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Impact of Hardness of Model Fresh Cheese on Aroma Release: In Vivo and in Vitro Study

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Using atmospheric pressure chemical ionization−mass spectrometry, aroma release was investigated in vivo and in vitro from three cheese-like gels with different hardnesses. In vivo, nosespace experiments were performed with 14 subjects. Results showed that the harder gel induced a greater and a faster release of all aroma compounds. In vitro, aroma release was followed in a mouth simulator where breakdown was mechanically produced. The same rate of stirring was applied to the three gels. In these conditions, we found that the amount of aroma released from the three gels was not discriminant. Thus, modification of gel structure had a strong impact on in vivo aroma release, but structural variations alone were not sufficient to induce a greater release. Natural breakdown provided by panelists during food consumption and adapted to the texture of the food was proposed to be the key parameter affecting in vivo aroma release.

KEYWORDS: Aroma release; structure; APCI-MS; nose space; mouth simulator; air/matrix partition coefficient

INTRODUCTION

The perception of aroma during food consumption is mainly dependent on the amount of aroma compounds reaching the sensory system as a function of time. The availability of these compounds results from a complex process involving their release from the food into the gas phase within the oral cavity and their transport via the retronasal route to the nasal cavity where they interact with olfactory receptors. As a consequence, aroma release is one of the critical factors governing aroma perception.

Aroma release is influenced by both thermodynamic and kinetic factors. Thermodynamic factors determine the partitioning of volatiles between the food and the air phases under equilibrium conditions. Kinetic factors influence the rate at which equilibrium is achieved and are connected to transport phenomena into the food matrix and to mass transfer at the air/matrix interface. Static headspace analysis is commonly carried out to determine the air/food partition coefficients of aroma compounds. However, the eating process is dynamic by nature, and equilibrium is not achieved in the mouth. Thus, dynamic studies are necessary for a more reliable description of release from food. In particular, several studies showed that a higher intensity of aroma perception was linked with a higher release rate of aroma compounds in the mouth (*1*, *2*).

Different approaches may be used to study the kinetics of aroma release. The most simple system monitors the headspace composition by gas chromatography before equilibrium is reached (*3*). However, this approach does not reflect the actual release in the mouth. Therefore, using atmospheric pressure chemical ionization−mass spectrometry (APCI-MS) or proton transfer reaction−mass spectrometry (PTR-MS), in-nose measurements have been developed (*4*, *5*). Their advantage is to take physiological factors affecting aroma release into account: dilution with saliva, dilution with air from breathing, and breakdown of food structure induced by chewing. Numerous studies showed that these techniques were sensitive enough to investigate aroma release (*6*−*8*). However, because of the "panelist-specific" physiological factors involved, in-nose data are subject to large interindividual differences and their analysis is complex. Thus, to avoid this complexity and to specifically study some factors influencing aroma release, systems simulating mouth conditions have been developed (*9*−*11*). They were shown to be useful tools to simulate a range of real conditions (*12*).

Aroma release is affected by the composition (nature and concentration of ingredients) and the rheological properties of food. In vitro, several studies showed that an increase in food viscosity induced a decrease in aroma rrate (*13*, *14*). These results were explained both by a decrease in the mobility of aroma compounds and by retention of aroma compounds through molecular interactions with macromolecules. Boland et al. (*2*) observed in vivo that an increase in pectin and gelatin gel hardness resulted in an increase in the quantity of aroma compounds released and a decrease in the rate of release. Mestres et al. (*8*) showed that the increase in whey protein gel

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hardness did not influence the quantity of aroma compounds released in vivo but induced a decrease in the rate of release. Lethuaut et al. (*15*) found that the amount of aroma released was largely unaffected by the texture of custard desserts. Saint-Eve et al. (*16*) observed a higher amount of aroma released from yogurts prepared with a higher mechanical treatment. The different results found in the above-mentioned works indicate that the effect of structural modifications on aroma release depends on the type food. It is thus still difficult to establish a common hypothesis to explain the influence of food structure on aroma release.

In this context, the aim of this work was to study the release of aroma compounds from model fresh cheese and to investigate how it is influenced by structural variations. Three mixed milk gels with identical protein concentrations and differing only in the amount of chymosin were considered in this study. This allowed us to study aroma release from products with differences in structure and in texture but without major modifications of the composition. First, air/matrix partition coefficients ($K_{G/M}$) were determined to evaluate the differences in aroma release under static conditions. Second, in-nose measurements were performed to study the release of aroma compounds during consumption. Finally, aroma release was analyzed using a mouth simulator to focus on the effect of food structure by suppressing inter- and intraindividual variations affecting aroma release. For both experiments conducted in-nose and in the mouth simulator, the release of aroma compounds was monitored using APCI-MS.

MATERIALS AND METHODS

Preparation and Characteristics of Model Fresh Cheeses. The first preparatory step consisted of reconstituting a "composition-controlled" protein dispersion, which we will name "reconstituted milk". In a beaker, micellar casein powder (Promilk 852 B, Ingredia), whey protein powder (PS 90, Cofranlait), and lactose (Jerafrance) were successively, and in that order, dispersed in pure water (Millipore, Milli-Q system, Bedford, MA) under stirring at 120 rpm with an Eurostar power control-visc agitator (Ika-Werke). The agitator was equipped with an anchor-shaped body. Each powder was added very gradually. Once all of the powders had been added, the reconstituted milk was pasteurized in a water bath at 85 °C for 5 min and then immediately placed in ice. Finally, the mixture was stored until the next day in a refrigerator at 4 °C.

After storage at 4 °C, the reconstituted milk was placed at ambient temperature for about 45 min. NaCl (Prolabo), yeast extract (Soredab), and a mineral solution (Soredab) were then added under magnetic stirring. The mixture was then inoculated with starter cultures (*Lactococcus lactis* ssp. *lactis biovar diacetylactis*) and renneted with different quantities of chymosin (Chymax, Chr Hansen). Finally, the aroma formulation (Soredab) was added. The preparation was poured into containers, which were closed and left for 24 h at 34 °C for gelation. Gels were then stored at 4 °C and analyzed within 5 days. For the determination of partition coefficients, samples were poured into headspace vials. The vials were then sealed using silicone septa in metallic caps (VWR International, Austria). For nosespace measurements, samples were prepared in ice cube molds in order to obtain 5 g gel cubes to be given to the panelist. For the mouth simulator study, samples were prepared in 54 mm diameter plastic cups according to the dimension of the simulator (*17*).

For safety reasons, prior to consumption, the absence of *Listeria*, *Salmonella*, and total coliform bacteria was verified from each preparation by the Laboratoire Départemental de Côte d'Or (Dijon, France). The compositions of the three gels are presented in **Table 1**. This composition was identical for the three products, except for chymosin. The concentrations of each aroma compound added in the products are reported in **Table 2**.

Rheological properties of the gels were analyzed by penetrometry tests. Measurements were performed at 5 °C (storage temperature) using a TA-XT2 Texture Analyzer (Stable Micro Systems) fitted with a 10 mm diameter cylinder probe, which penetrated the samples at 1 mm/s for a distance equivalent to 30% of the initial sample height. Five replicate measurements were performed for each gel. From the force−displacement curve, the maximum force (F_{max}), which represents the hardness of the gel, was extracted. Values of F_{max} are presented in **Table 1**. The rheological hardness was significantly different between the three gels: M0 was the softest gel, and M10 was the hardest one. The three gels had the same Dornic acidity (163 °D) and pH (4.6). The quantity of serum present at the surface of the gel was measured, and the rate of syneresis [% (w/w)] was expressed as the ratio between this quantity and the quantity of inoculated reconstituted milk. This rate was 0.48 for M0, 2.2 for M3, and 3.9 for M10. These properties did not change significantly over the 5 days during which the gels were used. Using Likens−Nickerson extraction coupled with gas chromatography analysis, we checked that the concentration of aroma compounds (relative to an internal standard) was not significantly different in the three gels.

Table 1. Quantity of Materials Used for the Preparation of 1 kg of the Three Gels and F_{max} Values of These Gels[a]

composition for 1 kg	M0	M3	M10
micellar casein (g)	75	75	75
whey protein (g)	25	25	25
lactose (g)	40	40	40
water (g)	q.s.p. 1 kg	q.s.p. 1 kg	q.s.p. 1 kg
NaCl (g)	10	10	10
yeast extract (g)	2	2	2
mineral solution (mL)	1	1	1
starter culture (mL)[b]	0.2	0.2	0.2
chymosin (μg)	0	3	10
blend of aroma (mL)	0.9	0.9	0.9
F_{max} (N)	0.53 a	1.51 b	2.11 c

[a] Different letters (a–c) indicate that means are different at $p < 0.05$ (SNK test). [b] 0.75×10^{10} CFU/mL.

Table 2. Aroma Compound Concentration Added in the "Reconstituted Milk" and Characteristic Ions (*m*/*z* Values) Used To Monitor Their Release by APCI-MS[a]

aroma compound	concentration (mg/kg)	*m*/*z* value
3-methylbutanal	1.27	69
oct-1-en-3-ol	2.54	111
octanal	0.038	ND
heptan-2-one	2.89	115
ethyl butanoate	0.95	117
hexanoic acid	24.5	117
nonan-2-one	2.89	143
ethyl hexanoate	0.95	145
diacetyl	23.69	ND
phenylacetaldehyde	9.5	ND

[a] ND means that the compound was not detected.

Determination of $K_{G/M}$ Values. $K_{G/M}$ values were determined at 30 °C using the phase ratio variation (PRV) method to investigate the intrinsic capacity of the gels to retain aroma compounds within their structure. $K_{G/M}$ was defined as the ratio of concentrations of the aroma compound in the gas phase and the matrix at equilibrium. The PRV method permitted the determination of partition coefficients without external calibration. Ettre et al. (*18*) established the following relation between the chromatographic peak area at equilibrium (A) and the ratio V_G/V_M (β):

$$1/A = a\beta + b \quad (1)$$

Equation 1 represents a linear equation where the only variables are the phase ratio (β) and the peak area (A). The parameters a and b are obtained by plotting $1/A$ against β, and the value of $K_{G/M}$ is calculated as the ratio a/b.

Increasing quantities (0.1, 0.2, 0.4, 0.7, 1, 2, and 5 g) of each sample were poured into glass vials of 20 mL (Supelco, United States) in order

to obtain different phase ratios β. Only one injection was performed per vial, and three vials were analyzed for each ratio to obtain three replicate measurements. Different media aromatized at 0.9 mL/kg were analyzed as follows: the three gels (M0, M3, and M10), water, and the reconstituted milk (composed of water, proteins, and lactose).

The vials were placed at 30 °C in the incubator of an automatic headspace sampler MultiSampler2 (Gerstel, RIC France). After a 2 h equilibrium period, 1 mL of headspace sample was automatically taken using a gastight syringe (Hamilton, Switzerland) preheated to 30 °C and injected at a rate of 200 μL/s in a G1530A gas chromatograph (Agilent Technologies, United States) equipped with a flame ionization detector (GC-FID). Injector and detector temperatures were set at 240 and 250 °C, respectively. Separations were performed on a capillary DB-Wax column (30 m × 320 μm, df = 0.25 μm, J&W Scientific, United States). The carrier gas was helium (velocity of 22.4 cm/s at 143 °C) at a constant pressure. The oven temperature was programmed from 40 to 240 °C at increments of 5 °C/min with initial and final hold times of 5 and 10 min, respectively. From each series of seven volumes, a value of $K_{G/M}$ was determined, and the mean of the three values was considered as the $K_{G/M}$ of the compound.

APCI-MS: Experimental Conditions. *General APCI-MS Conditions.* On-line measurements were performed in vivo and from a mouth simulator to study the influence of structural variations on the dynamic release of aroma compounds from flavored mixed milk gels. They were carried out using an ion trap mass spectrometer Esquire-LC (Bruker Daltonique, France) equipped with a laboratory-built interface designed to optimize gaseous sampling through a Venturi effect (*19*). The sample was introduced into the source via a fused silica capillary tubing (i.d. = 0.53 mm) inserted into a heated transfer line maintained at 150 °C to avoid water condensation. The fused silica tubing was inserted into the Venturi region via a capillary adjustment device that allowed the inlet flow rate to be precisely adjusted to 30 mL/min, a value corresponding to an optimal signal-to-noise ratio. Volatile compounds were ionized by a 4 kV positive ion corona pin discharge and detected at *m*/*z* values corresponding to their protonated molecular ions (MH^+) or, in the case of alcohols and aldehydes, to their dehydrated protonated molecular ions [$(MH - H_2O)^+$]. APCI-MS analysis of the headspace of aqueous solutions containing each compound individually allowed the assignment of characteristic ions (*m*/*z* values) for each aroma compound. These *m*/*z* values are reported in **Table 2** only for compounds detected during nosespace experiments from the gels.

For each study, standardization of APCI-MS signal was performed before each session. The headspace of a 250 mL aqueous solution of heptan-2-one (15 ppm) contained in a 20 L Teflon bag inflated with 17 L of nitrogen was analyzed.

Specific Conditions for in Vivo Measurements. Fourteen volunteers (nine females and five males aged between 19 and 53) participated in the study. They were instructed not to smoke, eat, drink, or use any persistent-flavored product for at least 1 h before the session.

For each panelist, two sessions were organized, and for each session, four replicates were performed for each of the three gels. Products were presented in random order. For each run, a gel cube of 5 g previously stored for 1 h at 15 °C was eaten. Between two samples, panelists were asked to clean their mouths with bread, apple, and water.

A fixed eating protocol was used in order to reduce interindividual variations. After positioning the glass tube in one nostril, panelists were asked to breathe regularly for 30 s. This period (breath-blank phase) was used to record the potential residual signal of the previous sample and to control the regularity of breathing. Indeed, acetone (*m*/*z* 59), present in human breath as a result of hepatic fat metabolism, was continuously monitored as an indicator of the panelist's breathing patterns. Panelists were then instructed to place the sample in their mouth and to keep their mouth closed for 12 s. They subsequently chewed the product for 20 s while keeping their mouth closed and without swallowing (chewing phase). Finally, they swallowed the bolus and the recording continued for 36 s (post-swallowing phase). At the beginning of the session, panelists were invited to perform a blank run to familiarize themselves with this protocol.

Specific Conditions for in Vitro Measurements in a Mouth Simulator. Gel cylinders (60 g) previously stored at 15 °C were removed from their mold and placed in the simulator. This simulator was developed in a previous study on yogurts and can be described as a 180 mL reactor. The size of the sample was previously optimized to obtain good stirring conditions and to have a ratio between the volume of the product and the volume of the simulator not so far from in-mouth conditions (*17*). Product breakdown was performed by using a rotor with six 45° angled blades in order to simulate shearing actions, forces applied between the tongue and the palate (*20*). Through a liquid circulating in its double jacket, the reactor was placed at 30 °C to simulate temperature in the mouth. At the beginning, the rotor was above the surface of the product. It was then set in motion at a speed of 200 revolutions/min and progressively lowered into the product. After introduction in the product at 6 min, stirring was maintained for 8 min. The shear rate was estimated at 110 s^{-1}, a value in the range of previous estimates of shear rates in the mouth (*21*, *22*). The headspace of the mouth simulator was continuously drawn at 30 mL/min and led into the ionization source. The release of aroma compounds was monitored over a period of 16 min. Four replicates were performed for each gel.

APCI-MS Data Analysis. *In Vivo Data Treatment.* Our objective was to analyze the whole set of individual curves and not to average curves from all of the panelists. From APCI-MS raw curves made of about four points per second, a simplification was first done by averaging intensities of three consecutive points without losing the profile resolution. Data were then imported in R software version 2.0.1 (http://www.R-project.org) for spline smoothing. A value of 20 was chosen for the equivalent number of degrees of freedom in order to only reduce fluctuations due to the breathing pattern. The smoothed curves obtained were predicted with one point every second.

Different parameters were extracted from each individual release curve: I_{max}, which corresponds to the maximum intensity; t_{max}, which corresponds to the time at which I_{max} occurs; and AUC, which corresponds to the area under the curve. These parameters were determined for two phases of the eating protocol: the chewing phase (I_{maxc}, t_{maxc}, and AUC_c) and the post-swallowing phase (I_{maxps}, t_{maxps}, and AUC_{ps}). The AUC was also determined at 5 and 10 s of the release profile (AUC_5 and AUC_{10}) and for the whole release run (AUC_t). AUC_5 and AUC_{10} were preferred to the ratio I_{max}/t_{max} to estimate the rate of aroma release because of the nonlinearity of the release curves between 0 and I_{max}.

In Vitro Data Treatment. Curves were analyzed between 4 and 9 min. They were fitted with a mathematical model developed by Lafarge et al. (*23*) for the analysis of curves obtained in similar conditions. Two parameters were used to describe the release of aroma compounds: rrate, the initial slope of the release curve; and ΔI, the difference between the intensity level after stirring and the initial intensity level.

Statistical Analysis. Analysis of Variance (ANOVA) was performed using the general linear model (GLM) procedure of SAS 9.1 (SAS Institute Inc., Cary, NC). For in vivo data, a two-way model (product, random subject) with interaction was applied to the different in vivo release parameters. For in vitro data, a one-way model (product) was applied to the different parameters (partition coefficient and mouth simulator release parameters). When significant differences were observed between products ($p < 0.05$), product mean intensities were compared using the Student−Newman−Keuls (SNK) multiple comparison test.

RESULTS AND DISCUSSION

***$K_{G/M}$* Values.** Static headspace measurements were performed using the PRV method to determine the $K_{G/M}$ values of compounds constituting the aroma mixture added in the three mixed milk gels. Five of the 10 aroma compounds were volatile enough to be detected in the headspace of the five media. Among these five compounds, the partition coefficient of oct-1-en-3-ol could not be determined because of its low volatility: The air/water partition coefficient of oct-1-en-3-ol is 3.1×10^{-3} at 30 °C (*24*). Indeed, as explained by Ettre et al. (*18*), when the partition coefficient is low, variations in chromatographic peak area induced by the different ratios β are too small and the PRV method is not suitable. Therefore, only the partition

Table 3. Air/Water Partition Coefficients ($K_{G/M} \times 10^2$) of Aroma Compounds at 30 °C in Comparison with Literature Values ($K_{G/M,lit} \times 10^2$) and Their Physicochemical Characteristics

aroma compound	$K_{G/M}$	$K_{G/M}$,lit	logP[a]	solubility in water at 25 °C (g/L)	binding constant with β-lactoglobulin (M^{-1})
heptan-2-one	1.2	0.57 [25 °C (*34*)]	2.2	4.3 (*34*)	330 (*3*)
nonan-2-one	2.0	1.5 [25 °C (*35*)]	2.9	0.47 (*36*)	2440 (*3*)
ethyl butanoate	2.4	1.83 [30 °C (*37*)] 2.80 [37 °C (*38*)]	1.8	5.6 (*39*)	55 (*40*)
ethyl hexanoate	4.8	3.02 [30 °C (*37*)] 4.80 [37 °C (*38*)]	2.8	0.52 (*39*)	543 (*40*)

[a] Hydrophobicity (logP) calculated by the method of Rekker (*33*).

coefficients of ethyl butanoate, heptan-2-one, ethyl hexanoate, and nonan-2-one were obtained. For these compounds, correlation coefficients R^2 higher than 0.98 were found for the linear regressions between $1/A$ and β (eq 1). Values obtained in water were comparable with data from the literature (**Table 3**). Ethyl hexanoate is the most volatile compound, and esters are more volatile than ketones. Within the same chemical class, volatility increased with the increasing number of carbons in the molecule (25). This is due to a decrease in polarity of the molecules illustrated by the increase in logP value and the decrease in water solubility (**Table 3**). When partition coefficients were compared between water, the reconstituted milk, and the three gels, two different behaviors were observed among the four aroma compounds (**Figure 1**). For ethyl butanoate and heptan-2-one, $K_{G/M}$ values were not significantly different between the five media. For ethyl hexanoate and nonan-2-one, the significantly highest value of partition coefficient was found for the water system. In the case of gel systems, the partition coefficient values were significantly lower than for water and reconstituted milk systems, and no significant differences between the values obtained for the three gels were found.

For the most hydrophobic compounds, ethyl hexanoate and nonan-2-one, the decrease in partition coefficient between the water and the reconstituted milk sample may be explained by molecular interactions with proteins. Indeed, it is largely known that milk proteins, β-lactoglobulin, or caseins (*26*, *27*) are susceptible to interact with aroma compounds at the molecular level thereby reducing their volatility. In addition, interactions between aroma compounds and proteins were shown to be hydrophobic because their intensities increased when the chain length increased (*28*). The hydrophobic nature of molecular interactions explains why the volatilities of ethyl butanoate and heptan-2-one, the less hydrophobic compounds, were not affected by the addition of proteins. It is corroborated by the lower values of the binding constant between β-lactoglobulin and heptan-2-one or ethyl butanoate in comparison with nonan-2-one and ethyl hexanoate (**Table 3**).

During acidification, the protein structure is highly modified and proteins aggregate to form a three-dimensional network. Therefore, both the modification of interactions between aroma compounds and proteins and the trapping of aroma compounds in the network may cause an increase in the retention of ethyl hexanoate and nonan-2-one in the gels.

On-line Study of the Release of Aroma Compounds by APCI-MS. Six ions were detected (*m*/*z* 69, *m*/*z* 111, *m*/*z* 115, *m*/*z* 117, *m*/*z* 143, and *m*/*z* 145) during APCI-MS experiments. Five of them were unequivocally allocated to one aroma compound. Ion *m*/*z* 117 was allocated to the mixture of ethyl butanoate and hexanoic acid. Using tandem MS, we checked that the contribution of hexanoic acid to the intensity of *m*/*z* 117 signal was negligible.

In Vivo Release of Aroma Compounds during Food Consumption. The three gels were analyzed by nosespace APCI-MS experiments in order to study the release of aroma compounds under real conditions of consumption. Release curves of heptan-2-one obtained from the three gels are presented in **Figure 2**: The M10 gel exhibits a higher release than the M3 and M0 gels. As also observed by Mestres et al. (*8*), different aroma release patterns were found between panelists. For this reason, we decided to extract aroma release parameters from each individual release curve and for two phases of the eating protocol, as described in the Materials and Methods section.

For each aroma compound, a two-way ANOVA (product, random subject) with interaction was performed to study differences between products. The observation from **Figure 2** was confirmed because, with the exception of AUC_5 and t_{maxps}, a significant product effect was found for all other parameters: Higher values of I_{max} (I_{maxc} and I_{maxps}), AUC (AUC_{10}, AUC_c, AUC_{ps}, and AUC_t), and t_{maxc} were obtained for the harder gel M10. The same results were obtained for all volatile compounds; therefore, differences between products will be presented using heptan-2-one data (**Figure 3**).

The higher $I_{max(c,ps)}$ and $AUC_{(c,ps,t)}$ values in the M10 gel as compared to the M0 gel means that the amount of aroma

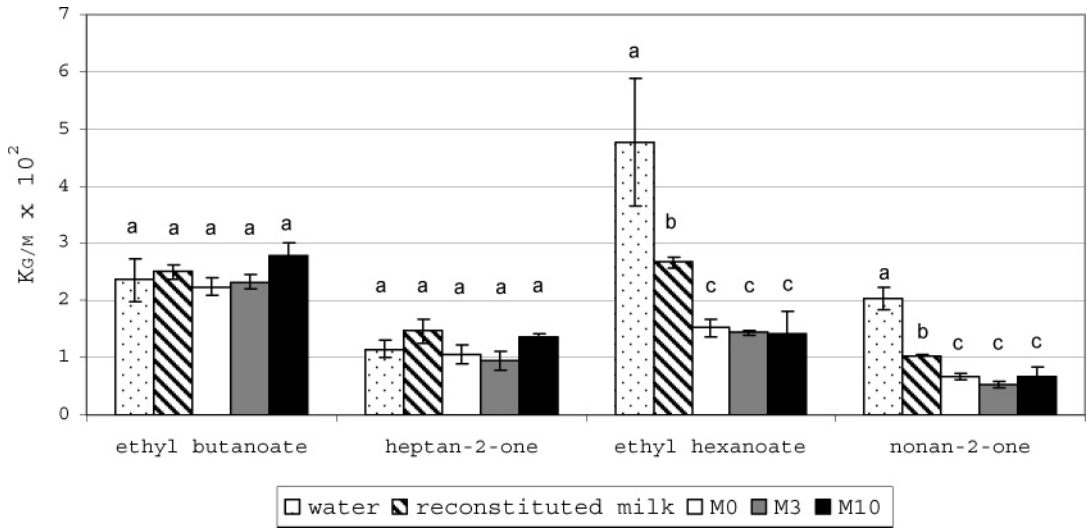

Figure 1. $K_{G/M}$ values determined at 30 °C using the PRV method. Different letters (a–c) indicate that means are different at $p < 0.05$ (SNK test).

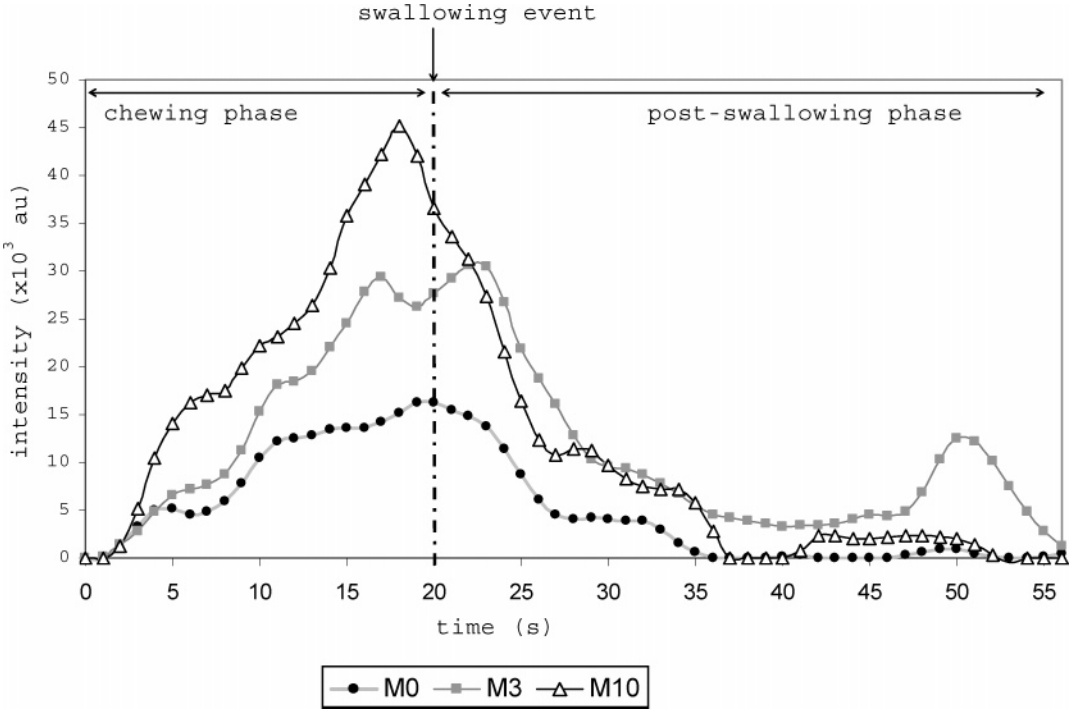

Figure 2. Aroma release profile of heptan-2-one obtained by nose space APCI-MS experiments with M0, M3, and M10 gels. Example of one replicate release curve of one panelist.

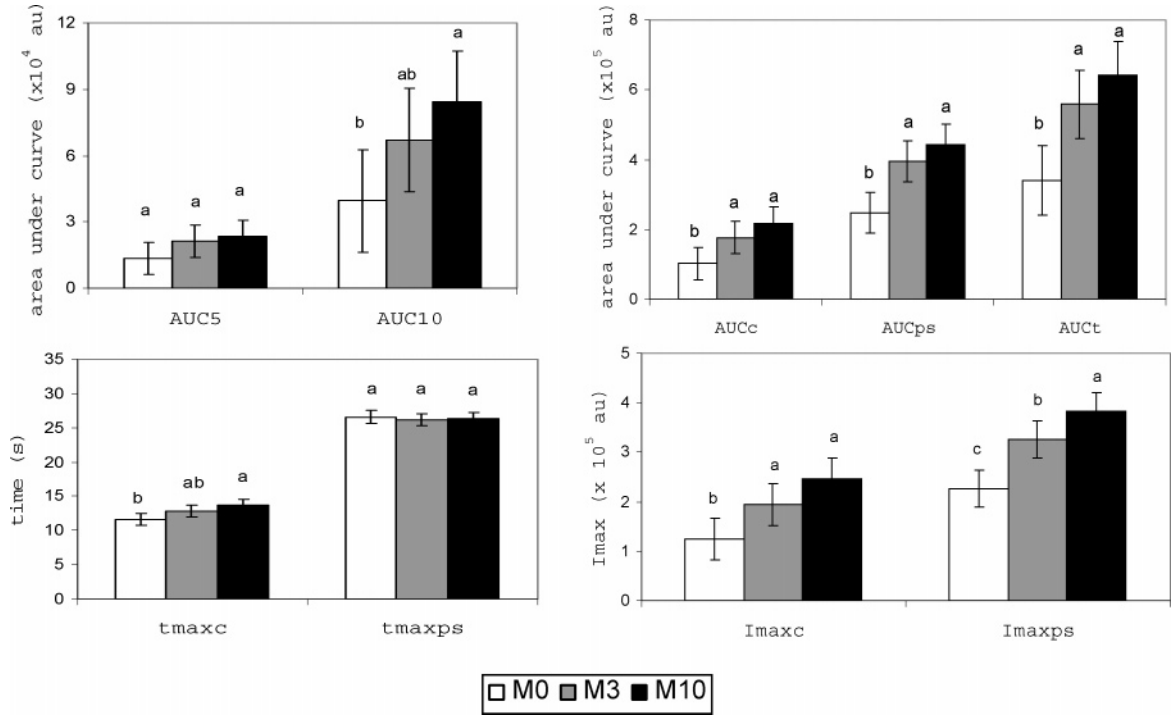

Figure 3. In vivo aroma release parameters: differences between the three gels. Mean values and 95% confidence intervals. Different letters (a–c) indicate that means are different at $p < 0.05$ (SNK test). Example of heptan-2-one.

released was higher for the harder gel, both for each individual phase of the eating process (c, chewing phase; ps, post-swallowing phase) and for the whole process (t, total). This result is in agreement with previous studies that showed a more extensive release of volatiles from the more rigid gels (*2*, *29*). The increase in the amount of aroma released from the M0 to the M10 gel is probably linked with the breakdown phenomena occurring in the mouth. Indeed, even if the duration of the chewing period was standardized for the three gels, it is likely that the harder gel required a more intense chewing than the softer gels, which would be in favor of a greater release of aroma compounds.

If we consider only the chewing phase, t_{maxc} was higher for M10. This may be linked to masticatory behaviors: A more intense chewing and a more progressive breakdown of the harder gel would result in a more progressive aroma release. On the contrary, the softer gel M0 could be rapidly transformed into a viscous solution mixed with saliva, a texture similar to a stirred

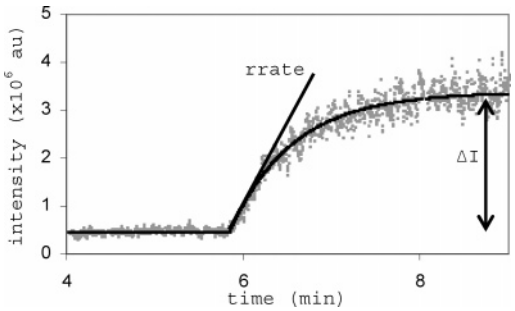

Figure 4. Release curve of ethyl hexanoate from gel M3 obtained by in vitro APCI-MS experiments: raw curve, modeled curve, and extracted parameters.

yogurt. Thus, the bolus state, ready to be swallowed, would be obtained faster for the softer gel. After, its structure probably does not evolve sufficiently to produce aroma release, which results in a lower value of t_{maxc}. During the post-swallowing phase, the three gels presented the same t_{maxps} value. This result may be attributed to the mechanism of swallowing. Indeed, recent studies showed that during the pharyngeal stage of swallowing, the velum is displaced from the back of the mouth to the bottom of the nasal cavity thereby breaking the respiratory flux (*6*, *30*). This allows the transfer of the bolus from the mouth to the pharynx and the esophagus, but it also displaces air charged with aroma compounds to the pharynx. Then, when the velum goes back to its initial position and the first exhalation takes place, air charged with aroma compounds, and present in the pharynx, is transported to the nose. A boost of aroma delivery occurs and is responsible for the maximum observed on the APCI-MS signal just after swallowing (*6*). In our study, as swallowing of the product was imposed on the panelists at 20 s, it is not surprising that the maximum occurs at 26 s, a few seconds later, whatever the structure of the product.

Finally, products were significantly different regarding the AUC_{10} parameter, which was higher for the M10 gel. In other words, the higher the hardness is, the higher the rate of release is. The modification of chymosin concentration resulted in the formation of gels with different structures (*31*, *32*). As a consequence, the gel with the highest amount of chymosin (M10) presented a heterogeneous breakdown in the mouth leading to the formation of several pieces in a continuous phase. Thus, this gel may exhibit a high exchange area with air. On the contrary, the M0 gel was more spreadable and formed a more homogeneous bolus. This difference in the heterogeneity of the bolus may explain the different rates of release observed in this study. Mestres et al. (*8*), also using a fixed eating protocol, observed the reverse phenomenon in the case of whey protein gels. They hypothesized that the softer gel, through a greater breakdown, induced a higher exchange area leading to a faster release. The difference with our study may be attributed to different breakdown of the products, and we may suppose, for instance, that their harder gel is less brittle than ours. Boland et al. (*2*) also observed a decrease in aroma release rate with gel hardness. However, these authors worked with a nonimposed protocol, which made the comparison with our data difficult.

In Vitro Release of Aroma Compounds in a Mouth Simulator. To specifically study the effect of food structure and understand in vivo results, the three gels were placed in a mouth simulator and broken using shearing forces. Aroma release was continuously monitored before and after stirring using APCI-MS. **Figure 4** shows an example of a release curve for ethyl hexanoate: at 6 min, at the beginning of the product breakdown, a significant increase in the release of aroma compounds was

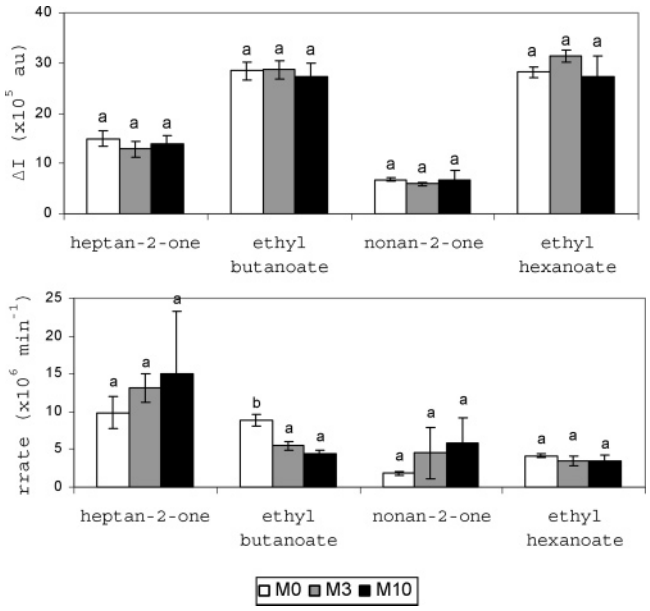

Figure 5. In vitro aroma release parameters: differences between the three gels. Mean values and 95% confidence intervals. Different letters (a and b) indicate that means are different at $p < 0.05$ (SNK test).

observed. Release curves were modeled to obtain relevant parameters (rrate, the slope at the beginning of the stirring; and ΔI, the difference in intensity after and before stirring). Correlation coefficients after application of the model were higher than 0.95 for esters and between 0.60 and 0.95 for ketones. They were considered as reliable values and indicated a good data fit.

During this study, the increase in APCI-MS signal of ions *m*/*z* 111 and *m*/*z* 69 due to stirring was not significant. As a consequence, data were obtained only for heptan-2-one, nonan-2-one, ethyl butanoate, and ethyl hexanoate.

For these four aroma compounds, differences between products are presented in **Figure 5**. For the first parameter ΔI, no differences were observed between products. Whatever the aroma compound, the quantity of aroma released was not influenced by structural variations of the gels. This result differs from those obtained during in vivo experiments where an increase in the quantity of aroma released was observed with an increase in gel hardness. One of our hypotheses was that panelists adapted their mastication patterns to the structure of the product. In particular, we supposed that panelists produced a more intense chewing of the harder gel, which induced a higher aroma release. In the model mouth, breakdown was simulated by applying the same rate of shearing to the three gels and the same amount of aroma was released from the three gels. Therefore, this result tends to confirm that in vivo differences were due to an adaptation of the panelist masticatory behavior rather than to a direct impact of the modification of gel structure.

The rate of release, rrate, was a significantly discriminating factor between the three gels only for ethyl butanoate. For this aroma compound, the slope of the curve decreases from the M0 gel to the M10 gel. In other words, the rate of release decreases with an increase in gel hardness. This result is different from the one obtained in our in vivo study where an increase in the rate of release with gel hardness was observed. It may be attributed to a higher destructuration of the M0 gel in vitro than in vivo. Indeed, in vitro, the softer M0 gel is probably better mixed than the harder M3 and M10 gels, and the surface layers are renewed more rapidly. The fact that only the rate of

ethyl butanoate was significantly different between the three gels may be due to its higher volatility in the gels (**Figure 1**). In vivo, because of the adaptation of the chewing pattern, structural modifications are on the contrary probably greater for the harder M10 gel. The absence of saliva in the model mouth, which affects both the breakdown of the product and the release of aroma compounds, may also contribute to the differences observed between in vivo and in vitro data.

As a conclusion, equilibrium data did not explain aroma release differences under breakdown conditions and when products are broken, change in food structure seems to have a predominant effect on aroma release. Moreover, the in vitro study was useful to underline one of the key parameters explaining in vivo aroma release: the adaptation of the masticatory behavior with food structure. Further investigations such as the characterization of the subjects' masticatory patterns would be needed to confirm our hypothesis.

ABBREVIATIONS USED

APCI-MS, atmospheric pressure chemical ionization−mass spectrometry; PTR-MS, proton transfer reaction−mass spectrometry; PRV, phase ratio variation; $K_{G/M}$, air/matrix partition coefficient; GC-FID, gas chromatography−flame ionization detector; I_{max}, maximum intensity; t_{max}, time to which maximum intensity occurs; AUC, area under curve; rrate, release rate; ΔI, difference between intensity levels; ANOVA, analysis of variance; GLM, general linear model; SNK, Student−Newman−Keuls.

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