  $E_{fi}$  ( $R^2 = 0.94$ ).  $F(\text{product})/t_s$  represents the dynamics of evolution of the contact area between the product and the saliva independent



**Figure 9.**  $F(\text{product})/t_s$  as a function of the fracture initiation energy  $E_{fi}$ .  $F(\text{product})/t_s$  represents the dynamics of evolution of the contact area between the product and the saliva independent of the panelist effect. Fracture initiation energy  $E_{fi}$  is defined as the energy necessary to initiate the fracture in the product during a compression test (data calculated from the experiments carried out in ref 14).



**Figure 10.** Experimental data and model of solutes release for two panelists with different masticatory performances ( $M_sP = 0.52$  (a);  $M_sP = 0.17$  (b)) and two products (250/40 and 250/0). Arrows indicate the equilibrium salt concentration in saliva at the first swallowing event.

of the panelist effect. This evolution is determined by the fracture initiation energy of the product.

**3.4.3. Final Model Adjustment.** Figure 10 shows the salt release kinetics fitted to the experimental data (from  $A_{\max}$  defined with eq 9) for two panelists (subjects 4 and 7) and two products varying in fat content (250/40 and 250/0). The comparison of these two panelists is interesting because they had different physiological parameters and different behaviors. In fact, their masticatory performances ( $M_sP$ ) were very different:



0.52 (subject 4) and 0.19 (subject 7). For panelist 4, the maximal contact area  $A_{\max}$  generated with product 250/40 was sufficiently large (281 cm<sup>2</sup>) so as not to limit salt release (Figure 10): the concentrations of salt between the product and the saliva were very close to the equilibrium at the first swallowing event. In contrast, panelist 7's masticatory behavior did not make it possible to generate a sufficiently large area, regardless of the product ( $A_{\max} < 75$  cm<sup>2</sup>; Figure 10b): the saliva/product equilibrium was never reached at the first swallowing event. The panelist's masticatory performance therefore limited the generation of contact area and salt release. Compared to the interindividual variability, the differences between the products were relatively low. However, a greater maximal contact area was generated with the fat product (250/40) than with the nonfat product. These differences can be explained by a longer mastication time for fat products that is related to the breakdown energy (Figure 5) and by the different breakdown properties between the two products (Figures 8 and 9).

Finally, because salt release can be described by a mass transfer coefficient and because salt concentration in the bulk product remains homogeneous during the small residence time of the product in the mouth, the formulation of the mathematical problem is simplified and the number of parameters to be known is reduced. These hypotheses have already been successfully validated with in vitro data by de Loubens et al.<sup>13</sup> In fact, only the product volume and the saliva/product contact area need to be known. The evolution of the contact area was assumed to be linear. It could be refined and improved if more detailed experimental data were available, namely, during the first seconds of the mastication period. New experimental methodologies must be developed to acquire such data without disturbing the natural mastication behavior of the panelists.

**3.4.4. Salty Perception and Salt Release Kinetics.** No direct relationships were observed between the salt release kinetics and the salty intensity of the time-intensity profiles on the basis of data from the present study. For example, the correlation coefficient between the area under the curves of the time-intensity profile and the concentration reached at the first swallowing event is 0.2. Morris et al.<sup>28</sup> suggested that the overall amount of delivered salt affects sensory perception. In the present study, the quantity of salt delivered during mastication was calculated using the mechanistic model. The relationships between this parameter and the area under the curves of time-intensity profiles and the maximal intensity are not satisfactory ( $R^2 < 0.1$ ).

However, specific studies on sensory receptors assume that receptor response depends on the type of stimulation: the rate of taste molecules transported to the taste receptors can influence the receptor response.<sup>29–31</sup> Busch et al.<sup>32</sup> investigated this hypothesis for salt perception in humans. On the basis of salt pulse experiments, they concluded that the frequency, timing, and concentration differences of salt stimuli can affect saltiness. For aroma compounds, Baek et al.<sup>33</sup> showed no correlation between the amount of volatile release and the sensory analysis, but found a good correlation between the rates of volatile release and the sensory data. To investigate the hypothesis that perception is related to the salt release rate, we simulated the salt release rate with the model developed and the parameters determined in the present study and calculated the maximal slope of the evolution of salt concentration in saliva (i.e.,  $\max(dC_s/dt)$ ).

A better correlation between the maximal salty intensity and the maximal slope of the evolution of salt concentration

( $R^2 = 0.6$ ) was observed. This result suggests that salt perception can be partly explained by the rate of salt release. However, this relationship between release and perception needs to be investigated further. Controlled salt delivery experiments should allow us to determine the links or the “transfer function” between release and perception.

To conclude, the model developed in the present study made it possible to better understand the mechanisms of salt release and perception during food consumption with physiological parameters (salivary flow rate and masticatory efficiency) and the rheological and breakdown parameters of food. This type of model could be a good tool to formulate products that satisfy both nutritional and organoleptic criteria.

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## REFERENCES

- (1) Jack, F.; Piggott, J.; Paterson, A. Cheddar cheese texture related to salt release during chewing, measured by conductivity – preliminary study. *J. Food Sci.* **1995**, *60*, 213–217.
- (2) Davidson, J.; Linforth, R.; Taylor, A. In-mouth measurement of pH and conductivity during eating. *J. Agric. Food Chem.* **1998**, *46*, 5210–5214.
- (3) Neyraud, E.; Prinz, J.; Dransfield, E. NaCl and sugar release, salivation and taste during mastication of salted chewing gum. *Physiol. Behav.* **2003**, *79*, 731–737.
- (4) Pionnier, E.; Chabanet, C.; Mioche, L.; Taylor, A.; Le Quere, J.; Salles, C. 2. In vivo nonvolatile release during eating of a model cheese: relationships with oral parameters. *J. Agric. Food Chem.* **2004**, *52*, 565–571.
- (5) Phan, V.; Yven, C.; Lawrence, G.; Chabanet, C.; Reparet, J.; Salles, C. In vivo sodium release related to salty perception during eating model cheeses of different textures. *Int. Dairy J.* **2008**, *18*, 956–963.
- (6) Harrison, M.; Campbell, S.; Hills, B. Computer simulation of flavor release from solid foods in the mouth. *J. Agric. Food Chem.* **1998**, *46*, 2736–2743.
- (7) Wright, K.; Sprunt, J.; Smith, A.; Hills, B. Modelling flavour release from a chewed bolus in the mouth: Part I. Mastication. *Int. J. Food Sci. Technol.* **2003**, *38*, 351–360.
- (8) Wright, K.; Hills, B. Modelling flavour release from a chewed bolus in the mouth: Part II. The release kinetics. *Int. J. Food Sci. Technol.* **2003**, *38*, 361–368.
- (9) Kobayashi, N.; Kohyama, K.; Sasaki, Y.; Matsushita, M. Statistical laws for food fragmentation by human mastication. *J. Phys. Soc. Jpn.* **2006**, *75*, 083001.
- (10) Kobayashi, N.; Kohyama, K.; Shiozawa, K. Fragmentation of a viscoelastic food by human mastication. *J. Phys. Soc. Jpn.* **2010**, *79*, 044801.
- (11) van der Bilt, A.; Olthoff, L.; van der Glas, H.; van der Weelen, K.; Bosman, F. A mathematical description of the comminution of food during mastication in man. *Arch. Oral Biol.* **1987**, *32*, 579–586.
- (12) Baragar, F.; van der Bilt, A.; van der Glas, H. An analytic probability density for particle size in human mastication. *J. Theor. Biol.* **1996**, *181*, 169–178.



(13) de Loubens, C.; Panouillé, M.; Saint-Eve, E.; Déléris, I.; Trélea, I. C.; Souchon, I. Mechanistic model of in vitro salt release from model dairy gels based on standardized breakdown test simulating mastication. *J. Food Eng.* **2010**, DOI: 10.1016/j.jfoodeng.2011.02.020.

(14) Panouillé, M.; Saint-Eve, A.; de Loubens, C.; Déléris, I.; Souchon, I. Understanding of the influence of composition, structure and texture influence on salty perception in model dairy products. *Food Hydrocolloids* **2010**, doi: 10.1016/j.foodhyd.2010.08.021.

(15) Drago, S.; Panouillé, M.; Saint-Eve, A.; Neyraud, E.; Feron, G.; Souchon, I. Relationships between saliva and food bolus properties from model dairy products. *Food Hydrocolloids* **2010**, doi: 10.1016/j.foodhyd.2010.07.024.

(16) Saint-Eve, A.; Lauverjat, C.; Magnan, C.; Déléris, I.; Souchon, I. Reducing salt and fat content: impact of composition, texture and cognitive interactions on the perception of flavoured model cheeses. *Food Chem.* **2009**, *116*, 167–175.

(17) Floury, J.; Rouaud, O.; Poullennec, M. L.; Famelart, M.-H. Reducing salt level in food: Part 2. Modelling salt diffusion in model cheese systems with regards to their composition. *LWT – Food Sci. Technol.* **2009**, *42*, 1621–1628.

(18) Cussler, E. Diffusion. *Mass Transfer in Fluid Systems*, 2nd ed.; University Press: Cambridge, U.K., 1997.

(19) Mishellany, A.; Woda, A.; Labas, R.; Peyron, M.-A. The challenge of mastication: preparing a bolus suitable for deglutition. *Dysphagia* **2006**, *21*, 87–94.

(20) Woda, A.; Nicolas, E.; Mishellany-Dutour, A.; Hennequin, M.; Mazille, M. N.; Veyrune, J. L.; Peyron, M. A. The masticatory normative indicator. *J. Dental Res.* **2010**, *89*, 281–285.

(21) Pionnier, E.; Chabanet, C.; Mioche, L.; Le Quere, J.; Salles, C. I. In vivo aroma release during eating of a model cheese: relationships with oral parameters. *J. Agric. Food Chem.* **2004**, *52*, 557–564.

(22) Dawes, C. Salivary flow patterns and the health of hard and soft oral tissues. *J. Am. Dental Assoc.* **2008**, *139*, 18S–24S.

(23) Muller, K.; Figueroa, C.; Martinez, C.; Medel, M.; Obreque, E.; Pena-Neira, A.; Morales-Bozo, I.; Toledo, H.; Lopez-Solis, R. O. Measurement of saliva volume in the mouth of members of a trained sensory panel using a beetroot (*Beta vulgaris*) extract. *Food Qual. Pref.* **2010**, *21*, 569–574.

(24) Boland, A.; Delahunty, C.; van Ruth, S. Influence of the texture of gelatin gels and pectin gels on strawberry flavour release and perception. *Food Chem.* **2006**, *96*, 452–460.

(25) Gierczynski, I.; Laboure, H.; Semon, E.; Guichard, E. Impact of hardness of model fresh cheese on aroma release: in vivo and in vitro study. *J. Agric. Food Chem.* **2007**, *55*, 3066–3073.

(26) van Ruth, S.; Buhr, K. Influence of mastication rate on dynamic flavour release analysed by combined model mouth/proton transfer reaction-mass spectrometry. *Int. J. Mass Spectrom.* **2004**, *239*, 187–192.

(27) Lauverjat, C.; Déléris, I.; Trélea, I. C.; Salles, C.; Souchon, I. Salt and aroma compound release in model cheeses in relation to their mobility. *J. Agric. Food Chem.* **2009**, *57*, 9878–9887.

(28) Morris, C.; Koliandris, A.-L.; Wolf, B.; Hort, J.; Taylor, A. J. Effect of pulsed or continuous delivery of salt on sensory perception over short time intervals. *Chemosens. Perception* **2009**, *2*, 1–8.

(29) Meiselman, H. L.; Bose, H. E.; Nykvist, W. E. Effect of flow rate on taste intensity responses in humans. *Physiol. Behav.* **1972**, *9*, 35–38.

(30) Halpern, B. P.; Kelling, S. T.; Meiselman, H. L. An analysis of the role of stimulus removal in taste adaptation by means of simulated drinking. *Physiol. Behav.* **1986**, *36*, 925–928.

(31) Alberts, B.; Lewis, J.; Raff, M.; Roberts, K.; Watson, J. D. *Cell signalling*, in *Molecular Biology of the Cell*; Garland Publishing: New York, 1994; pp 721–785.

(32) Busch, J. L. H. C.; Tournier, C.; Knoop, J. E.; Kooyman, G.; Smit, G. Temporal contrast of salt delivery in mouth increases salt perception. *Chem. Senses* **2009**, *34*, 341–348.

(33) Baek, I.; Linforth, R.; Blake, A.; Taylor, A. Sensory perception is related to the rate of change of volatile concentration in-nose during eating of model gels. *Chem. Senses* **1999**, *24*, 155–160.