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1. In Vivo Aroma Release during Eating of a Model Cheese: Relationships with Oral Parameters

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This study aims to follow the kinetics of aroma compound release during model cheese consumption in order to clarify the relationships between flavor release and some oral parameters. Eight subjects participated in the study. Breathing, salivation, chewing, and swallowing were monitored during the eating process. Temporal nosespace analyses were performed using on-line atmospheric pressure ionization−mass spectrometry (API-MS) and off-line solid-phase Micro extraction−gas chromatography−mass spectrometry (SPME-GC-MS). Flavor release profiles were obtained only for ethyl hexanoate, heptan-2-one, and heptan-2-ol. Among them, only the concentrations of ethyl hexanoate and heptan-2-one could be determined by API-MS. Absence of competition between the aroma compounds was checked for both techniques. In-nose maximum concentration (C_{max}) varied with aroma compounds. However, C_{max} was reached at the same time (T_{max}) for the three compounds. Interindividual differences were observed for most of the parameters studied and for all of the aroma compounds. They were related to the interindividual differences among the oral parameters. The aroma release parameters C_{max} and AUC (area under the curve) could be related to respiratory and masticatory parameters. In most cases, the same relationships were observed whatever the nature of the aroma compound.

KEYWORDS: Aroma release; saliva; breath; chewing; electromyography (EMG)

INTRODUCTION

Flavor release is an important issue in food science and has been extensively studied in recent years (*1*, *2*). The systems considered in most studies were often very simple, consisting generally of an aroma compound within a simple medium, usually an aqueous solution (*3*, *4*). The methodologies used generally were static or dynamic headspace. More recently, breath-by-breath analyses have been developed to follow volatile flavor release in the expired air of people during eating (*5*). Very few studies have been conducted on the release of volatiles from real solid or semisolid foods in the mouth. This, no doubt, is due to the variety and complexity of even the simplest solid foods and the complexity of the mastication process (*6*).

In vivo, the aroma stimulus depends on the concentration of aroma compounds in the nasopharynx, which, in turn, is affected by release rates of the compounds from the food in the mouth (*2*). In-mouth flavor release is known to be affected by food matrix and composition and by the mastication process. The impact of food composition and particularly interactions of aroma compounds with each other and with other food components have been extensively studied (*7*−*9*), especially within the EU COST Action 96 (Interaction of food matrix with small ligands influencing flavor and texture). These interactions were shown to be significant factors for aroma release. A number of devices have been proposed to study the impact of the mastication process on flavor release (*10*). They simulate, more or less precisely, the eating behavior, which is known to be very complex. Indeed, all of the phenomena that occur between food intake and swallowing (destructuration of the food matrix, increase of the available surface area, dilution in saliva with partial dissolution, and air flow in the upper airways) affect the release of volatile compounds (*11*). Videofluorography has provided the essential basis for visualizing and analyzing the processes by which food is moved to the cheek teeth and is subsequently manipulated (*12*), and videofluoroscopy and real-time magnetic resonance imaging (MRI) methodologies provided information about transfer of the volatile compounds during the swallowing process (*13*, *14*). All of these events that affect flavor release can influence flavor perception.

"Model-mouth" systems, which allow us to control chewing parameters, could be a powerful tool to investigate the effect of one or several oral parameters without individual variability. Van Ruth et al. (*15*) have studied three types of mouth model

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Table 1. Ingredients for the Production of the Processed Cheese

ingredient		quantity (g/500 g of cheese)	supplier
water	Milli-Q water	228.8	Millipore, Bedford, MA
proteins	casein rennet[a]	125	Eurial Poitouraine, Nantes, France
lipids	anhydrous milk fat	115	Cormans, Goe-Limbourg, Belgique
amino acids	leucine	0.7	Dolder, Basel, Switzerland
	phenylalanine	0.4	
	glutamic acid	1.3	
acids	lactic acid	2	Merck, VWR, Strasbourg, France
	citric acid	2.2	
minerals	$CaCl_2$, $2H_2O$	3.68	Merck
	$MgCl_2$, $6H_2O$	0.85	
	KCl	0.95	
	NaH_2PO_4, $2H_2O$	4.06	
	trisodium citrates	15.12	
aroma compounds[b]	propionic acid	0.5	Sigma Aldrich, St-Quentin, France
	butyric acid	0.2	
	diacetyl	0.0015	
	heptan-2-one	0.0025	
	ethyl hexanoate	0.0025	
	heptan-2-ol	0.0025	

[a] Casein rennet contained 7.5% minerals: Ca = 26; Na = 0.16; K = 0.26; Mg = 1; P = 15.8 g/kg of casein. [b] Quantity of aroma compounds introduced before the heating process.

systems for the flavor release of rehydrated bell pepper cuttings. Roberts and Acree (*16*) have designed a so-called "retronasal aroma simulator" (RAS) to investigate the effect of saliva, temperature, shearing, and fat on flavor release. However, although these "chewing machines" have provided useful data to better understand flavor release during eating and despite some success in comparing RAS data with nosespace data on a model cheese (*17*), it has not been generally possible to reproduce the complexity of the real mouth. Moreover, direct measurement of the concentration of volatiles in the breath expired from the nose during eating seems to be the only way to investigate the influence of human physiology on aroma release.

This study was undertaken to investigate the release profiles of three aroma compounds during the consumption of a complex flavored model cheese and to relate these profiles to subject's oral parameters.

MATERIALS AND METHODS

Flavored Model Cheese. *Preparation.* A model cheese made with fat and proteins was used to simulate a complex food system. The cheese contained proportions of volatile and nonvolatile compounds, selected on the basis of available literature data for various cheeses and added at approximate levels found in Comte cheese (*18*, *19*). The ingredients and quantities used are listed in **Table 1**. Minerals, acids, and amino acids were dissolved in pure water (128.8 g) (Millipore, Milli-Q system, Bedford, MA) under stirring for 1 h at 65 °C. This solution, the casein rennet, the fat, and the aqueous aroma solution (100 g), kept at 65 °C for 1 h, were subsequently stirred vigorously for 7.30 min at 65 °C with a cutter mixer (R3VV, Robot Coupe, Montceau en Bourgogne, France). The temperature (64 ± 1 °C) and pH (5.4) of each preparation were measured at the opening of the lid. The flavored model cheese was immediately poured into a plastic bag, placed at −20 °C during 25 min, vacuum sealed, and preserved at 4 °C for 1 week before consumption. One cheese was prepared per subject. For safety reasons, the absence of *Escherichia coli*, *Staphylococcus*, and *Salmonella* was checked for each cheese.

Aroma Loss Control. Loss of aroma occurred during heating and packaging of the cheeses. Slurries A−H were made by grinding 2.5 g of each cheese, mixing the cheese with 5 g of Milli-Q water, and stirring for 1 h at 25 °C. The headspace concentration of these slurries was measured by solid-phase microextraction−gas chromatography−mass spectrometry (SPME-GC-MS) and compared to the headspace concentration of slurry Z, which contained the same flavor concentration as a slurry in which cheese would not lose aromas. Slurry Z was made by mixing 2.5 g of an unflavored "control" cheese with 0.5 g of an aroma solution and 4.5 g of water and then stirring for 1 h at 25 °C. The aroma compounds present in the headspace above each slurry were extracted over 60 s with a 100 μm poly(dimethylsiloxane) (PDMS) fiber (Supelco, Bellefonte, PA). It can be noticed that the peak areas obtained in GC-MS starting from the sorption by the SPME fiber used for these experiments were found to be in the linear response range of the fiber. The following GC-MS conditions were used to analyze the SPME samples: 3 min of desorption at 250 °C in the injection port of a GC 6890 (Agilent Technologies, Palo Alto, CA) equipped with a splitless/split injector and coupled with a mass selective detector 5973 (Agilent Technologies). The column was a DB-Wax (30 m; 0.25 μm i.d., 0.25 μm film thickness; J&W Scientific, Agilent Technologies), with helium as carrier gas (35 cm/s). The oven temperature was initially raised from 50 to 140 °C at 6 °C/min, then raised from 140 to 220 at 15 °C/min, and finally kept at 220 °C for 5 min. The mass spectrometer acquired data in the selected ion monitoring (SIM) mode: *m*/*z* 43 and 86 for diacetyl; *m*/*z* 43, 58, and 114 for heptan-2-one; *m*/*z* 88, 99, and 101 for ethyl hexanoate; *m*/*z* 45, 55, and 83 for heptan-2-ol; *m*/*z* 45, 73, and 74 for propionic acid; *m*/*z* 60 and 73 for butyric acid. Data were recorded and analyzed with the HP-Windows Chemstation software (Hewlett-Packard, Palo Alto, CA). Relative quantification allowed the determination of the losses in each flavored cheese in comparison with the "control" cheese. Three replicates were done for each cheese.

Atmospheric Pressure Ionization−Mass Spectrometry (API-MS) and SPME Methodology for Nosespace Sampling. *API-MS.* Nosespace experiments were performed using API-MS with continuous gaseous sample introduction. Acetone (*m*/*z* 59), heptan-2-one (*m*/*z* 115), and ethyl hexanoate (*m*/*z* 145) release measurements were carried out using an Esquire mass spectrometer (Bruker, Daltonik, Wissembourg, France) fitted with a modified probe designed to allow gaseous sampling (*20*) due to the presence of a venturi system. Air from the nose was sampled at a flow rate of 55 mL/min through a deactivated stainless steel tubing (i.d. = 0.53 mm) (Silcosteel, Evry, France) heated to 150 °C. An auxiliary gas (nitrogen) was used at a flow of 9 L/min. This inert heated transfer line prevented vapor condensation and minimized unwanted chemical reactions. The volatiles studied were detected at *m*/*z* values corresponding to their protonated molecular ions (MH^+). Prior to each session, the dynamic headspace (N_2 flow rate = 200 mL/min) of a solution of hexan-2-one (9.3 ppb), which provided a signal of the same order of magnitude as the signal observed during the nosespace sessions, was analyzed by API-MS. The signal obtained for

the protonated molecular ion (*m*/*z* 101) allowed the calibration of the system to be performed. To study possible competitions between the aroma compounds for the ionization process, the headspaces of several solutions of heptan-2-one and ethyl hexanoate with the five other aroma compounds were analyzed in API-MS (either as separate components or as mixtures). The concentration range used (from 0.0043 to 1.07 ppm for heptan-2-one and from 0.06 to 2.4 ppm for ethyl hexanoate) represented the range of intensities observed during the nosespace analyses.

As the API-MS system used did not allow the detection of the other aroma compounds because of their too low concentration in the nosespace and/or their higher detection threshold, we used a complementary SPME method (*21*). A Y-junction was set up between the entry of the API-MS capillary, the entry for the SPME fiber, and the nose of the subject.

SPME. The release of heptan-2-ol and butyric acid was measured in a discontinuous way by inserting a SPME fiber at different moments of the mastication in the Y-junction and sampling expired air for 8 s. A "blank" was sampled before the product was introduced into the mouth, and several sampling periods were used during eating: 1−9, 6−14, 16−24, 26−34, 36−44, 56−64, 86−94, 116−124, and 176−184 s. Two successive mastications in the same session were necessary to obtain one replicate due to an overlapping of sorption times. Ten fibers were needed to perform these experiments. Throughout the experiments, each fiber was randomly attributed to a particular given period of each mastication. After extraction of the nosespace samples, the first fiber was immediately desorbed and the aroma compounds were analyzed as described above. The nine other fibers were stored at room temperature until they could be analyzed by GC-MS. Fibers were stored in glass tubes hermetically sealed just after the extraction phase to avoid aroma compound loss due to exchanges between the fiber and the laboratory air.

Nosespace Sampling. Eight subjects, five males and three females, between 23 and 51 years of age (average = 32.6 years) participated in this study. They were instructed not to eat or drink 2 h before the experiments. Four sessions of 30 min over 4 consecutive days were conducted for each panelist to obtain three replicates of SPME profiles and eight replicates for API-MS. At each session, the panelist ate two samples of 5 g of cheese previously stored for 1 h at 20 °C. They were asked to eat in their own way, mouth closed, and to breathe into a plastic tube connected to the heated transfer line. The regularity of their respiratory rhythm was checked by charting the acetone permanently present in human breath. Total breath sampling lasted 3 min. Panelists were asked to clean their mouths by bread and apple chewing and by water drinking in order to eliminate any after-feel perception. They started the second mastication several minutes later, after verification with API-MS of the absence of heptan-2-one and ethyl hexanoate in their expired air.

Oral Measurements. *Electromyography (EMG) Recordings.* The EMG recordings were conducted according to the method of Mioche et al. (*22*). The left and right superficial masseters and anterior temporalis muscles of each volunteer were located by palpation when they clenched their teeth. After careful cleaning of the overlying skin, two surface electrodes (Bionic) coated with conductive paste were fixed 2 cm apart, lengthwise along each muscle, with an adhesive. An additional ground electrode was attached to the subject's ear lobe.

Each subject was instructed to eat 5 g of cheese (2 × 1.5 × 1 cm), chewing as naturally as possible. The experiments were done in triplicate.

After signal rectification, several variables were analyzed for the complete sequence of mastication starting at the moment of food intake and ending at the last swallow: chewing time (total sequence duration before the last swallow), number of chews during the chewing time, chewing rate per minute, the mean voltage of each burst, the sum of the integrated areas of all individual bursts in the sequence (burst duration multiplied by its mean voltage expressed in V·s), previously called muscle work (*23*), and the mean work (total work divided by the number of bursts).

Breath Parameters. Simultaneously with the EMG recordings, the whole nasal air flow was monitored using a flow meter (Pulmo System II, MSR, Rungis, France). Each subject was instructed to breath as naturally as possible in a nasal mask (MSRe) connected to the flow meter. The whole nasal air flow rate was measured at rest and during the mastication. The respiratory rate was calculated for both conditions.

Swallowing Events. During chewing, deglutition was controlled according to the procedure of Mioche et al. (*24*) using a necklace strain gauge, which provides a deviation in the baseline when a swallow is triggered.

Masticatory Performances. Each subject was instructed to chew standardized cylinders of Optosil (Perrigot et Cie, Dijon, France) (∅ = 1.4 cm, height = 1.8 cm, weight = 3.3 ± 0.05 g) and to count their chews. After 20 s, they were asked to spit the sample into a coffee filter and to rinse the mouth with water. Rinsings were also collected in the filter. This procedure was repeated four times. 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 mesh size of 4 mm. The masticatory performance of each subject over 20 s was defined as the amount of sample that passed through the sieve versus the amount of chewed sample.

Salivation. Whole saliva flow rate was measured via absorption by cotton rolls (Roeko, Longenau, Germany). The total flow rate was determined by calculating the weight difference of each cotton roll before and after the experiment. A pretest of 1 min was carried out to clear extra salivation produced by the introduction of the cotton rolls. To determine the salivation with mechanical stimulation, two cotton rolls were placed on the aperture duct of the parotid glands and the subjects were instructed to chew 0.5 ± 0.01 g of Parafilm (American National Can, Menasha, WI) over a 1-min period without swallowing. Then the experimenter removed the cotton rolls, and the subject spat out the remaining Parafilm and saliva into a tare glass. All of the measurements were done in triplicate and at the same time of day for all of the subjects.

Data Analyses. API-MS data were smoothed after all of the peaks corresponding to exhalation events had been selected. SPME-GC-MS data were not smoothed, and each point on the curve represented a measurement time. To summarize the information contained in the curves, some parameters were defined and studied by analysis of variance. These parameters included C_{max}, the maximum intensity of the curve corresponding to the greatest amount of aroma in nosespace; T_{max}, the time when C_{max} is reached; slope, the initial slope of the curve measured between 0 and 10 s; and, finally, AUC, the area under the curve corresponding to the total aroma release over a 3-min period. ANOVAs, mean comparisons using Newman−Keuls test, *t* tests, correlations, and linear regressions were performed with SAS software version 8.01 (SAS Institute Inc., Cary NC). The statistical models used are listed below:

(a) Competitions. The two-way ANOVA involving concentration and preparation factors (one or six aroma compounds) was performed on the intensities obtained from API-MS measurements (model: intensity = concentration + preparation + concentration × preparation).

(b) Average of Release Profiles. ANOVAs with each parameter, T_{max}, log C_{max}, and log AUC (model: parameter = compound + subject) were performed to investigate the release differences among the aroma compounds (C_{max} and AUC parameters were log transformed to obtain variance homogeneity).

(c) Regression analyses were performed between flavor release parameters and oral variables: parameter = *a* [PCA axis1] + *b* [respiratory rate] + *c* (for PCA axis 1, see **Figure 2**).

RESULTS AND DISCUSSION

Optimization of the Procedure. *API-MS and SPME Sampling.* The API-MS system allows continuous detection of acetone, heptan-2-one, and ethyl hexanoate during eating. APCI is a difficult process to control. Nonquantitative results and selectivity in the ionization of particular compounds have been described (*5*). Thus, the possible competitions are between ethyl hexanoate and/or heptan-2-one and the other aroma compounds present in the cheese were studied. Several solutions prepared with one or six aroma compounds in the concentration range found in the nosespace data were analyzed. The two-way

Table 2. Individual Release Parameters (T_{max}, C_{max}, AUC, and Slope)[a] Obtained from Each Aroma Release Curve

	heptan-2-one				ethyl hexanoate				heptan-2-ol			
subject	T_{max}	C_{max}	AUC $\times 10^{-3}$	slope	T_{max}	C_{max}	AUC $\times 10^{-3}$	slope	T_{max}	C_{max}	AUC $\times 10^{-3}$	slope
1	52.5	8594	859	167	47.5	1266	117	29.9	52.5	7509	823	198
2	24	2784	184	183	18	312	24.1	21.6	23.3	3776	337	203
3	38.6	4577	401	225	30	598	54.5	41.5	27.5	3903	399	165
4	21.2	4312	273	287	22.5	577	33.5	38.6	27.4	2782	281	133
5	43.3	2385	212	53	41.6	312	26.8	8.9	40	2519	356	134
6	37.1	3968	328	147	34.3	554	42.6	30.2	37.3	3077	307	146
7	25	5776	516	393	20	919	76.2	76.7	32.9	6321	607	256
8	53.3	934	108	45.9	53.3	112	14.7	8.2	90	4531	625	153
av	**36.9**	**4166**	**360**	**188**	**33.4**	**581.3**	**48.7**	**32**	**41.4**	**4302**	**467**	**174**
SD	12.6	2329	239	116	13.1	368.2	33.7	21.9	21.7	1766	195	42.6

[a] T_{max}, time to reach the maximum concentration; C_{max}, maximum concentration; AUC, area under the curve; slope, initial gradient of the curve measured between 0 and 10 s.

ANOVA indicated that neither the preparation effect nor the concentration × preparation interaction was significant ($p > 0.05$) for heptan-2-one and ethyl hexanoate. The concentration effect was highly significant ($p < 0.0001$) for both compounds. Thus, the differences observed in the intensities were due only to the use of different concentrations. In the concentration range tested, no significant difference was observed in the intensities for both compounds when they were prepared alone or mixed with the five other aroma compounds. It can be concluded that no competition between the aroma compounds occurred for the ionization. A comparative and complementary SPME method (*21*), carried out simultaneously with nosespace API-MS, was developed to follow, in a discontinuous way, the changes in the concentration of aroma compounds released in expired air during chewing. The absence of competition for absorption on the SPME fiber was also verified.

Sensory analysis showed that propionic acid ("pungent" flavor) and diacetyl ("butter" flavor) could be perceived by the subjects while eating the cheese, although concentrations in their nosespaces were too low to be detected either by API-MS or SPME-GC-MS in all of the samples. Release of butyric acid present in the expired air of the subjects (*21*) was quantified only at 30 and 60 s due to the absence of linearity using some SPME fibers for some concentrations. Hence, only data concerning heptan-2-ol, heptan-2-one, and ethyl hexanoate, detected either by API-MS or by SPME-GC-MS, were completely analyzed. The calibration, performed prior to each session with a solution of hexan-2-one, allowed the different aroma release profiles to be compared. In our conditions, API-MS gave some quantitative results, and the amounts of heptan-2-one and ethyl hexanoate released over 3 min of mastication were quantified by GC-MS.

Losses of Aroma Compounds during Production of Cheeses. Due to heating and transfer of the hot cheese for packaging, some losses of aroma compounds occurred. It was important to quantify these losses in order not to bias the nosespace analyses. First of all, when the eight cheeses were globally compared to the "control" cheese, no significant difference was observed for butyric acid and propionic acid ($p > 0.05$). That means no loss occurred for these two aroma compounds during the cheese production. However, some significant differences were observed for diacetyl ($p < 0.0001$), heptan-2-one ($p < 0.0001$), heptan-2-ol ($p = 0.002$), and ethyl hexanoate ($p = 0.004$). The losses were different according to the aroma compound: 99% for diacetyl, 58% for heptan-2-one, 36% for ethyl hexanoate, and 32% for heptan-2-ol. Furthermore, we observed that the quantity of aroma compounds present in the cheese after the process was not totally repeatable. Indeed, the results of the analyses of variance and the mean comparison tests (Newman−Keuls) revealed some significant differences among the eight products concerning ethyl hexanoate ($p = 0.0009$) and propionic acid ($p = 0.03$). Propionic acid was not investigated further in the nosespace experiments, but in the case of ethyl hexanoate we wanted to know if these differences could skew the analysis of the individual nosespace release profiles. In the extreme cases, the cheese eaten by subject 3 contained 1.4-fold of ethyl hexanoate in comparison with the cheese eaten by subject 6. Delahunty reported that, in many cases, even if there are interindividual differences between the aroma release profiles, the proportion of all volatile compounds during consumption was similar from one consumer to another. An index termed the Aroma Stimulus Index (ASI) can be used to quantify this relationship (*25*). This index is postulated to be constant in time during consumption. Taking into account C_{max} and AUC, we calculated the ASI for heptan-2-one (no significant difference between the model cheeses) and ethyl hexanoate (significant difference) by comparing subjects 3 and 6 for whom the respective model cheeses contained the same quantity of heptan-2-one but different quantities of ethyl hexanoate. For both parameters, the ASI between subjects was very similar for the two aromas: C_{max}(heptan-2-one) subject 3/C_{max}(heptan-2-one) subject 6 = 1.15 and C_{max}(ethyl hexanoate) subject 3/C_{max}(ethyl hexanoate) subject 6 = 1.08. In terms of AUC, we found 1.22 and 1.28, respectively. Thus, it can be concluded that the differences observed in the cheeses concerning the concentration of ethyl hexanoate were not sufficient to bias the nosespace analyses and may be of the same order of magnitude as differences in the composition of commercial cheeses.

Aroma Release during Eating. *Average of Release Profiles.* The ANOVA performed showed that the subject effect was highly significant ($p < 0.0001$) for all of the analyses. We focus on the compound effect in the following discussions. For C_{max} and AUC parameters, the results showed a significant effect of the type of compound ($F = 2709$ and $p < 0.0001$ for C_{max}, $F = 10601$ and $p < 0.0001$ for AUC). In the expired air, the maximum concentration of heptan-2-one was ~10 times higher (average $C_{max} = 4166$ ng/L of aspirated air and AUC = 360000) than that of ethyl hexanoate (average $C_{max} = 581$ ng/L of aspirated air and AUC = 48700) (**Table 2**). The concentration of heptan-2-ol, analyzed by only SPME, could not be determined. Similar trends were found for each subject. However, due to losses, the cheeses contained less heptan-2-one (2.1 ppm) than ethyl hexanoate (3.2 ppm). The physicochemical properties of the aroma compounds and the properties of the matrix progressively mixed with saliva explained the differences observed in the release patterns. Many studies have explored

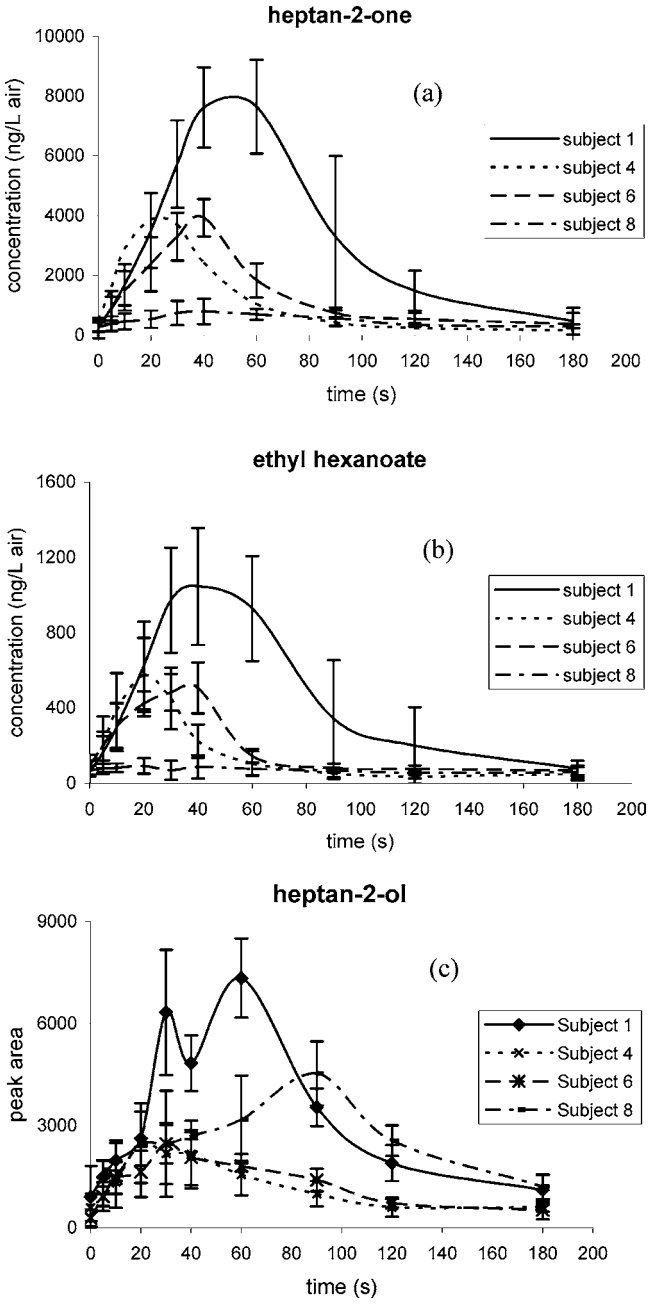

Figure 1. Volatile release patterns observed for heptan-2-one (a), ethyl hexanoate (b) (both analyzed continuously by API-MS), and heptan-2-ol (c) (analyzed by SPME-GC-MS in a discontinuous way) for subjects 1, 4, 6, and 8 over a 3-min period. Data obtained from SPME-GC-MS were not smoothed, and standard deviations were represented for each time sampled. Data obtained by API-MS were smoothed, and standard deviations were reported for each sampling time corresponding to SPME sampling.

the influence of such physicochemical variables on flavor release, albeit more generally in simple systems. The flavored model cheese in this study is a complex medium as it is a gelified oil-in-water emulsion. Voilley et al. (*26*) studied complex media and explained that flavor release depends on the affinity of the odorants for the food product and, therefore, on their availability for the vapor phase. From a physicochemical point of view, key features influencing transfer and release are the presence of the interface between the aqueous and lipid phases, the surface area of the interface, and the nature of the surface active agent absorbed at this oil−water interface.

Table 3. Correlation Coefficients between the Release Parameters[a] of Heptan-2-ol (Hol), Heptan-2-one (Hon), and Ethyl Hexanoate (EH)

correlation	T_{max}	C_{max}	AUC	slope
Hon/Hol	0.78*[b]	0.71*	0.66	0.62
EH/Hon	0.96***	0.99***	0.99***	0.95***
EH/Hol	0.85**	0.75*	0.69	0.67

[a] T_{max}, time to reach the maximum concentration; C_{max}, maximum concentration; AUC, area under the curve; slope, initial gradient of the curve measured between 0 and 10 s. [b] $p < 0.1$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; NS, $p > 0.1$.

Conducting these experiments in-mouth during chewing would be necessary to understand the factors influencing flavor release during mastication. Emulsification also affected the release of the aroma compounds, including volatility and mass transfer factors (*27*). The less important release observed with ethyl hexanoate could be explained by its higher hydrophobicity: Log *P* is 2.8 for ethyl hexanoate and 1.82 for heptan-2-one (*28*). Thus, ethyl hexanoate may occur preferentially in the lipid phase. Caseins used as the emulsifier in the oil/water system also play a role in these interactions and generally lead to a decrease in the volatility of the flavor compounds (*29*).

No significant effect of the aroma compound could be observed for the T_{max} parameter among the three aroma compounds ($F = 2$ and $p = 0.16$; mean values = 41.4 s for heptan-2-ol, 36.9 s for heptan-2-one, and 33.4 s for ethyl hexanoate) (**Table 2**). There was thus no significant delay of release for any aroma compound in comparison with the others. Van Ruth et al. (*30*) found similar results concerning the T_{max} of butan-2-one, diacetyl, ethyl butyrate, hexanal, and heptan-2-one contained in sunflower oil. In contrast, Harvey et al. (*31*) observed some considerable variation in T_{max} for citral, limonene, decanal, ethyl hexanoate, and hexenol during the mastication of flavored gelatin/pectin gels, but these experiments were carried out with only one operator (five replicates). The authors hypothesized that this variation was due to the physicochemical properties of aroma compounds. In experiments on mints (*32*), the high boiling point compounds with the greatest polarity were the most persistent in the breath. Therefore, the combination of polarity/boiling point for the compound could play a role in the time course of aroma release. In the present study, the physicochemical properties of heptan-2-one, ethyl hexanoate, or heptan-2-ol may not be different enough to observe such a phenomenon, so we can hypothesize that the nature of the food matrix affects the time to the maximum release of the three compounds in the same way.

Interindividual Differences. Individual aroma release parameters are reported in **Table 2**. Analysis of variance indicates a significant effect of the subject factor whatever the aroma compound for T_{max}, C_{max}, AUC, and log slope. The *p* values concerning 11 of the 12 parameters were highly significant ($p < 0.001$).

Release profiles for heptan-2-one, ethyl hexanoate, and heptan-2-ol are presented in **Figure 1** for four subjects. Subject 1 had the highest C_{max} for the three aroma compounds. T_{max} varied also according to the subject. Finally, we could observe that for all of the subjects, the quantity of each aroma compound present in the expired air is negligible after a 3-min period of flavor release monitoring. As explained above, we could not discuss all of the data obtained. For butyric acid, however, the chromatographic peak areas obtained at 30 and 60 s informed us about the rate of the release according to each subject, and some significant differences among the subjects appeared at 30 s ($F = 21$, $p < 0.0001$) and at 60 s ($F = 3$, $p = 0.03$). As we

Table 4. Oral Parameters[a] Measured on Each Subject

subject	SF	Ch	CT	CR	MV	SR	TW	MW	MP	RR	RF
1	2.36	57	57.5	59.2	0.06	3.7	4.3	0.07	15.7	21.9	0.208
2	0.99	33	23.9	83	0.035	8.5	1.3	0.04	0.69	15	0.219
3	0.37	35	50.1	42.3	0.028	4	2.1	0.06	16.3	14.9	0.214
4	2.32	34	23.3	89.3	0.047	11.6	1.8	0.05	26.5	12.7	0.229
5	0.56	26	31.3	49.6	0.025	9.3	0.8	0.03	5.46	15	0.203
6	1.45	20	22.9	53.5	0.02	3.4	0.4	0.02	1.57	21.7	0.312
7	0.32	41	55.5	44.4	0.052	3.2	2	0.05	1.74	17.3	0.223
8	1.49	52	53.5	59.7	0.018	5.5	0.7	0.02	6.74	12.3	0.224
av	**1.23**	**37.2**	**39.8**	**60.1**	**0.036**	**6.2**	**1.7**	**0.04**	**9.34**	**16.4**	**0.229**
SD	0.82	12.4	18.8	17.3	0.02	3.2	1.23	0.02	9.24	3.67	0.03

[a] SF, salivary flow; Ch, number of chews; CT, chewing time; CR, chewing rate; MV, mean voltage; SR, swallowing rate; TW, total work; MW, mean work; MP, masticatory performances; RR, respiratory rate; RF, respiratory flow rate.

have shown above using the ASI, even if different patterns of release were observed for different panelists, the total quantities of heptan-2-one and ethyl hexanoate were released in similar proportions from one consumer to another during consumption. Each panelist presented a different profile, but the shape of the curve was not dependent upon the compound, as observed from correlations between parameters (**Table 3**). Correlations with butyric acid could not be established as C_{max} could not be determined for this aroma compound.

Relationships between Aroma Release and Oral Parameters. We investigated the relationships between aroma release profiles and oral parameters in order to explain the interindividual variation of aroma release by the physiology.

Three main processes have been put forward to explain in vivo flavor release (*33*). First, mastication breaks the product down and thereby enhances the release of the flavor. Second, volatile components are transported through the upper airways to the olfactory epithelium driven by respiratory air flow. Third, specific losses occur in the mouth and the upper airways due to absorption. These complex phenomena have been only sparsely described. To explore some of the possible parameters affecting the in-mouth flavor release, we considered the first two processes only, mouth losses being out of control.

Salivary, chewing, and respiratory parameters have been measured on each subject (**Table 4**). As several chewing and salivary parameters were closely correlated (data not shown), we performed a principal component analysis (PCA) (done on the correlation matrix) on these variables and kept the two first axes, which explained 75% of the variance (**Figure 2**). The first axis represented the amplitude of muscle activities (mean and total work, the mean voltage of each burst) and number of chews. The second axis represented (positive side) chewing rate, salivary flow, masticatory performances, and swallowing rate and (negative side) chewing time. Regression analyses were then performed between aroma release parameters and oral parameters using new noncorrelated variables (the coordinates of the subjects on the two first axes of the PCA) and the respiratory variables. Respiratory flow rate and respiratory rate were also included in the regression models as they are said to take part in the transport of the aromas from the mouth to the olfactory epithelium. Significant results are summarized in **Table 5**. The model that significantly explained some of the variations observed in the aroma release data was composed of the first axis of the PCA and the respiratory rate. The regression coefficients were positive. Interestingly, subjects with high C_{max} and/or AUC for heptan-2-one and ethyl hexanoate had also high values of respiratory rate, number of chews, and muscle activity. High muscle activity, expressed by muscle work, for example, might be in favor of a better degradation of the bolus. A

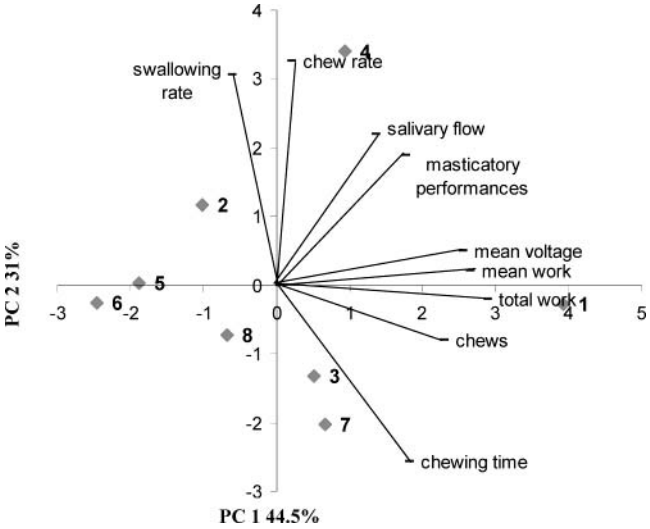

Figure 2. Principal component analysis (PCA) performed on masticatory and salivary parameters. The eight subjects are represented by the symbol ◆.

Table 5. Relationships between the C_{max} of Heptan-2-ol (Hol), Heptan-2-one (Hon), Ethyl Hexanoate (EH), the AUC of Heptan-2-one and Ethyl Hexanoate, and the First Axis of the PCA (Involving Masticatory and Salivary Variables) and Respiratory Rate[a]

	P value	
	PCA axis 1	respiratory rate
C_{max}–Hol	0.04	NS
C_{max}–Hon	0.004	0.012
C_{max}–EH	0.0057	0.013
AUC–Hon	0.0008	0.0018
AUC–EH	0.0019	0.0046

[a] The first axis represented the amplitude of muscle activities (mean and total work, mean voltage of each burst) and number of chews. Model tested: parameter = *a* PCA axis 1 + *b* respiratory rate + *c*. NS, $p > 0.05$. C_{max}, maximum concentration; AUC, area under the curve.

significant correlation was found between muscle work and chewing efficiency (data not shown). Consequently, the food surface in contact with the vapor phase increases, and the transfer of the aroma compounds from the matrix to the vapor phase is higher. Furthermore, we found that a high respiratory rate also involved a high C_{max} or AUC for heptan-2-one and ethyl hexanoate. We can hypothesize that a greater respiratory rate contributes to bringing more volatiles to the upper air ways, and consequently more volatiles are present in the expired air of the panelists. A similar trend was observed by Hanaoka et al. (*34*) using GC−olfactometry, where subjects who were asked

to breathe more rapidly rated odors more intensively, suggesting a possible individual influence of breathing.

However, neither T_{max} nor slope parameters were significantly related to the oral parameters. Concerning butyric acid, the interindividual variation observed at 30 and 60 s was not related to any oral parameter.

In the literature, influence on flavor release of one or several oral variables taken one at a time while the other variables were controlled was often studied using model mouth systems. For instance, van Ruth et al. (*30*) investigated the effects of saliva in the system (0, 20, 40, 60, and 80% v/v) and the mastication rates (0, 26, 52, and 78 cycles/min). They showed higher release of all compounds with higher mastication rates. However, this relationship was not linear, and it varied among compounds. In the present study, no relationship was observed between chewing rate and the maximum concentration (C_{max}). The chewing rate varied from 42.3 to 89.3 cycles/min among subjects (**Table 4**). This range may not be sufficient to observe such an effect. Whatever the compound, aroma release was similarly influenced by masticatory parameters.

Although the influence of saliva flow rate on flavor release has been shown in some studies using model mouth systems (*17*, *30*), such an effect was not observed in this in vivo study. Odake et al. (*35*) showed that saliva affected differently the release of heptan-2-one and diacetyl in emulsion and cream-style dressings. The volume of added saliva ranged from 0 to 10 mL mixed with 5 g of product, which was much higher than the in vivo variation we found (0.3−2.36 g/min with 5 g of product) (**Table 4**). During mastication, dilution with saliva and swallowing leads to a continuous change in volume, composition, and food bolus viscosity. These changes, associated with interindividual variability, could explain differences with mouth models (*35*).

Using a retronasal aroma simulator, Deibler et al. (*17*) studied the influence of air flow rate, temperature, saliva ratio, and blending speed on flavor release of an imitation cheese. They found that a 5-fold increase of the air flow rate reduced the release of ethyl acetate, ethyl hexanoate, ethyl butanoate, and isoamyl acetate. Again, we failed to find such a relationship in vivo, as maximum variations among panelists were only 1.5. Harrison and Hills (*37*) increased the air flow rate by 20-fold, so once again the variation was higher than that observed in vivo.

In conclusion, this paper has examined how, during the eating process of a flavored model cheese, aroma release can be explained by differences between individuals and volatile compounds. Interindividual differences in temporal release of heptan-2-one, ethyl hexanoate, and heptan-2-ol could be explained by interindividual differences in numerous oral parameters. Aroma release (C_{max} and AUC) parameters could be related to respiratory rate and masticatory parameters (chew number and muscle activity). In the literature, the influences of a number of separable processes in mastication and transport through the upper airways on flavor release have been reported. However, the variation range of the oral variables used in vitro, in model mouth systems, is often higher than the variation range measured in vivo. That may explain the differences between the observations reported here and those in the literature. Furthermore, until now, the effects of oral variables on flavor release have been investigated separately. Yet several oral variables need to be considered simultaneously in order to observe the global effect of all the oral parameters implicated in the chewing process. The same study has been carried out with a focus on the nonvolatile compounds present in the flavored model cheese (*38*) and with respect to perception (*39*).

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