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Flavored Yogurt Complex Viscosity Influences Real-Time Aroma Release in the Mouth and Sensory Properties

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The influence of flavored yogurt texture on aroma perception and in-nose aroma release measured by atmospheric pressure chemical ionization mass spectrometry analysis was investigated. The study was carried out on six yogurts varied by protein composition and mechanical treatment. For the same matrix composition, the complex viscosity of yogurts influenced in-nose release and perception. After swallowing, aroma release and intensity of olfactory perception were stronger in low-viscosity yogurts than in high-viscosity yogurts. Moreover, the protein composition influenced aroma release only when yogurts exhibited wide variations of complex viscosity and consequently texture. In mouth, aroma release and perception were influenced more by yogurt mechanical treatment than by protein composition. On the basis of mass transfer analysis, the main physical mechanism which could explain the difference in aroma release would be the surface exchange area developed in the mouth and in the throat.

KEYWORDS: Dairy proteins; aroma release; texture; perception; APCI-MS

INTRODUCTION

Aroma release during food consumption has been acknowledged in numerous studies as a key factor in flavor perception. Indeed, aroma compounds must reach the olfactory epithelium via the retronasal or the orthonasal pathway to be perceived by the consumer. The retronasal pathway delivers the aroma released from food products during chewing and swallowing. Several methods for measuring aroma compound quantity in real time in the nose space of subjects during eating have been developed. The two most commonly used techniques for on-line analysis of air in the nasal cavity during eating are atmospheric pressure chemical ionization mass spectrometry (APCI-MS) and proton transfer reaction mass spectrometry (PTR-MS) (*1*, *2*). In vivo aroma analysis has been used to show the effect of food formulation on aroma release during consumption: effects of fat (*3*), proteins (*4*, *5*), and thickening agents or sweeteners (*6*, *7*). Moreover, in vivo aroma analysis has been used to study the impact of physiological factors such as mouth volume, saliva flow rate, and air flow on the aroma compound profile that reaches the receptors (*8*, *9*). These factors can lead to considerable interindividual differences. These differences have been investigated both by working with a great number of subjects and replicates and by using a mouth model or a throat simulator to control oral physiological variations (*10*, *11*).

Flavor release is one of the mechanisms that may explain differences in flavor perception of products with different structures, leading to differences in texture perception. Some studies have investigated the relationship between the behavior of aroma compound in food matrices (physicochemical interactions and release) and the perception. For that, these studies modified the rheological properties of matrices by composition variation (thickener, fat, or protein). It was shown that nonvolatile constituents can specifically interact with aroma compounds. These physicochemical interactions partly explain the associated modulation of olfactory perception (*12*−*14*), but sensory interactions between texture and aroma can also play a role (*15*). These cognitive interactions have been identified more specifically in studies investigating both on line aroma release and perception during consumption. Thus, although product thickening decreased the perceived flavor intensity, it does not necessarily result in any change in the aroma compound concentrations measured in-nose (*4*, *15*). The perceived texture rather than in-nose flavor concentrations determines the perceived aroma intensity (*4*). Sensory interaction cannot be ruled out when considering the results of the recent study of Boland (*7*). These authors observed that increasing the physical strength of the hydrocolloid gel (gelatin and pectin) decreased the rate of flavor release during in-nose analysis. Consequently, the sensory changes can also be directly explained by the available

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Table 1. Premix Composition for Yogurt Preparation Varying in Protein Composition

ingredients (suppliers)	CAS yogurt	MPO yogurt	WP yogurt
water (Volvic, Danone)	1 L	1 L	1 L
low-heat skim milk powder (Ingredia)	100 g	135 g	100 g
lactose (Ingredia)	21 g	–	21 g
sodium caseinate (Ingredia)	14 g	–	–
whey protein concentrate (Ingredia)	–	–	14 g
anhydrous milk fat (Lactalis)	40 g	40 g	40 g
sugar (sucrose) (Daddy)	50 g	50 g	50 g
protein total content (% in w/w)	5.4%	5.4%	5.4%
dry matter (% in w/w)	22.50%	22.50%	22.50%

aroma compound concentration (*7*). The authors explained their different observations compared to those of Weel et al. (*4*) and Hollowood et al. (*15*) by a difference in protocol with a free chewing procedure. Indeed, mastication could induce a breakdown of the food structure, an increase in the surface area, and, therefore, an increase in the rate of aroma release.

Thus, the influence of the physical properties of model food on flavor perception is not yet completely understood. To the best of our knowledge, previous studies have primarily focused on texture differences due to composition variations and not on mechanical treatment variations, to maintain constant composition and to dissociate the effects.

In a previous study, we showed that the microstructure, the rheological properties, and the aroma release under equilibrium conditions in yogurts vary with a modification of the protein composition (*16*). Variations in these physicochemical characteristics partly explain variations in texture and olfactory perceptions of the flavored yogurts (*17*). However, food consumption is not a static experience. The resulting olfactory perception is based on initial impact, perception during chewing, and perception after swallowing, termed persistence. To take into account this dynamic process and understand its impact on aroma release and olfactory perception, we assessed aroma release in vivo and perception during consumption.

In this context, the objective of this study was to investigate the effect of the complex viscosity on the in-nose flavor release and on the temporal olfactory perception during consumption of a stirred yogurt flavored with a complex aroma. Complex viscosity was varied by using two factors. The first was the protein composition of the yogurt, at a constant protein level. The second was the mechanical treatment that enabled us to modify the complex viscosity while keeping the composition constant.

MATERIALS AND METHODS

Flavored Yogurt Preparation. Six flavored stirred yogurts were prepared. They had the same dry matter (22.5%), fat (4%), and protein (5.4%) content (**Table 1**). To modify the complex viscosity of the products, two variation factors were studied: milk protein composition and mechanical treatment. Three yogurts were obtained by varying only the protein fraction used to fortify the premix: enrichment with sodium caseinate (CAS yogurt), enrichment with milk powder (MPO yogurt), and enrichment with whey protein (WP yogurt). To obtain the three other yogurts, an additional mechanical treatment was applied to the first three products to produce less viscous yogurts. After the fermentation, all six yogurts were pumped from the fermenter through a pipe (1.5 m in length and 6 mm in diameter). This pumping corresponded to the lowest degree of mechanical treatment (MT− yogurts or yogurts with a weak mechanical treatment). A second pumping was applied to three of yogurts through the same type of pipe but ending with a conical tip (0.8 mm in diameter with an angle of 6°) at 4 °C, 1 day after the fermentation. This second pumping led to the highest degree of mechanical treatment (MT+ yogurts or yogurts with a strong mechanical treatment). Yogurt preparation and fermentation conditions were described in detail by Saint-Eve et al. (*16*).

Table 2. Aroma Compound Concentrations in Yogurts and Their *m*/*z* (molecular weight per charge) Values Corresponding to Their Protonated Molecular Ions

aroma compound	concentration in yogurt (mg/kg)	*m*/*z* (MH+)
butanoic acid	2.21	89
decanoic acid	1.11	173
hexanoic acid	1.12	117
diacetyl	4.34	87
ethyl acetate	17.88	89
ethyl butyrate	27.24	117
ethyl hexanoate	22.44	145
ethyl octanoate	1.14	173
methyl cinnamate	2.2	163
4-hydroxy-2,5-dimethyl-3(2*H*)-furanone	18.47	129
γ-decalactone	2.52	171
hexanal[a]	1.01	101/83
(*Z*)-3-hexenol[a]	23.68	101/83
limonene	2.23	137
linalool[a]	1.88	155/137
3-hydroxy-2-methyl-4*H*-pyran-4-one	32.53	127
vanillin	15.72	153

[a] The $[MH - H_2O]^+$ ion was followed.

Table 3. Complex Viscosities Determined at Low Shear Stress (0.1 Pa) and Thickness Sensory Properties Evaluated by the Eight Subjects for the Six Yogurts[a]

	CAS yogurt		MPO yogurt		WP yogurt	
	MT−	MT+	MT−	MT+	MT−	MT+
complex viscosity (Pa.s)	158.9a	18.9e	109.3b	41.5d	60.3c	25.2ed
thickness intensity	5.6a	2.4d	4.2b	3.0cd	3.7bc	3.2cd

[a] Values with different lowercase letters appended are significantly different ($p < 0.05$) (SNK test).

Yogurts were flavored to 0.1% (m/m) with a strawberry flavor containing 17 aroma compounds mixed with propylene glycol (Aldrich). Concentrations of aroma compounds ranged from 1.01 to 32.53 mg/kg of yogurt (**Table 2**).

pH measurements, rheological properties using a controlled-stress rheometer, and aroma compound quantities determined by solid-phase microextraction analysis in yogurt were performed and considered as manufacturing checkpoints (details given in ref *16*). No significant variation of pH and aroma compound concentration between products was observed. The final pH of yogurts when consumed reached 4.30 $\pm$ 0.02. The complex viscosity of the six products tested at a low shear stress (0.1 Pa) is presented in **Table 3**.

Instrumental Methods for in Vivo Aroma Compound Release. Nose-space experiments using APCI-MS on-line analysis (atmospheric pressure chemical ionization mass spectrometry) were performed on the six products with eight experienced subjects (four female and four male, ages 22−52). They were able to recognize and classify various sensory properties according to their nature and their intensity. All subjects participated in one training session, performing at least three practice runs prior to analysis to familiarize themselves with the protocol.

Experiments were performed using APCI-MS with gaseous sample introduction. Aroma compound release measurements were carried out using an Esquire-LC mass spectrometer (Bruker Daltonique, Wissembourg, France) fitted with an APCI-MS source, to allow a gaseous sampling introduction due to the presence of a Venturi system. Air was sampled at a flow rate of 35 mL/min through a deactivated fused silica tubing (0.53 mm inside diameter) (Supelco, Saint Quentin

Fallavier, France) heated to 150 °C. A glass nosepiece in the form of a Y-junction was set up between the entry of the APCI-MS capillary and the subject's nose.

The protonated molecular ion from each molecule was detected at its corresponding *m*/*z* value (**Table 2**). Some compounds with the same molecular weights of the strawberry aroma that were studied had the same *m*/*z* values. In this case, the signal measured corresponded to the sum of both compounds.

Prior to each session, the dynamic headspace (N_2 flow rate of 35 mL/min) of a solution of heptan-2-one (15 ppm), which provided a signal on the same order of magnitude as the signal observed during the nose-space sessions, was analyzed by APCI-MS. The purpose of this procedure was to control the potential derivation of the APCI-MS signal during experiments.

Two MS acquisition methods were used with regard to the molecular weight of volatile compounds. The first one used an optimization procedure of the signal on the protonated molecular ion (*m*/*z* 89) and allowed the calibration of the system to be performed for ions with *m*/*z* values between 59 and 117. The other one used the protonated molecular ion (*m*/*z* 145) and allowed the calibration of the system to be performed and to detect ions with *m*/*z* values from 129 to 173.

In-nose signals were expressed as peak heights and were proportional to aroma quantities and, therefore, to aroma concentration.

For each product, four replicates were performed, and for each replicate, two acquisition modes for the APCI-MS signal (corresponding to the two calibrations) were carried out. Four sessions of 90 min over two consecutive days were organized for each subject. During a session, the subjects ate 12 yogurt samples of 5 g at 10 °C according to a defined procedure. They had to swallow after having kept the yogurt in their mouth for 12 s. This time was chosen with regard to the literature (*18*) and to the yogurt structure after preliminary tests to limit temperature variability during the swallowing event between panelists. At the end of the 12 s period, they were asked to eat as they normally did with usual swallowing, mouth closed, and to breathe into the nosepiece. The nose-space APCI-MS signal was measured for 1 min after introduction of the yogurt into the mouth so that complete information about aroma release by yogurt in the oral cavity could be captured. Yogurt samples were presented in random order, and subjects were instructed to clean their palate with bread, apple, and water between the samples. The subjects rested for a minimum of 3 min between samples. All the experiments were performed for 9 days on the same products (from the same preparation). During this time, the evolution of products based on pH and complex viscosity measurements was controlled, and products were considered to be stable.

Software developed in the laboratory was used to automatically draw the breath according to the breath curve for each ion and to extract the main parameters quantitatively representing the curve. The nose-space aroma release curves were divided into two phases. The first phase (P_1) corresponded to the "oral" phase of consumption during chewing until swallowing, and the second phase (P_2) corresponded to the phase after swallowing until end time (**Figure 1**). For the two phases of the release profile, calculated parameters involved the areas under the curves (AUC_1 and AUC_2) and the maximum intensities of the release profile (I_{max1} and I_{max2}). At the end of the second phase, the area under the last 10 s of the curve was calculated (S_{50-60}).

Sensory Analysis. Difficulties in producing consistent data with time−intensity sensory evaluation have been reported many times (*19*). Therefore, in this study, to reduce possible biases, subjects evaluated the overall olfactory intensity of the products at the different key discreet times during consumption. At the same time as the nose-space aroma release measurement, the subjects scored the perceived intensity at three times during the tasting: (i) upon introduction of the yogurt into the mouth, (ii) when swallowing (12 s after introduction of yogurt into the mouth), and (iii) 60 s after introduction of yogurt into the mouth (persistence). The subjects scored the perceived overall aroma intensity of the attributes on an unstructured scale anchored with the terms "very weak" and "very intense". Each sample was evaluated eight times by each subject. Data acquisition was assisted by FIZZ (*20*). At the end of the evaluation, the subjects evaluated the thickness of the yogurts. The yogurts with different protein compositions and weak mechanical treatment exhibited clear differences in their perceived thickness and rheological properties (**Table 3**).

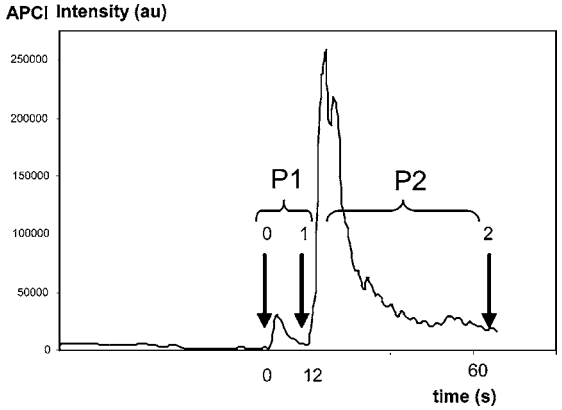

Figure 1. Release curves for ethyl hexanoate from CAS yogurt, when consumed by a subject using in-nose/APCI-MS analysis: (0) upon introduction of the yogurt into the mouth, (1) at swallowing time, and (2) at persistence time. P1 is phase 1, when the product was in the mouth, and P2 is phase 2, after the product was swallowed.

To validate tendencies observed on temporal sensory measurements, the effect of mechanical treatment on "thickness" and "overall aroma intensity" was investigated separately with 20 subjects and in a manner independent of any APCI-MS measurements.

The sessions were carried out in an air-conditioned room under white light in individual booths. Three tests (three protein compositions) were performed. For each test, three products of the same protein composition were simultaneously presented to the subjects: one reference sample (MT+ yogurt) and two test samples [MT+ yogurt (product identical to the reference) and MT− yogurt]. For both sensory attributes, the subjects scored the intensity of the two samples by comparing them to the reference. The samples were arranged for each subject according to a Latin square. Each test was replicated.

Data Analysis. A three-way analysis of variance (ANOVA) (composition, mechanical treatment, random subject) with interactions was applied to the four sensory variables (overall aroma intensity at introduction, at swallowing, and at persistence and thickness) and the five APCI-MS variables (AUC_1, AUC_2, I_{max1}, I_{max2}, and S_{50-60}) for each aroma compound. Within a mechanical treatment, the effect of the protein composition was assessed by a two-way ANOVA (composition, random subject) with interactions on sensory data and APCI-MS parameters. When significant product differences were observed ($p < 0.05$), product mean intensities were compared using the Student Newman Keuls (SNK) multiple-comparison test. Principal component analysis (PCA) was then performed to visualize the sample differences and the variable correlations. APCI-MS data were used for the construction of the PC dimension. Sensory data (intensity at swallowing time and at persistence time) were used as supplementary variables. For sensory analysis results, independent of any APCI-MS measurements, the yogurts varying by mechanical treatment were assessed with a Student's *t*-test. ANOVA and Student's *t*-tests were performed using SAS, version 9.1 (*21*), and PCA analysis using Statistica (*22*).

RESULTS

Sensory Analysis. *Temporal Sensory Properties*. The overall aroma intensity was significantly higher at swallowing time than when the food was introduced into the mouth (30% higher) and than after swallowing (50% higher), regardless of the yogurt (**Figure 2**). With regard to the effect of the mechanical treatment on perception at introduction in mouth and swallowing, a trend for a slightly higher intensity for MT+ was observed (**Figure 3**), but the difference was not significant ($p = 0.15$). However, at the persistence time, a significant effect of the mechanical treatment on overall strawberry aroma intensity was observed ($p < 0.05$). Yogurts with a weak mechanical treatment

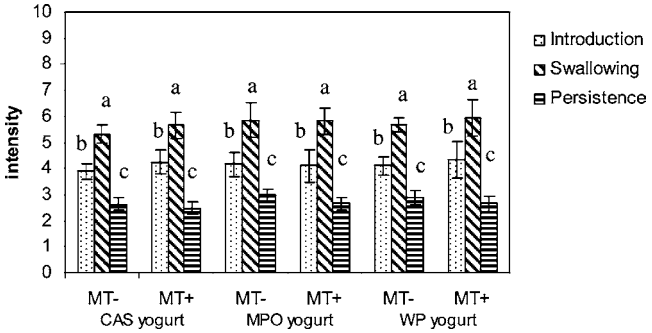

Figure 2. Overall intensity perceived for the six yogurts at the three consumption times: (i) upon introduction of the sample into the mouth, (ii) at swallowing time, and (iii) at persistence. Means with the standard deviation. The letters a–c indicate means that significantly differ at $p < 0.05$ (SNK test).

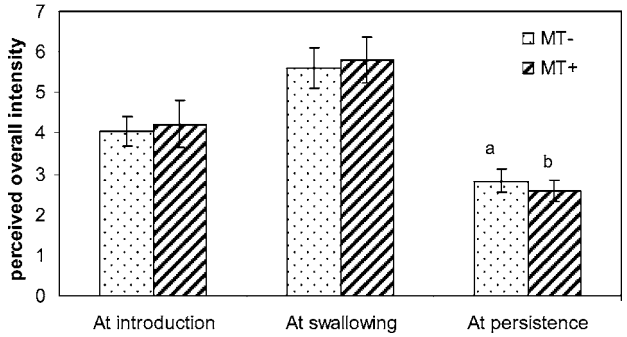

Figure 3. Overall aroma intensity perceived by the subjects for the yogurts varying by their mechanical treatment and averaged by protein composition type (CAS, MPO, and WP yogurts). Means with the standard deviation. The letters a and b indicate means that significantly differ at $p < 0.05$ (SNK test).

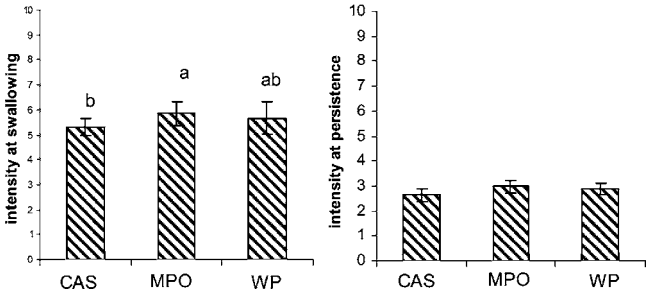

Figure 4. Overall intensity perceived for the six yogurts varying by protein composition (CAS, MPO, and WP yogurts) with weak mechanical treatment (MT− yogurts) on the significant sensory attributes by ANOVA. Means with the standard deviation. The letters a and b indicate means that significantly differ at $p < 0.05$ (SNK test).

were perceived as being more intense than yogurts with a strong mechanical treatment at persistence (**Figure 3**).

Protein composition had a varying influence on the olfactory perception of yogurts, depending on the level of mechanical treatment. The overall perception of the strawberry aroma of the MT− yogurts differed at swallowing time according to the protein composition ($p = 0.06$), but no significant effect ($p > 0.4$) of the protein composition for MT+ yogurts was observed, regardless of the considered time during consumption. The overall aroma intensity was perceived as being lower in MT− yogurt enriched with caseinate than in yogurt enriched with milk powder (**Figure 4**). No significant effect of protein composition in MT− yogurts was observed at persistence time. However, one trend could be observed ($p = 0.13$): yogurts enriched with caseinate were slightly less intense than the two others.

Effect of Mechanical Treatment on Perception. Mechanical treatment influenced sensory properties. The yogurts with a weak mechanical treatment were perceived as being significantly thicker than yogurts with a strong mechanical treatment ($p < 0.0001$). The greatest variation of thickness due to the additional mechanical treatment applied to the MT− product was 75% and was observed for yogurt enriched with caseinate with the percent variation defined as (MT− yogurt − MT+ yogurt)/(MT+ yogurts) × 100. The weakest impact of mechanical treatment on thickness was observed for yogurt enriched with whey proteins (55% variation).

Moreover, the Student's *t*-test highlighted differences in olfactory perception between MT+ yogurts and MT− yogurts. For the three protein compositions (CAS, MPO, and WP yogurts), the yogurt with a strong mechanical treatment was perceived as being more intense in flavor than the one with a weak mechanical treatment ($p < 0.05$). The variation of olfactory intensity between the two levels of mechanical treatment lies between 10 and 20%, the widest variation being observed for the CAS yogurt. Consequently, yogurts with identical composition, which were perceived as being thicker, were also perceived as being significantly less intense in flavor.

APCI-MS Analysis. In-nose analysis was carried out by APCI-MS analysis. Among the 17 aroma compounds of the studied aroma, only seven ions (*m*/*z* 87, 89, 101, 117, 137, 145, and 173) revealed a response above noise level. The low concentration of aroma compounds in yogurts (**Table 2**), the presence of fat in products leading to low volatility of the majority of hydrophobic flavor compounds, and the "in vivo" measurement explained the relatively low level of performances of APCI-MS analysis obtained in this study. However, these performance levels were relatively higher than those of other studies already published (*6*, *7*). The seven ions followed in the nasal cavity during yogurt consumption corresponded to ten aroma compounds of the strawberry flavor (**Table 2**). For the four couples of compounds detected at the same *m*/*z*, one of the compounds in each couple had a higher concentration and a higher volatility in the yogurt than the other (*16*). We could therefore consider that the APCI-MS signal for each *m*/*z* detected was mainly due to one compound: ion at *m*/*z* 89 (ethyl acetate), ion at *m*/*z* 117 (ethyl butyrate), ion at *m*/*z* 173 (ethyl octanoate), and ion at *m*/*z* 101/83 [(*Z*)-3-hexenol]. Moreover, considering the odor threshold values (*23*), these four compounds had probably more sensory impact than the others in each couple (butanoic acid, decanoic acid, hexanoic acid, and hexanal, respectively). For a large majority of subjects, as illustrated in **Figure 1**, we observed that the in-nose aroma release quantity was greatest after swallowing. Indeed, I_{max2} values for the second phase (after swallowing) were higher than I_{max1} values for the first phase (before swallowing) for the large majority of aroma compounds followed by APCI-MS analysis. The main influence on yogurt structure concerned the effect of mechanical treatment, which affected in-nose aroma release. The MT+ yogurts had significantly ($p < 0.05$) higher values for I_{max2} and for AUC_2 than the MT− yogurts for five ions (ions at *m*/*z* 87, 117, 137, 145, and 173) (**Figure 5**). An increase of ∼20−30% in the magnitude of the APCI-MS signal was observed for less viscous yogurts (MT+ yogurts) compared to that of MT− yogurts. For the three yogurts with a weak mechanical treatment, the protein composition effect influenced the in-nose release during consumption of four ions ($p < 0.05$). Indeed, the maximal intensity after swallowing (I_{max2}) significantly differentiated the three

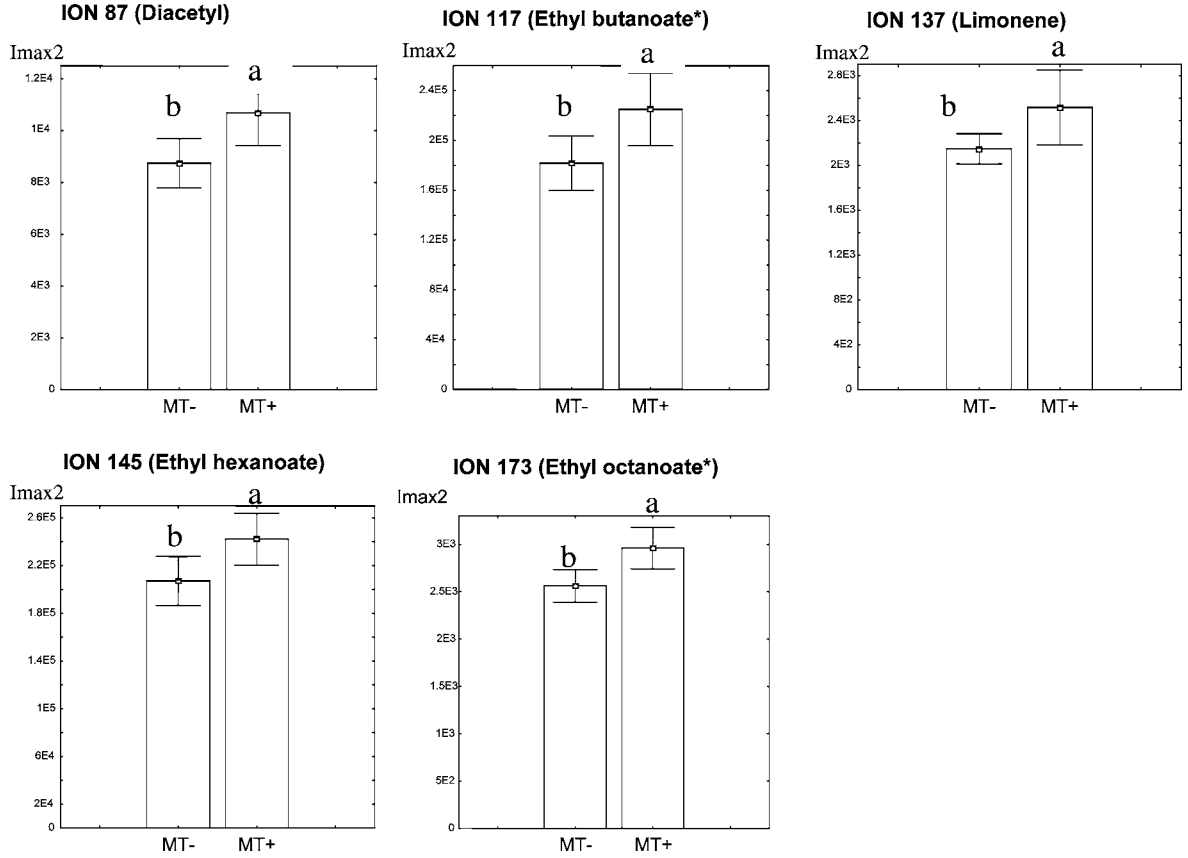

Figure 5. Effect of mechanical treatment (MT– for weak mechanical treatment and MT+ for strong mechanical treatment) on the average maximum in-nose quantities of the three yogurts and averaged across subjects for the maximum intensity of aroma release after swallowing for the significant aroma compounds by ANOVA. Means with the standard deviation. For ethyl butanoate and ethyl octanoate, this is the main compound in the strawberry flavor responsible for the signal of this ion. The letters a and b indicate means that significantly differ at $p < 0.05$ (SNK test).

Table 4. Average Parameter Values for the Release Curves of Yogurts with a Weak Mechanical Treatment (MT– Yogurts) Varying by Their Protein Composition with a Significance of 5 or 10% Determined by ANOVA[a]

parameter	ion (*m*/*z*)	probability (%)	CAS yogurt	MPO yogurt	WP yogurt
I_{max1}	173	5	1244b	1512a	1369ab
I_{max2}	87	5	9680a	9217a	7336b
	89	5	518316a	500310a	407022b
	101	5	2925ab	3540a	2186b
	117	5	203240a	182404ab	159697b
	173	10	2400b	2541ab	2748a
AUC_2	87	5	178511a	164906ab	142689b
	89	5	5458643a	4548190ab	4011386b
	145	10	2776091b	3267825a	3030554ab

[a] Values with lowercase letters appended are significantly different ($p < 0.05$ or 0.1) (SNK test).

yogurts for the ion at *m*/*z* 87 (diacetyl), the ion at *m*/*z* 89 (ethyl acetate), the ion at *m*/*z* 117 (ethyl butyrate), and the ion at *m*/*z* 101 [(*Z*)-3-hexenol] (**Table 4**). Smaller quantities of these four aroma compounds were released after swallowing yogurt enriched with whey protein (WP). Greater amounts of diacetyl, ethyl acetate, and ethyl butyrate were released from the yogurt enriched with caseinate (CAS) and greater amounts of (*Z*)-3-hexenol from the yogurt enriched with MPO. A reverse trend was observed for the maximal release (I_{max2}) of more hydrophobic compounds such as ethyl octanoate (ion at *m*/*z* 173) and for the area under the curve (AUC_2) of ethyl hexanoate (ion at *m*/*z* 145) (**Table 4**) ($p < 0.1$). For these two compounds, the aroma release was lower in CAS yogurt than in WP yogurt. Finally, with regard to the different release parameters for consumption of the three MT+ yogurts, no difference between these yogurts was observed.

The first factorial plot of PCA on APCI-MS and sensory parameters of yogurts showed that yogurts with different mechanical treatments formed three groups along the first principal component (PC1): MT+ yogurts (CAS yogurt, MPO yogurt, and WP yogurt), MT− yogurt enriched with caseinate, and MT− yogurt enriched with whey protein and milk powder (**Figure 6a**). MT+ yogurts can be described as (i) being more intense at swallowing time, (ii) having higher aroma release for some ions (*m*/*z* 137, 145, and 173), and (iii) leading to a lower release for the ion at *m*/*z* 89 (**Figure 6b**). Examination of the correlations between variables related to APCI-MS parameters and sensory data revealed that swallowing was correlated with the AUC_1 parameters of the ion at *m*/*z* 173 ($r = 0.73$), I_{max2} of the ion at *m*/*z* 145 ($r = 0.74$), and S_{50-60} of the ion at *m*/*z* 89 ($r = -0.85$) and persistence with the AUC_1 parameters of the ion at *m*/*z* 137 ($r = 0.79$), AUC_1 of the ion at *m*/*z* 173 ($r = 0.70$), AUC_2 of the ion at *m*/*z* 173 ($r = 0.79$), and S_{50-60} of the ion at *m*/*z* 117 ($r = -0.88$) and the ion at *m*/*z* 89 ($r = -0.85$).

DISCUSSION

Maximum in-Nose Flavor Release Intensity and Perception When Swallowing. The release of aroma compounds into the nose did not occur until the sample was swallowed. This observation was in agreement with others (*7*, *24*, *26*). These results indicate that the mouth cavity can be considered to be more or less a closed system until swallowing occurs. The velum

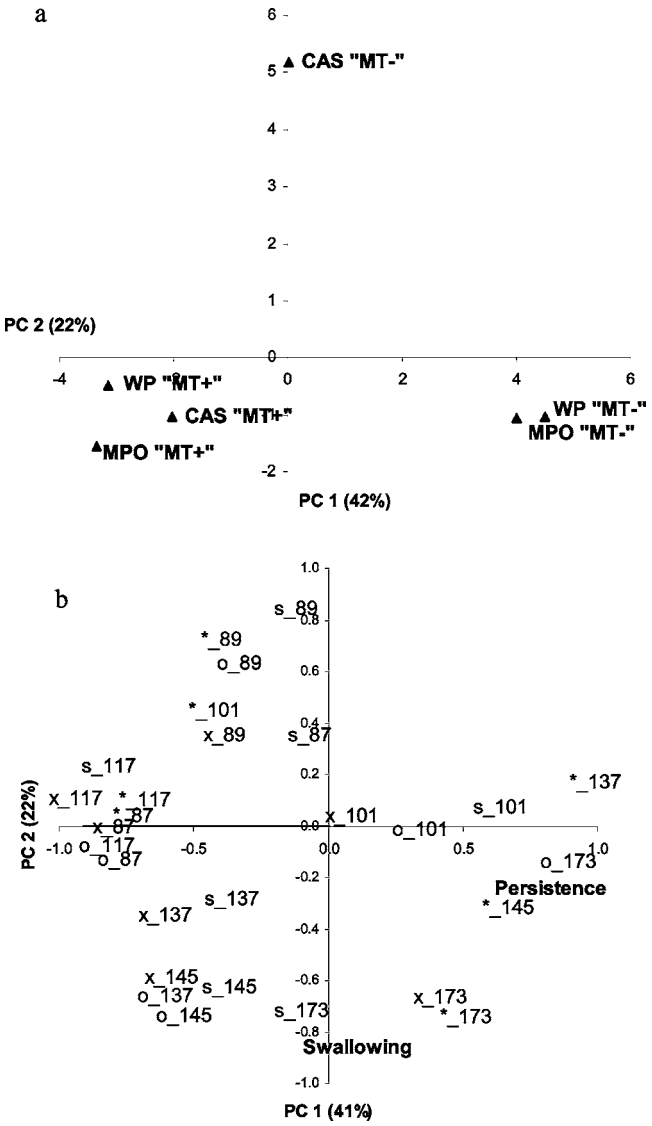

Figure 6. PCA plots [(**a**) individuals plot and (**b**) variables plot] on the sensory (at swallowing time and at persistence time) and APCI-MS (ions at *m*/*z* 87, 89, 101, 117, 137, 145, and 173) scores of the six yogurts [CAS, WP, and MPO with weak mechanical treatment (MT–) or strong mechanical treatment (MT+)]. The shape of the symbol differs with APCI-MS parameters: (*) AUC_1, (○) AUC_2, (×) I_{max2}, and (s) S_{50-60}.

between the oral cavity and the pharynx produces a closure capable of more or less effectively blocking the transfer of aroma compounds to the nasal cavity via the retronasal route into the nasal cavity and olfactory epithelium (*27*). When swallowing occurs, the soft palate is displaced. This allows the consumed sample to pass into the esophagus and also opens the nasal cavity for the passage of aroma compounds into the nose (*24*). That is why the APCI-MS signal was strongest at swallowing time, regardless of the product and for all eight subjects.

As already observed by Buettner et al. (*24*, *25*), information extracted from the in-nose release curve was in agreement with that collected on aroma perception: the higher the magnitude of the nose-space APCI-MS signal, the stronger the perception. APCI-MS parameters after swallowing, as well as the olfactory perception at swallowing time, were significantly higher than the same parameters measured at other consumption times, regardless of the yogurt (**Figures 1** and **2**). Moreover, in-nose release parameters of the second phase of consumption (i.e., after swallowing) led mainly to the discrimination of yogurts according to their protein composition or mechanical treatment.

Influence of the Complex Viscosity of Yogurt on Temporal Perception and in-Nose Flavor Release. *Effect at Swallowing.* The additional mechanical treatment on MT− yogurt led to a more "liquid" product (MT+) with a lower complex viscosity. At swallowing time, a trend of a lower perceived intensity in MT− yogurts than in MT+ yogurts was observed (**Figure 3**). This trend was confirmed when yogurt thickness and overall intensity were scored separately in a manner independent of any APCI-MS measurements. Under classical conditions of sensory measurements, subjects evaluate products under conditions more simple and comfortable than those used during nose-space analysis. Under these classical conditions, the aroma intensity of MT+ yogurts was significantly higher than that of MT− yogurts. Moreover, for the same composition, but with variable rheological properties, the least viscous yogurts (MT+) significantly released a greater aroma quantity than the most viscous yogurts (MT−) after swallowing (**Figure 5**). This result associated with the sensory one showed that the released quantity in the nasal cavity at swallowing time could explain the perceived intensity evaluated by the subjects at the same time. A possible explanation for these differences between MT+ and MT− yogurts may be that during consumption, the low-viscosity MT+ yogurts can more extensively cover the mucous membranes of the mouth and the throat. MT+ yogurts could thus develop a greater exchange surface area available for the mass transfer of aroma compounds from the product to the air flow of breath. Since the quantity of flavor compound transferred from the product to the air phase is directly proportional to the exchange area, the higher the surface exchange, the higher the release after swallowing.

Structure variation could also have an additional effect on the kinetic aspects of flavor release by modifying flavor transport through the food product. This could be confirmed by diffusion coefficient determination of aroma compounds in these yogurts. However, Paçi Kora et al. (*18*) did not observe any significant effect of mechanical treatment on hexanal mass transfer in fat-free stirred yogurt. The variation of complex viscosity of a gel with a similar composition seems not to modify significantly the aroma mass transfer of hexanal in the yogurt gel (*18*). The diffusion coefficient may also not be a key factor in explaining olfactory perception.

The variation in aroma release quantity between MT+ and MT− yogurts seems to depend on the difference in complex viscosity between both products. A greater decrease in complex viscosity due to the additional mechanical treatment led to a greater increase in aroma release (**Table 3**). The greatest viscosity deviation induced by mechanical treatment was observed for CAS yogurt, and this yogurt led to the greatest aroma release variation in-nose. As an example, for the ion at *m*/*z* 145 (ethyl hexanoate), the difference between MT+ and MT− yogurts in aroma release quantities in the nasal cavity after swallowing (AUC2) was 27% for CAS yogurt, only 17% for WP yogurt, and 15% for MPO yogurt. This result was in accordance with complex viscosity differences. These results were in agreement with numerous studies that reported a decrease in the olfactory intensity of aroma compounds for an increase in viscosity. However, these studies generally included the addition of a thickening agent (*28*, *29*). Boland et al. arrived at similar conclusions concerning the relationship between in-nose flavor release by PTR-MS and sensory properties of pectin gels with different structure and texture (*7*). Nevertheless, a clear link between the intensity of flavor perception and the quantity

of aroma compounds released has not been systematically observed in the literature. It has been shown that an increase in gel viscosity led to an increase in gel thickness but did not significantly change the in vivo in-nose aroma concentration, although there were significant changes in the perceived odor and taste (*4*, *5*, *15*). This result led the authors to suppose the presence of a sensory interaction between texture and flavor. In the study presented here, olfactory perception was explained by physicochemical results, but our results did not exclude the possible cognitive interactions between texture and aroma perceptions.

Effect after Swallowing. Whereas the application of an additional mechanical treatment to yogurt tended to increase the intensity of flavor perception at swallowing time, the reverse effect was observed on perception at persistence time (**Figure 4**). We can hypothesize that the low viscosity of MT+ yogurts could facilitate the elimination of yogurt traces in the mouth and throat by successive swallowing, thus leading to less persistence after 60 s in the mouth than for MT− yogurt. The MT− yogurt may interact or adhere more with oropharyneal mucosa than the less viscous MT+ yogurt. According to Buettner et al. (*25*), the durability and the thickness of the mucosa coating are highly dependent on the structure of the food material. MT− yogurts may lead to a thicker film coating the pharynx after swallowing than MT+ yogurts, leading to a longer release after swallowing. However, in this study, the S_{50-60} parameter from the APCI-MS signal representing the flavor release in the nose at persistence time did not differ between MT+ and MT− yogurts. This parameter makes it difficult to explain the olfactory differences from the aroma compound release at persistence time. No parameter from the flavor release curve could validate the hypothesis of a difference in the mucosa coating for either yogurt. Brauss et al. (*3*) also reported that aroma release after swallowing is not a key factor in discriminating yogurts with and without fat.

Another hypothesis can be proposed to explain the difference between physicochemical measurements and sensory data at persistence. The ions followed by APCI-MS analysis mainly included the aroma compounds responsible for fruity notes (*30*). However, no aroma compound with a high molecular weight and low volatility such as maltol or vanillin was followed by this method, whereas they are responsible for sweet notes and contributed to the perceived overall intensity of strawberry flavor. It could be suggested that they participated in aroma persistence but could not be measured.

Protein Composition Influenced Temporal Sensory Properties and in-Nose Flavor Release. *Yogurts with High Complex Viscosity (MT−).* Yogurt enriched with caseinate was perceived as being the least intense at swallowing time (**Figure 4**). The temporal olfactory perception was in agreement with a previous sensory study (*17*), showing by quantitative descriptive analysis that MT− yogurts enriched with caseinate were perceived as being less intense for a majority of olfactory notes than WP yogurts. These results were in agreement with aroma analysis carried out under static conditions, except for (*Z*)-3-hexenol (ion at *m*/*z* 101). The release (determined by SPME analysis) of many aroma compounds (seven aroma compounds among the 17 compounds of the strawberry mixture) under static conditions was lower in caseinate-enriched yogurts than in whey protein-enriched yogurts at 4 °C (*16*), but in vivo results were in agreement with in vitro static measurements for only one aroma compound, (*Z*)-3-hexenol. The in-nose release of ions at *m*/*z* 87 (diacetyl), *m*/*z* 89 (ethyl acetate), and *m*/*z* 117 (ethyl butyrate) varied in the opposite direction of the sensory observations: higher rates of release in CAS yogurt than in WP yogurt (**Table 4**). This disagreement between in vivo and in vitro results has already been observed and was in accordance with studies on custards and gels (*7*, *31*). Van Ruth et al. revealed that firmer custards demonstrated higher in-nose flavor concentrations than softer custards, whereas the latter had higher static headspace flavor concentrations (*31*). Boland et al. found similar results on pectin gels with the same disagreement between static and in-nose measurements (*7*). One hypothesis could be proposed to explain the difference between in-nose and static measurements and especially the higher in-nose release rates in CAS yogurt than in WP yogurt. The most viscous yogurt (CAS yogurt) may have been sheared more extensively and consequently released a greater quantity of aroma compounds than WP yogurt with the lowest complex viscosity of the three yogurts. This hypothesis was in agreement with the work of Boland et al. (*7*).

Another interesting result, observed with MT− yogurt, concerned two hydrophobic esters: ion at *m*/*z* 145 (ethyl hexanoate) and ion at *m*/*z* 173 (ethyl octanoate). For both compounds, the lowest in-nose release rate was observed for CAS yogurt ($p < 10\%$), corresponding to a reverse trend of the one described above, for the four ions (*m*/*z* 87, 89, 101, and 117). This result was in agreement with the release under static conditions above the three yogurts (*16*): CAS yogurt retained these esters to a greater degree than WP yogurt. In this case, physicochemical interactions between aroma compounds and proteins could partially explain the in-nose release differences between the yogurts. Moreover, the overall intensity perceived at swallowing time for CAS yogurt was the lowest and in agreement with hydrophobic ester in-nose release.

The overall olfactory perception of strawberry aroma was due to a mixture of aroma compounds, but ethyl hexanoate was a key compound of the strawberry flavor (*32*). This could explain the agreement between sensory and release (in-nose and static) measurements. The wide variability of the APCI-MS measurements (low aroma concentrations and aroma mixture in yogurt) could explain that the ester variations among the three MT− yogurts were only significant with a probability of 10%. The differences in velum movements between the subjects may be considered as an additional variable to explain the variability in flavor release (*33*). Finally, we could hypothesize that the four aroma compounds presenting a significant release variation had a limited contribution to the perceived intensity, contrary to ethyl hexanoate. The positive and high correlations between ions at *m*/*z* 145 and 173 and the perception at swallowing time confirmed this hypothesis.

Yogurts with Low Complex Viscosity (MT+). In-nose aroma release, as well as the overall intensity perceived at swallowing time, did not show any significant difference among the three MT+ yogurts. No effect of protein composition on temporal perception or in-nose aroma release quantities was observed. This result could be explained by similar exchange areas of yogurts developed in the mouth whatever the composition. The level of viscosity seems to play a major role. Indeed, variations of complex viscosity among MT+ yogurts were quite low.

Comparison between MT+ Yogurts and MT− Yogurts. For MT− yogurts, protein composition influenced both complex viscosity and physicochemical interactions with aroma compounds. In this case, significant changes in aroma release and perception were observed. For MT+ yogurt, there was a weak impact of protein composition on complex viscosity. In this case, no difference in aroma release and perception was observed among yogurts with different protein compositions. Conse-

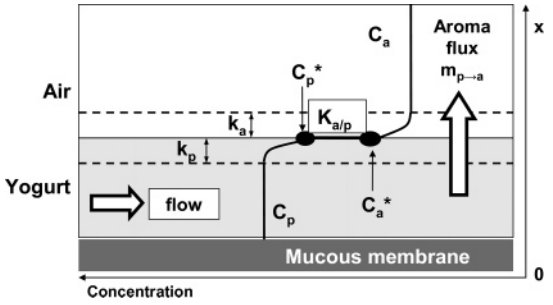

Figure 7. Profile concentration of aroma compounds in the mouth or in the throat during yogurt consumption, where k_p and k_a are the local mass transfer coefficients (meters per second) of the product phase and air phase, respectively, C_a and C_p are the concentrations (kilograms per cubic meter) of the compound in the air phase and in the product, respectively, $K_{a/p}$ is the dimensionless partition coefficient, and C_p^* is the concentration at the product–air interface at equilibrium (kilograms per cubic meter).

quently, complex viscosity variation seemed to prevail over physicochemical interaction in the in-nose aroma release and subsequent perception.

Conclusion. This study contributes to a better understanding of the impact of yogurt complex viscosity on in-nose aroma release and temporal sensory properties. Complex viscosity variations induced by mechanical treatment seem to have a greater impact on in vivo aroma release and olfactory perception than protein composition alone. Even if interindividual physiological differences led to different opening and/or closure of the velum and chewing and/or swallowing behaviors of subjects, the effect of product complex viscosity on perception in this study clearly explained the flavor release difference during consumption.

Physical mechanisms which could explain these differences between MT− and MT+ yogurts in aroma transfer were investigated. The mass transfer of flavor compound from the yogurt to the air phase, under convective and isothermal conditions, illustrated in **Figure 7**, can be described by the following equation (*34*):

$$\dot{m}_{p\rightarrow a} = K_{ov}A(C_p - C_p^*) \quad (1)$$

where $\dot{m}_{p\rightarrow a}$ is the mass transfer flux of a flavor compound (kilograms per second), K_{ov} is the overall mass transfer coefficient of the compound (meters per second), A is the surface area of mass transfer (square meters), C_p is the concentration of the compound in the product (kilograms per cubic meter), and C_p^* is the concentration of the compound at the product−air interface (kilograms per cubic meter). C_p^* is an equilibrium concentration in the product phase and can be determined from the thermodynamic equilibrium at the product−gas interface through the partition coefficient ($K_{a/p}$), defined as

$$K_{a/p} = \left(\frac{C_a}{C_p}\right)_{\text{at equilibrium}} \quad (2)$$

where C_a and C_p are the concentration (kilograms per cubic meter) of the compound in the air phase and in the product, respectively, and $K_{a/p}$ is the dimensionless (kilograms per cubic meter/kilograms per cubic meter) partition coefficient.

On the basis of this mass transfer equation (eq 1), differences in flavor release can be explained by each term of the equation: (i) a difference in the driving force of mass transfer $(C_p - C_p^*)$ expressed as $(C_p - C_p^*) = (C_p - C_a/K_{a/p})$ (eq 3), (ii) a difference in the overall mass transfer coefficient, K_{ov}, and (iii) a difference in the surface area of mass transfer, A.

The overall mass transfer resistance ($1/K_{ov}$) can be expressed as two local mass transfer resistances (product and air) as follows:

$$\frac{1}{K_{ov}} = \frac{1}{k_p} + \frac{1}{k_a K_{a/p}} \quad (4)$$

where k_p and k_a are the local mass transfer coefficients (meters per second) of the product phase and air phase, respectively. Each local mass transfer coefficient depends on the hydrodynamic conditions of the respective phase, on the geometry of the system, and on the diffusion coefficient of the compound.

Considering eqs 1, 3, and 4, the main parameters that explain mass transfer are (i) the partition coefficient at the air−product interface, (ii) the local mass transfer coefficient in the product, and (iii) the surface area of mass transfer. We can reasonably consider that the local mass transfer in the gas phase is not dependent on the complex viscosity of the product. Step by step, these three hypotheses have been investigated.

First, the interactions between the aroma compounds and the matrices were investigated by headspace measurements under static conditions. No significant difference in aroma compound retention between MT+ and MT− yogurts has been observed for a very large majority of compounds (16 of 17 of the strawberry flavors for MPO yogurt) (*35*). Second, the diffusivities of some key aroma compounds of the strawberry flavor in yogurts were determined. Even if higher apparent diffusion coefficients were obtained in MT+ yogurt than in MT− yogurt, their variations between yogurts were quite low and could not explain the in-nose aroma release differences observed between the yogurts (*36*). These results were in agreement with the study of Gostan et al. (*37*), who investigated aroma compound self-diffusion measurements by NMR on carrageenan gel with different structure.

The influence of the shear rate was studied on specific equipment simulating the shear rate in mouth conditions (*38*). This equipment permits us to study the influence of shear rate on flavor release without any modification of the mass transfer surface area. Results showed that no difference in aroma release under shear rate was observed between the yogurts of different complex viscosities at 10 °C and at 25 °C (*35*). However, when yogurt is consumed, the mass transfer is not really in isothermal condition during the first seconds. The heat transfer was studied on yogurts with different complex viscosity (induced by mechanical treatment and by addition of a thickener) by Paçi Kora et al. (*18*) on low-fat yogurts. Results showed no difference in heat transfer between yogurts.

By eliminating the various assumptions successively, we find it seems that the difference in surface area could be the main mechanism which could explain the difference in flavor release.

During yogurt consumption, the low-viscosity yogurts (MT+) can cover more extensively the mucous membranes of the mouth and the throat. Consequently, these yogurts could develop a greater exchange surface area for the mass transfer of aroma compounds from the product to the air flow of breath. Since the quantity of flavor compound transferred from the product to the air phase is directly proportional to the exchange area (eq 1), the higher the surface exchange, the higher the rate of release after swallowing.

The hypothesis of the role of the exchange area was also confirmed with the study of yogurts with different protein compositions. Protein composition modifications induced less significant effects, whatever the mechanical treatment was,

suggesting that complex viscosity variation seemed to prevail over physicochemical interaction in the in-nose aroma release and subsequent perception.

The in vivo measurement variability suggests that in vitro methods (artificial mouth) could be a useful experimental tool in identifying the limiting step of mass transfer under controlled conditions. Further experiments are in progress to validate our hypothesis about the mechanisms involved in flavor release during yogurt consumption. For this, the equilibrium between adhesion and flow forces on mucous membrane will be investigated in an attempt to better understand the development of surface exchange area in mouth and in throat and thus to predict the aroma release.

ABBREVIATIONS USED

CAS yogurt, caseinate-enriched yogurt; MPO yogurt, milk powder-enriched yogurt; WP yogurt, whey protein-enriched yogurt; APCI-MS, atmospheric pressure chemical ionization mass spectrometry; MT−, weak mechanical treatment; MT+, strong mechanical treatment; I_{max1}, maximum intensity of phase 1 of the release profile; I_{max2}, maximum intensity of phase 2 of the release profile; AUC_1, area under the curve of phase 1 of the release profile; AUC_2, area under the curve of phase 2 of the release profile; S_{50-60}, area under the final 10 s of the curve; ANOVA, analysis of variance; SNK, Student Newman Keuls; PCA, principal component analysis.

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