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2. In Vivo Nonvolatile Release during Eating of a Model Cheese: Relationships with Oral Parameters

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This study deals with the release kinetics of nonvolatile compounds (NVC) (leucine, phenylalanine, glutamic acid, citric acid, lactic acid, propanoic acid, sodium, potassium, calcium, magnesium, chloride, and phosphates) during the eating of a model cheese and the relationships to some oral (salivary and masticatory) parameters. The aroma release has previously been characterized in similar conditions [Pionnier, E.; Chabanet, C.; Mioche, L.; Le Quéré, J.-L.; Salles, C. *J. Agric. Food Chem.* **2004**, *52*, xxx−xxx (*1*)]. Saliva samples were collected from the tongues of eight assessors at different times during and after the chewing sequence. Atmospheric pressure ionization−mass spectrometry and/or high-performance liquid chromatography analyses have been performed on these samples in order to quantify the 12 NVC released in saliva. The maximum concentration (C_{max}) in saliva varied significantly according to the compound. However, there was no significant effect of the compound on the time to reach maximum concentration (T_{max}). Interindividual differences were observed for most of the parameters and for all of the NVC studied. The parameters extracted from the release profiles of the NVC were closely correlated. High T_{max} and AUC (area under the curve) values could be related to high chewing time and low saliva flow rates, low chewing rates, low masticatory performances, and low swallowing rates.

KEYWORDS: Nonvolatile release; saliva; chewing; electromyography

INTRODUCTION

In the field of flavor analysis, volatile compounds have been more extensively studied than nonvolatile compounds (NVC) responsible for tastes. One reason for this is that aroma compounds are more easily sampled (by taking a gas sample from the headspace for instance) and analyzed than NVC (*2*). Although the NVC have been identified and quantified in a wide range of foods, far less is known about their release from the food matrix during the chewing process. Because the food NVC need to be diluted in saliva in order to stimulate taste receptor cells, the study of nonvolatile release during mastication involves the time course analysis of the components present in saliva. Furthermore, as in the case of aroma compounds where nosespace sampling is performed at a location that seems more relevant for comparison with sensory analysis (i.e., as close as possible to the olfactory receptors), saliva sampling is performed on the surface of the tongue, as close as possible to the taste receptor cells.

Only a few studies focused on nonvolatile release during mastication. In-mouth sensors were used to measure conductivity and pH during chewing (*3*, *4*). However, the measurements were nonspecific and could be affected by the water content and textural properties of the food as sensors need to be placed in a certain amount of saliva. Electronic tongues could lead to more precise data, but they cannot be placed in the mouth for direct measurement (*5*, *6*). Moreover, they require rather high volumes of clear liquid, and it remains difficult to relate a specific signal to a particular compound. Davidson et al. (*7*) followed sucrose release during gum chewing using another methodology. They analyzed saliva samples at several moments during the chewing sequence, using a direct liquid mass spectrometric procedure.

Flavor is a combined perception linked to volatile and nonvolatile compounds release. In a previous paper (*1*), the same model cheese was investigated with respect to release of volatile compounds release during chewing. We showed that this release depended on physicochemical properties of the compounds and also on the subjects. Additionally, interindividual variation was shown to be related to variation in physiology. In particular, C_{max} and AUC (area under the curve) parameters for each volatile compound were influenced by respiratory rate, number of chews, and masticatory muscle activity. Indeed, high C_{max}

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values were related to high values for these oral variables. However, the T_{max} parameters did not seem to be influenced by the oral parameters measured.

This study aims to investigate the release of NVC during the eating process of a complex model cheese in relation to individual saliva and chewing parameters in order to clarify interindividual variability observed in flavor release.

MATERIALS AND METHODS

Model Cheese Preparation, Subjects. The preparation of the model cheese is described in part 1 (*1*), reporting the quantity of each compound incorporated in the cheese. The subjects participating in this study were the same as in part 1.

Saliva Sample Preparation. Saliva samples were collected from the panelists' tongues at different moments during the eating of 5 g of cheese via the cotton bud technique as described by Davidson et al. (*4*).

Three 1-h sessions for each panelist were conducted. From each chewing sequence, saliva was collected at different times: 10, 20, 30, 40, 60, and 90 s. The subjects were told to eat in their own way ("free" chewing). In addition, at the beginning of the session, one sample of saliva was collected before the first cheese sample was eaten (blank). In order not to interfere with the chewing behavior, only one saliva sample was collected per mastication. Each release profile of the taste compounds during eating is thus the result of six successive mastications. Panelists rinsed their mouths with some bread, apple, and water, and a 9-min delay was observed between sequences. The experiments were performed in triplicate.

After collection and weighing, saliva was extracted from cotton buds with 1.5 mL of water by sonication (5 min), filtered (∅ =13 mm, Cluzeau, Sainte Foy la Grande, France), and divided in two parts: two-thirds of the volume for atmospheric pressure ionization−mass spectrometry (API-MS) analysis and one-third for high-performance liquid chromatography (HPLC) analysis. Each sample was diluted with an equal volume of methanol (for API-MS) or acetonitrile (for HPLC).

Saliva Analysis. *API-MS.* A mass spectrometer equipped with a liquid chromatographic interface (Platform LCZ, Micromass, Manchester, U.K.) was used. A mobile phase of methanol/water (50:50, v/v) was continuously pumped into the interface, at a flow rate of 0.4 mL/min. The source block and desolvation temperatures were, respectively, 150 and 400 °C. A 10 μL aliquot of the saliva extracts or standards was injected via a Rheodyne injection loop (Rheodyne, Cotati, CA). All NVC were monitored with electrospray ionization−mass spectrometry (ESI-MS) in negative or positive ionization (according to the nature of the compound) with selected ion mode. The value of the cone voltage used for this analysis was 25 V.

HPLC: Ionic Chromatography. The analyses were conducted with a Dionex DX300 ion chromatographic system (Dionex, Voisins le Bretonneux, France). The individual components included a quaternary AGP1 pump, an electrochemical detector used in conductance mode, and a Dionex AS50 autosampler. Loop injection volumes of 25 μL were used throughout the experiments. System control and data acquisition were accomplished using Dionex UCI-100 Chromeleon software.

The anions were separated using a Dionex AS11-HC column, an IonPac ATC-3 trap column, and an IonPac AG11-HC guard column at 20 °C and a flow rate of 1.5 mL/min. Eluents were prepared by adding the appropriate amount of a 50% (w/w) sodium hydroxide (J. T. Baker, Sodipro, Echirolles, France) solution to 1 L of degassed, filtered (0.45 μm filter) 18 MΩ Milli-Q water. Elution was achieved in a gradient run (**Table 1**).

The cations were separated using a Dionex IonPac CS12-A column and an IonPac CG12-A guard column at 20 °C. Elution was achieved in an isocratic mode with 22 mN sulfuric acid (Aldrich) at a flow rate of 1 mL/min.

All experiments were performed using suppressed conductivity detection with a Dionex (CSRS-ultra 4 mm for cations and ASRS-ultra 4 mm for anions) suppressor and a SRS controller.

Table 1. Gradient Elution Program Used for Analysis of Anions by Ion Chromatography[a]

time (min)	% A	% B	% C
0	60	0	40
7	60	0	40
15	0	15	85
20	60	0	40
25	60	0	40

[a] Eluents A, B, and C were, respectively, 2 mM NaOH, 200 mM NaOH, and pure water.

Table 2. Method Used for the Analysis of the Nonvolatile Compounds Present in Saliva during Eating of the Processed Cheese

compound		API-MS	HPLC
amino acids	leucine	X	
	phenylalanine	X	
	glutamic acid	X	
acids	citric acid	X	
	lactic acid	X	X
	propanoic acid	X	X
minerals	Na	X	X
	K	X	X
	Ca		X
	Mg		X
	Cl	X	X
	phosphates	X	

Table 2 illustrates the methodology used for the analysis of each compound. Standard mixtures were used for calibration, allowing the quantification of each compound.

Oral Measurements. Electromyography (EMG) recordings, masticatory performances, salivary flow rate, and swallowing events were measured as described in Pionnier et al. (*1*).

Data Analyses. 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 NVC in saliva; 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 NVC release over a 90-s period. ANOVAs, *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) Comparison of the Methodologies. Regression analyses were performed between release parameters (T_{max}, C_{max}, AUC, and slope) obtained from API-MS and from HPLC: API parameter = a (HPLC parameter).

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

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

RESULTS AND DISCUSSION

Optimization of the Procedure. The sampling of saliva from the mouth during eating involved very small volumes of liquid. Analysis then required sensitive methods to quantify NVC. Two complementary methods were used to analyze the NVC present in the saliva at different stages of the eating process (**Table 2**). In these experiments, ionic chromatography by HPLC represented the reference method because it is quantitative and repeatable. It has been used to determine anions in milk (*8*); it

Table 3. Regression Coefficients (*a*) Obtained from the Regression Model API-MS Parameter = *a* (HPLC Parameter) for the Four Parameters (T_{max}, C_{max}, AUC, and Slope)[a] (24 Observations) [T_{max}(API-MS) = 0.77/T_{max} HPLC for Cl]

	T_{max} (API–MS/HPLC)	C_{max}(API–MS/HPLC)	AUC(API–MS/HPLC)	slope(API–MS/HPLC)
Cl	0.77***[b]	0.54***	0.50***	0.47***
Na	0.92***	0.54***	0.51***	0.56***
K	0.44**	0.58***	0.49***	0.47***
lactate	0.78***	0.5***	0.57***	0.62***

[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] **, $p < 0.01$; ***, $p < 0.001$.

enabled simultaneous identification and quantification of chloride, phosphate, and citrate ions with good repeatability and good sensitivity (<20 μg/kg). Ionic chromatography was also used to identify and quantify some organic acids and in particular citric, lactic, propionic, and butyric acid in sugar factory products (*9*), as well as inorganic cations such as Na^+, K^+, Ca^{2+}, and Mg^{2+} in brine solution (*10*). Finally, this method also allowed the determination of Cl^-, NO_3^-, SO_4^{2-}, and PO_4^{3-} anions in some microvolumes of human tear fluid and serum (*11*). Thus, it appeared to be a reliable and accurate technique to analyze and quantify the cations and anions present in the saliva samples collected during chewing. However, this method was very time-consuming. Direct mass spectrometry techniques such as API-MS, on the other hand, use no chromatography but sample the mixture of compounds directly into the source and resolve the ions by their *m*/*z* value only. The key problems are potential suppression of ionization in the source leading to nonquantitative results and equivocal identification of compounds solely on the basis of their *m*/*z* values (*2*). This method is very rapid compared to ionic chromatography (1 to 15 or 30 min/sample, respectively, for cations or anions). This technique was first used to analyze taste compounds released in-mouth such as sucrose, calcium, and potassium present in saliva samples (*4*). However, this study is based only on the observations obtained from one panelist. We applied this methodology to a more complex model with several taste compounds of different chemical properties incorporated into a model cheese that can be considered as a gelled emulsified matrix, and we used several subjects.

The release profiles obtained by API-MS were compared to those obtained by ionic chromatography in order to validate the API-MS methodology used in our study. Regression analyses were performed for Cl^-, Na^+, K^+, and lactate (24 observations) between the two methods for the four parameters extracted from each curve. Positive and significant relationships were observed between the two methods for all of the parameters (**Table 3**). Thus, the API-MS method yields patterns of release for Cl^-, Na^+, K^+, and lactate similar to those obtained by ionic chromatography. We observed from the regression coefficients (<1) that the analysis by API-MS slightly underestimated the quantity of each compound present in the saliva sample. Regarding the AUC parameter, which represented the total quantity released over 90 s, API-MS resulted in values that were 2 times smaller (the regression coefficients are, respectively, 0.5, 0.51, 0.49, and 0.57 for Cl^-, Na^+, K^+, and lactate). The presence of cations may often cause formation of adducts in the source, resulting in the appearance of an ion at another *m*/*z* value (M + 23 for a sodium adduct, for instance) (*2*). This phenomenon may cause underestimation of the quantity of "free" cations. Another effect is that of signal suppression of organic compounds due to the presence of minerals, which has also been reported for samples containing biological fluids. The same authors showed that even small amounts of minerals in saliva caused highly significant decreases in the signal intensity of organic compounds. Separation of salts from saliva could be achieved by using a C_{18} capillary column between the Rheodyne valve and the source (*2*). This created sufficient retention of the organic compounds so that most of the salt was eluted rapidly and effectively removed. In this study, we also used this solution, which may reduce, although not completely resolve, the problem. Considering that API-MS is more a semiquantitative method than a quantitative one, we can conclude that when a great number of samples are required for analysis, it appears as an interesting semiquantitative method to perform a rapid and reliable screening of the interindividual differences. However, although the use of HPLC multiplies analysis time by a factor of 15 or 30, it remains the reference method to obtain reliable quantitative results. Thus, for Cl^-, Na^+ and K^+, we present data obtained by HPLC. In the case of lactic acid, we present data obtained by API-MS because variation coefficients were smaller than with HPLC.

NVC Release during Eating. Contrary to the work of Pionnier et al. (*1*), in which some aroma compounds of insufficient concentration in the expired air of the subjects could not be analyzed, all of the nonvolatile compounds present in higher concentration in the product were analyzed and quantified either by HPLC or by API-MS during the mastication of the model cheese. In general, the quantity of the NVC in the saliva increased at the beginning of the chewing process, reached a maximum, and decreased more or less rapidly at the end of the mastication even if particular patterns (with two maxima) could be observed for few curves. These profiles were similar to those previously observed (*4*).

Some NVC such as sodium, potassium, calcium, chloride, bicarbonate, and inorganic phosphate are naturally present in saliva (*12*) and therefore cannot be strictly related to the quantity released from chewed food. For such compounds, the quantity measured was the sum of the quantity initially present in saliva and the quantity released from the model cheese and diluted in the saliva. However, we cannot exclude the concentration variation in saliva of these taste compounds during eating; consequently, we decided for all data not to subtract the initial offset of concentration in saliva.

Average of Release Profiles. The subject factor of the analysis of variance was highly significant ($p < 0.0001$) for all analyses. We focus on the compound effect in the following discussions. Considering the T_{max} parameter, no significant effect ($F = 0.54$, $p = 0.87$) of the type of nonvolatile compound could be observed. As in the aroma release study (*1*) (where T_{max} ranged from 33.4 to 41.3 s), this parameter belonged to the same range order in the NVC release study (from 40 s for potassium to 31 s for glutamate) (**Table 4**). The T_{max} parameter observed was in the same order range. Our results are in agreement with those of Davidson et al. (*4*), who found no difference in T_{max} ($\approx$47 s) among in-mouth sodium, calcium, and potassium during the chewing of Cheddar cheese or in-mouth sucrose, glucose, fructose, citric acid, and malic acid during the chewing of fresh orange segments. We can conclude that the NVC are all released from the cheese and diluted in saliva in the same way whatever the physicochemical properties (solubility, hydrophobicity, etc.) of the compounds. We can hypothesize that the nature of the

Table 4. Individual Release Parameters (T_{max}, C_{max}, AUC, and Slope) Obtained from Each NVC Release Curve

	Ca				Ci				Cl				Gl				K				La		
subject	T_{max}	C_{max}	AUC	slope $\times 10^3$	T_{max}	C_{max}	AUC	slope $\times 10^3$	T_{max}	C_{max}	AUC	slope $\times 10^3$	T_{max}	C_{max}	AUC	slope $\times 10^3$	T_{max}	C_{max}	AUC	slope $\times 10^3$	T_{max}	C_{max}	AUC
1	33.3	0.28	20.4	4.3	40	0.26	18	6.3	26.7	0.17	13.5	14	40	0.07	5.3	2.5	23.3	0.08	6.8	5.9	30	0.15	11.3
2	23.3	0.09	6.2	3.4	33.3	0.12	8	3.3	36.7	0.36	25.6	22	20	0.05	2.9	2.3	20	0.10	7.6	8.1	26.7	0.15	10.4
3	70	0.16	8.64	1.9	63.3	0.07	3.6	3.2	46.7	0.31	24.9	24	63.3	0.03	1.6	1.9	63.3	0.16	10	6.7	80	0.13	9.2
4	13.3	0.26	9.25	21	13.3	0.33	12	28	16.7	0.44	28.4	42	13.3	0.11	4.2	9.7	13.3	0.09	6.5	8	10	0.22	13.7
5	36.7	0.38	4.2	15	36.7	0.44	31	14	53.3	0.46	37.1	33	36.7	0.16	11	6.8	80	0.12	9.8	8.6	46.7	0.33	24.2
6	13.3	0.1	20.1	8.7	13.3	0.12	3.7	12	20	0.32	21.6	28	10	0.07	2.9	6.8	10	0.11	8	11	30	0.16	11.1
7	40	0.23	14.8	10	26.7	0.28	18	14	23.3	0.67	39.5	38	16.7	0.10	6.2	7.2	56.7	0.14	9.5	8	16.7	0.26	17.4
8	80	0.32	20.1	10	90	0.44	28	13	46.7	0.43	33.7	25	46.7	0.13	9.3	7	53.3	0.11	9.2	8.2	46.7	0.24	18.9
av	**38.7**	**0.22**	**13**	**9.2**	**39.6**	**0.25**	**15.3**	**11.7**	**33.8**	**0.4**	**28**	**28.2**	**31**	**0.09**	**5**	**5.5**	**40**	**0.11**	**8.4**	**8.06**	**35.9**	**0.20**	**14.5**
SD	24.6	0.1	6.7	6.4	25.9	0.1	10	8	14	0.1	8.6	9.1	18.8	0.04	3.3	2.9	26.4	0.03	1.4	1.5	21.9	0.1	5.2

	Le				Mg				Na				Ph				Pho				Pr		
subject	T_{max}	C_{max}	AUC	slope $\times 10^3$	T_{max}	C_{max}	AUC	slope $\times 10^3$	T_{max}	C_{max}	AUC	slope $\times 10^3$	T_{max}	C_{max}	AUC	slope $\times 10^3$	T_{max}	C_{max}	AUC	slope $\times 10^3$	T_{max}	C_{max}	AUC
1	43.3	0.025	1.7	8.3	43.3	7.5	0.55	1.4	43.3	0.12	9.7	4.6	33	0.016	1.1	0.5	43.3	0.13	9.2	8.5	46.7	0.03	2.57
2	30	0.015	1	7.1	20	3.5	0.23	1.7	20	0.09	6.2	4.7	40	0.010	0.7	0.4	60	0.16	10.6	5.8	30	0.047	2.67
3	63.3	0.010	0.5	5.3	20	11	0.51	9.1	63.3	0.11	7.9	5.8	63	0.005	0.3	0.3	63.3	0.10	6.8	7.1	80	0.065	4.33
4	13.3	0.036	1.4	34	13.3	8.7	0.28	6.5	13.3	0.14	8.2	13	13	0.019	0.8	1.6	13.3	0.27	14.6	26	16.7	0.048	3.11
5	46.7	0.054	3.8	26	36.7	13	0.91	5.6	40	0.19	15	12	47	0.03	2.4	1.5	46.7	0.54	34.5	13	46.7	0.062	5.06
6	10	0.016	0.8	16	10	3.4	0.16	3.4	10	0.09	5.8	9.1	13	0.010	0.6	1	13.3	0.11	6.7	28	13.3	0.037	2.21
7	33.3	0.032	2.1	21	40	13	0.92	4.1	33.3	0.15	10.4	7.6	33	0.019	1.1	1	26.7	0.35	20.3	14	13.3	0.094	4.62
8	46.7	0.048	3.4	20	70	8.9	0.61	3.8	46.7	0.15	12	8.5	47	0.031	2.2	1.3	43.3	0.34	24.7		46.7	0.054	4.06
av	**35.8**	**0.03**	**1.84**	**17.2**	**31.7**	**8.6**	**0.52**	**4.45**	**33.7**	**0.13**	**9.4**	**8.16**	**36**	**0.018**	**1**	**0.95**	**38.7**	**0.25**	**15.9**	**14.6**	**36.7**	**0.055**	**3.58**
SD	18	0.016	1.2	10	19.9	3.8	0.3	2.6	18.3	0.03	3.1	3.2	17	0.01	0.8	0.5	19.3	0.2	9.9	9	23	0.02	1.1

[a] Ca, calcium; Ci, citric acid; Cl, chloride; Gl, glutamic acid; K, potassium; La, lactic acid; Le, leucine; Mg, magnesium; Na, sodium; Ph, phenylalanine; Pho, phosphates; Pr, propanoic acid; T_{max}, time to reach maximum concentration; C_{max}, maximum concentration; AUC, area under the curve; slope, initial gradient of the curve measured between 0 and 10 s.

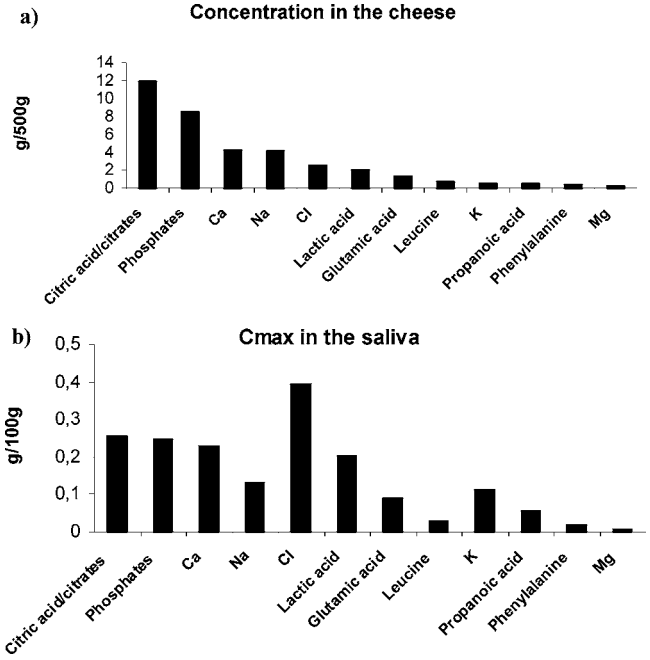

Figure 1. Concentration of each nonvolatile compound in the cheese (grams per 500 g of model cheese) and in the saliva (C_{max}) (grams per 100 g of saliva).

food matrix affects in the same way the time to reach the maximum concentration in saliva for the 12 compounds analyzed.

In contrast, C_{max}, AUC, and slope parameters showed a significant effect of the nature of the compound ($F = 103$, $p < 0.0001$, for C_{max}; $F = 115$, $p < 0.0001$, for AUC; and $F = 106$, $p < 0.0001$, for slope). During the eating of the model cheese, the maximum concentration of each nonvolatile compound of the model cheese present in saliva was as follows: chloride, 0.396; citrates, 0.254; phosphates, 0.250; calcium, 0.228; lactate, 0.202; sodium, 0.132; potassium, 0.114; glutamate, 0.09; propanoate, 0.055; leucine, 0.039; phenylalanine, 0.018; and magnesium, 0.008 g/100 g of saliva (**Table 4**). Chloride was the most concentrated compound in saliva and magnesium the least concentrated. **Figure 1** illustrates the quantity of each nonvolatile incorporated into the cheese and the C_{max} present in the saliva. We observe that it is not necessarily the most highly concentrated compound in the cheese that is the most concentrated in the saliva. The permanent presence of some taste compounds in saliva could explain this observation. Moreover, that could be also explained by some specific distributions of each NVC in the different phases of the bolus (water, proteins, and fat).

Concerning sensory perception of the NVC, detection thresholds were measured for each panelist for Na^+ (using NaCl) and lactic acid (largely responsible for salty and sour tastes, respectively). Values ranged from 0.003 to 0.027 g/100 g of water for NaCl and from 0.0036 to 0.0147 g/100 g of water for lactic acid among subjects. The nonvolatile concentration collected from saliva during chewing was thus higher than detection thresholds; therefore, the compounds could be detected by the panelists during sensory analysis of the cheese.

Interindividual Differences. Great interindividual variation in release profiles was observed between subjects. Subject effect was significant ($p < 0.1$) for all compounds and all subjects except for T_{max} of Cl^- and slope of Mg^{2+}. The individual release parameters for each NVC are reported in **Table 4**. **Figure 2** represents chloride, calcium, leucine, phosphates, and potassium release profiles for four subjects. Considering chloride, calcium, leucine, and phosphates, subjects 4 and 8 were the two for whom the C_{max} in saliva was the highest; the opposite behavior was observed for subject 3. Ranking order for the subjects was different depending upon whether C_{max} values from the aroma release profiles (*1*) or the NVC release profiles were examined. For instance, considering subject 8, the aroma release was very low in comparison with the other subjects, whereas this is not the case for the NVC release. We can thus hypothesize that different oral mechanisms are involved in the aroma and nonvolatile releases. The quantity of each nonvolatile present in the saliva was not always negligible after a 90-s period of nonvolatile release monitoring although the chewing process ended at 37.2 s on the average. In the case of potassium, it seemed that the quantity in saliva did not change during the eating process. The concentration of K^+ released during chewing is likely lower than the concentration of K^+ in saliva.

Although different patterns of release were observed according to the panelist, nonetheless, for a given panelist, the shapes of the curves were similar for most of the 12 NVC (**Figure 3**). This similarity is observed on the principal component analysis (PCA) (**Figure 4**), showing the numerous correlations between the nonvolatile molecules concerning each release parameter (C_{max}, T_{max}, AUC, and slope). It was probably due to their rather good solubility in aqueous medium.

Relationships between Nonvolatile Release and Oral Parameters. We investigated the relationships between nonvolatile release profiles and some oral parameters: salivary flow, swallowing frequency, chewing rate, chewing time, mean work, total work, masticatory performance, mean voltage, and number of chews during the mastication of the model cheese. The results of the oral parameters measured on each subject are reported in part 1 (*1*). Because breathing was not hypothesized as taking part in the release of the nonvolatile molecules (contrary to aroma compounds), we did not include respiratory flow and frequency in the variables potentially involved in the nonvolatile compound release.

Due to certain correlations between the masticatory and salivary parameters, some difficulties in the interpretation of the results of the regression analyses could appear. Thus, we first performed a PCA (done on the correlation matrix) on the masticatory and salivary variables representing the chewing process and retained the first two axes, which explained 75% of the variance (*1*). Regression analyses were then performed using new noncorrelated variables, the coordinates of the subjects on these axes. The first axis represented the amplitude of muscles activities (mean and total work of the masticatory muscles and mean voltage of each burst) and number of chews. The second axis represented chewing rate, salivary flow, masticatory performances, and swallowing rate (on the positive side) opposed to chewing time (on the negative side). We present all of the variables for which the p value was <0.15 (**Table 5**). The model explaining some of the variations observed in the T_{max} values was composed of the second axis of the PCA with a negative regression coefficient: indeed, for Na^+, Ca^+, K^{2+}, and leucine, the subjects for whom the T_{max} was high were also the subjects for whom we observed high values for chewing time and small values for salivary flow, chewing rate, swallowing frequency, and masticatory performances. A trend was found where a long chewing time leads to a longer duration to reach the maximum concentration associated with low chewing, low swallowing rate, and low salivary flow. Indeed, a low

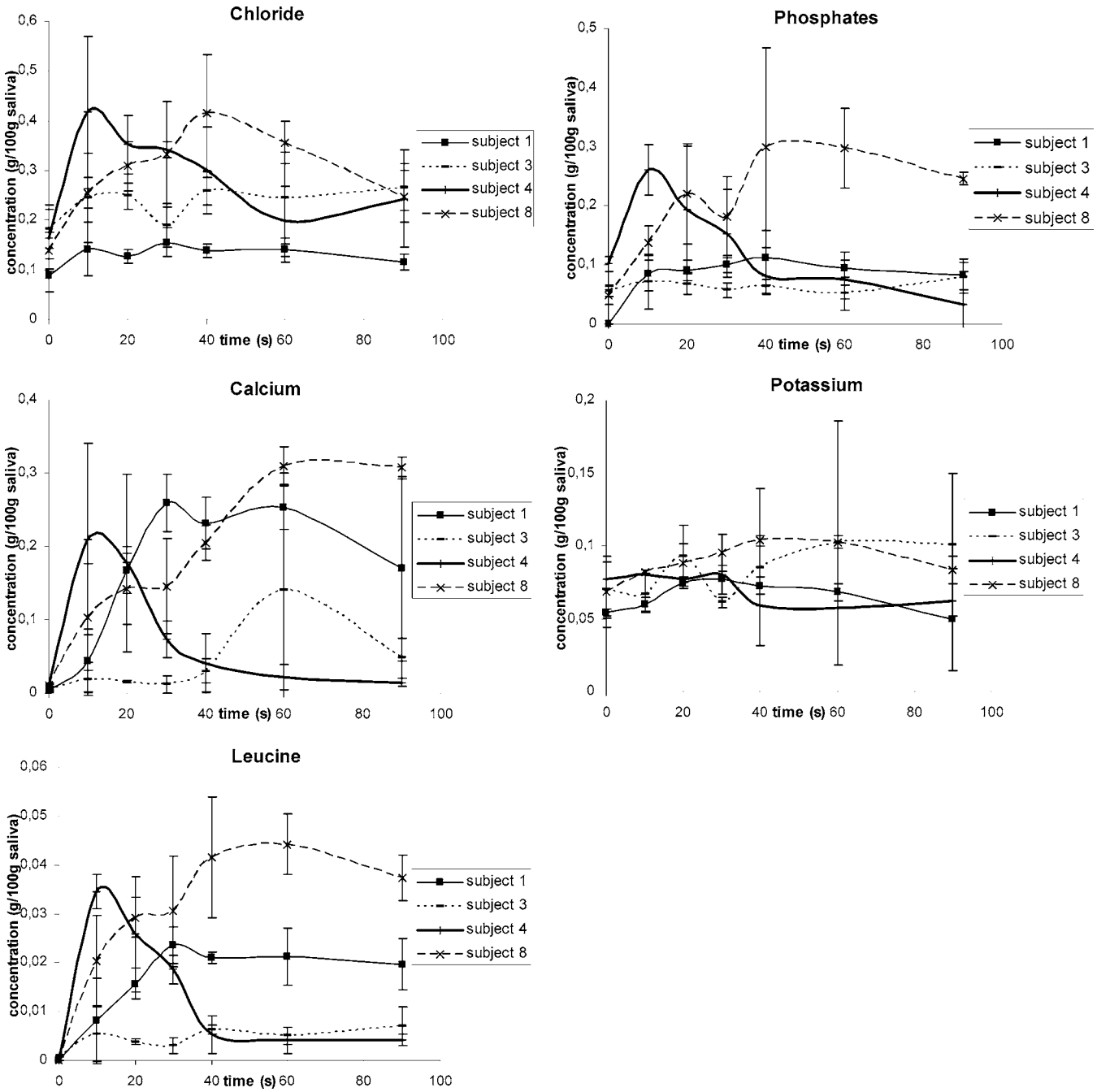

Figure 2. Variation in the concentrations of chloride, calcium, leucine, phosphates, and potassium in saliva during the eating of the processed cheese. Data obtained were not smoothed. Standard deviations were reported for the times corresponding to the saliva sampling times.

chewing rate involves a long chewing time, and a low salivary flow involves a low swallowing rate. If the salivary flow is low, more time is needed to extract the maximum concentration of NVC from the matrix during eating; therefore, T_{max} is longer.

Additionally, the AUC of K^+ was also negatively related to the second axis of the PCA. As AUC represented the total release of nonvolatiles over a 90-s period, this suggested that the total release of this compound was higher in some subjects who have long chewing times and low chewing frequencies, low salivary flows, low swallowing frequencies, and low masticatory performances.

A trend was found for the slope of Ca^{2+} to be positively related to the second axis ($p = 0.15$), meaning high release gradient (between 0 and 10 s) involved low chewing times and high chewing rates, high salivary flows, high swallowing rates, and high masticatory performances. However, this last relationship seemed to be due to one particular observation.

No relationship was found between C_{max} and some oral parameters. The few studies investigating the influence of oral parameters on flavor release have focused on aroma release; thus, it was difficult to make relevant comparisons with the literature.

In conclusion, the release of NVC during the eating process of a complex model cheese in relation to individual saliva and chewing parameters was investigated to clarify interindividual variability observed in flavor release. As in the aroma release study (*1*), the great interindividual variation observed in the release parameters could be related to the interindividual variation observed in several oral parameters. However, different oral parameters were involved in aroma and nonvolatile release.

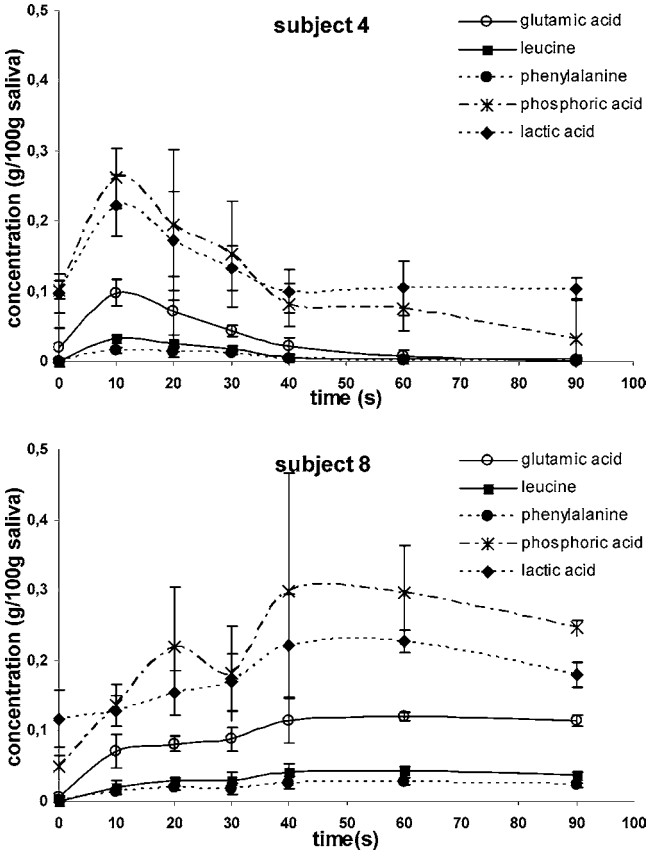

Figure 3. In-mouth phenylalanine, glutamic acid, leucine, phosphoric acid, and lactic acid concentrations (grams per 100 g of saliva) recorded for two panelists during eating of the processed cheese. Data points are the mean of three replicates. Standard deviations were reported for the times corresponding to the saliva sampling times.

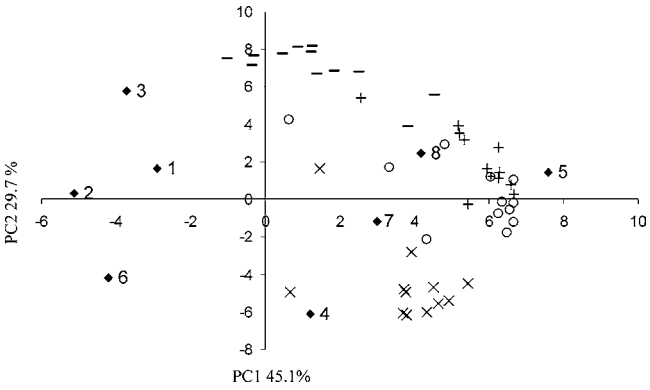

Figure 4. Principal component analysis (PCA) performed on the release parameters [T_{max} (–), C_{max} (○), AUC (+), and slope (×)] concerning the 8 subjects (□) and the 12 NVC.

Concerning NVC release, high T_{max} and AUC values could be related to high chewing time and low saliva flow rate, low chewing rates, low masticatory performances, and low swallowing rates. The relationships between flavor release and flavor perception during eating of the model cheese will be discussed in Pionnier et al. (*13*).

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Table 5. Relationships between the NVC Release Parameters and the Regression Model Constituted by PCA Axis 2[a]

parameter	PCA axis 2 *P* value
T_{max}(Ca)	0.12
T_{max}(K)	0.14
T_{max}(Le)	0.15
T_{max}(Na)	0.11
AUC(K)	0.038
slope(Ca)	0.15

[a] This axis represented chewing rate, salivary flow, masticatory performances, and swallowing rate (on the positive side) as opposed to chewing time (on the negative side). Model tested: parameter = *a* PCA axis 2 + *b*; T_{max}, time to reach maximum concentration; AUC, area under the curve; slope, initial gradient of the curve measured between 0 and 10 s.

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