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Complex Viscosity Induced by Protein Composition Variation Influences the Aroma Release of Flavored Stirred Yogurt

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Dairy protein composition is known to influence the structure and the texture characteristics of yogurts. The objective of the present work was therefore to investigate the impact of protein composition, at a constant protein level, on the physicochemical properties of 4% fat flavored stirred yogurt and, more specifically, on the rheological properties, the microstructure, and the aroma release. The results showed that caseinate-enriched yogurts generally presented changes in their microstructure network and had a higher complex viscosity than whey protein-enriched yogurts. To a lesser extent, the release of the majority of aroma compounds was lower in caseinate-enriched yogurts. It was therefore possible to quantify physicochemical interactions between aroma compounds and proteins. The influence of gel structure on the flavor release was observed and was in agreement with sensory characteristics previously studied for these products (Saint-Eve, A.; Lévy, C.; Martin, N.; Souchon, I. Influence of proteins on the perception of flavored stirred yogurts. *J. Dairy Sci.* **2006**, *89*, 922−933.

KEYWORDS: Milk proteins; yogurt; microstructure; rheological properties; aroma release

INTRODUCTION

Yogurt is a fermented milk product widely consumed around the world. Flavor and texture are essential aspects of the quality and the consumer acceptability of these products. One way to improve the texture of yogurt consists of fortifying the milk base. The addition of whey protein concentrate, sodium caseinate, or milk powder is usually employed in yogurt manufacture. In a recent study, we showed that milk protein composition influences the sensory properties of 4% fat flavored stirred yogurts (*1*). Indeed, the variation of yogurt protein composition generated large texture differences and also some olfactory differences between the yogurts. To better understand and control the flavoring of yogurt and to adapt it to its structure and composition, it is necessary to identify and quantify the physicochemical properties responsible for the sensory stimuli. Improved knowledge of the influence of the structure and the composition on flavor release will provide a better understanding of the mechanisms that govern flavor release and perception. In the present study, we investigated the influence of protein composition on the microstructure, the rheological properties, and the aroma release of stirred yogurts to develop a better understanding of the determinants of texture and flavor perception variations in yogurts.

It is well-known that type and content of milk proteins are of significant importance for the physical properties and the perceived texture of fermented products (*2*, *3*). Microstructure studies of dairy products using scanning electron microscopy (SEM) have revealed the structural modifications of the gel network when composition changes. Yogurts enriched with whey protein concentrate exhibit a structure with a very fine network containing very small pores. In contrast, the addition of sodium caseinate to the milk base results in a rather coarse and loose structure (*4*−*6*). When considering the influence of the whey protein/casein concentration ratio on physical viscosity, data from the literature appear to be conflicting (*3*). Most of the authors observed that increasing the level of whey protein concentrate in yogurt results in greater gel strength (*5*, *7*). On the contrary, other studies reported that at similar protein levels, the addition of caseinate instead of skim milk powder to the milk base strongly increases yogurt viscosity (*8*−*11*). These observations can be explained by the nature of the interactions between denatured whey proteins and casein micelles achieved in the formation of the gel during acidification.

Moreover, variations of the structure and the composition of the food matrix (due to the nature of proteins) can be responsible for the modification of flavor retention. Proteins are known to interact with the aroma compounds and often cause their volatility to decrease (*12*). Numerous studies have been conducted on the interaction between β-lactoglobulin and aroma compounds (*12*, *13*). For example, β-lactoglobulin binds with esters, methyl ketones, alcohols, and aldehydes but not with methylpyrazyne or shorts acids (*14*, *15*). The most probable binding site for these compounds is the hydrophobic pocket of

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Table 1. Premix Composition for Preparation of the Three Yogurts

ingredients (suppliers)	yogurt CAS	yogurt MPO	yogurt WP
water (Volvic, Danone) (L)	1	1	1
skim milk powder (Ingredia) (g)	100	135	100
lactose (Ingredia) (g)	21		21
sodium caseinate (Ingredia) (g)	14		
whey protein concentrate (80% w/w) (Ingredia) (g)			14
anhydrous milk fat (Lactalis) (g)	40	40	40
sugar (sucrose) (Daddy) (g)	50	50	50
protein total content (% in w/w) (%)	5.4	5.4	5.4
dry matter (% in w/w) (%)	22.50	22.50	22.50

β-lactoglobulin belonging to the family of the lipocalin protein (*12*). Contrary to β-lactoglobulin, the physicochemical interactions between aroma compounds and sodium caseinate were not as extensively studied.

Few studies have investigated the effect of yogurt structure on aroma release by adding a thickening agent. In low-fat flavored stirred yogurt, Paci Kora et al. and Nongonierma et al. showed a slight influence of composition (addition of a thickening agent) on aroma retention (*16*, *17*). No study was previously made of the effect of protein composition on both structure and flavor release properties of a dairy complex matrix.

In this context, the aim of the present work was to investigate and to quantify the effect of protein composition on the physical properties and the aroma release of 4% fat flavored stirred yogurts. To characterize its organization, the gel was examined at both the microscopic level (microstructure of the protein system) and the macroscopic level (rheological properties). The flavor release was quantified in yogurt by two methods. All results will be discussed in order to establish links between the structure of the gel and the flavor release in order to develop a better understanding of yogurt perception (*1*).

MATERIALS AND METHODS

Yogurt Manufacture. Three flavored stirred yogurts were studied. They had the same dry matter (22.5% w/w), fat (4% w/w), and protein (5.4% w/w) contents (**Table 1**). Only a protein fraction used to fortify the premix varied as follows: enrichment with sodium caseinate (CAS yogurt), enrichment with low heat skim milk powder (MPO yogurt), and enrichment with whey protein concentrate (WP yogurt). The MPO yogurt was considered the reference product.

The first stage of the manufacture of yogurts was the milk base reconstitution starting from the three different emulsions with a two-stage homogenizer (APV1000, APV, France). Each milk base was heated at 92 °C for 5 min. The fermentation was carried out in a 7 L fermenter (SGi, France) and thermostated at 44 °C. The milk was inoculated with *Lactobacillus delbrueki* ssp. *bulgaricus* (LB18 incorporated in 0.005% vol/vol in milk) and *Streptococcus thermophilus* (ST7 and ST143 in 0.01% vol/vol) provided by Chr Hansen (Arpajon, France). Fermentation was stopped when the pH reached 4.6, and the yogurts were pumped and then stored at 4 °C. After 9 days of storage (corresponding to the age of yogurts for all of the analyses), the yogurt pH was determined at 4 °C with a pH meter (Mettler Toledo, France). This pH measurement was considered as one of the manufacturing checkpoints.

After 1 day of storage at 4 °C, all of the yogurts were flavored to 0.1% (w/w) with the strawberry flavor containing 17 aroma compounds mixed with propylene glycol (Aldrich, France). The flavoring step was performed with a food processor (Kenwood, United States) under controlled conditions (2 kg of yogurt per batch, 30 s, 2.5 power). Concentrations of aroma compounds ranged from 1.01 to 32.53 mg/kg of yogurt (**Table 2**). These concentrations corresponded to the ones used in the sensory study (*1*).

Table 2. Aroma Compound Composition of Strawberry Aroma Mixed with Propylene Glycol and Their Physicochemical Properties[a]

aroma compounds	concn in yogurt (mg/kg)	Log P[a,b]	air–water partition coefficient at infinite dilute $\times 10^3$ (dimensionless, 25 °C)
butanoic acid	2.21	0.79	0.0394[b]
decanoic acid	1.11	4.09	1.7[b]
hexanoic acid	1.12	1.92	0.294[b]
Z-3-hexenol	23.68	1.61	
diacetyl	4.34	–1.34	0.547[c]
ethyl acetate	17.88	0.73	5.48[b]
ethyl butanoate	27.24	1.85	16.3[b]
ethyl hexanoate	22.44	2.83	29.5[b,d]
ethyl octanoate	1.14	3.81	65[b]
4-hydroxy-2,5-dimethyl-3(2*H*)furanone	18.47	0.82	0.000349[b]
γ-decalactone	2.52	2.72	
hexanal	1.01	1.78	8.61[e]
limonene	2.23	4.57	
linalol	1.88	2.97	0.879[b]
3-hydroxy-2-methyl-4H-pyran-4-one	32.53	0.09	
methyl cinnamate	2.2	2.62	1.05[b]
vanillin	15.72	1.21	0.000987[b]

[a] Log P = Log of the partition coefficient of the compound between water and octanol, calculated values. [b] EPI Estimation Programs Interface v3.10. [c] Ref *37*. [d] Ref *22*. [e] Ref *36*.

Yogurt Microstructure. Sample observations and photomicrography were performed with SEM. After 1 week of storage at 4 °C, three yogurt samples were taken with a 0.2 mL positive displacement microdispenser and prepared for SEM, according to the method adapted from Kalab (*18*). The yogurt samples were fixed for at least 4 h in a glutaraldehyde solution (12.5% vol/vol). Samples were then rinsed five times for 15 min in ultrafiltered water. The samples were further dehydrated in a graded acetone series (500, 750, and 950 mL L^{-1}) and then rinsed in anhydrous acetone. Dehydrated samples were dried by the critical point method under CO_2 with an Emscope CPD 750 apparatus (Ashford, Great Britain). The dehydrated cylinders of samples were stuck onto stubs with an epoxy resin (Araldites) and were then broken up with a scalpel in order to exhibit sample microstructure. A final 40 nm thick coating of gold–palladium was applied in a Polaron E 5100 coater (West Sussex, Great Britain). Sample observations and photomicrography were performed in a Hitachi S-3000N SEM (Tokyo, Japan) operating at a voltage of 4 kV. Six fields were observed for each sample.

Rheological Properties. The rheological properties of yogurts were measured using a controlled-stress rheometer (model RS1, Haake, Germany), equipped with a cone and plate geometry (60 mm diameter, 2° angle, and 117 μm gap). The measurement was controlled by RheoWin Pro software, version 2.84 (Haake, Germany). After gentle standardized mixing by three up and down movements of a spoon in the yogurt cup, the samples were deposited on the plate of the rheometer with a syringe (5 mL). Three replications were carried out with yogurt from the same cup. Measurements were made at 10 °C. Three types of tests were conducted. The first one was a frequency sweep test. A constant stress (2 Pa) was imposed on the product at an increasing frequency (ω = 0.1−100 rad/s) in order to describe the degree of organization of the yogurts. Measurements were verified to ensure that they were performed in the linear viscoelastic domain. The second test measured the complex viscosity by the stress sweep. A slope of constraints from 0.1 to 100 Pa, with a frequency of 1 Hz, was exerted. For data treatment, only complex viscosity values at 0.1 and 50 Pa were used to discriminate the products. The correlation between constraints and perception was investigated in the literature. When evaluating texture with a spoon, a low shear rate was used. When evaluating the texture in the mouth, viscosity at higher constraints was used (*19*, *20*). That is why 0.1 Pa was chosen to be correlated to texture with a spoon and 50 Pa was chosen to be correlated to texture in mouth. The third test measured the flow curve of stirred yogurt by increasing

the shear rate from 0 to 100 s^{-1} (upward curve), followed by decreasing the shear rate from 100 to 0 s^{-1} (downward curve). Three cycles were carried out with a time lag of 30 s between each loop. Three minutes was allotted for the sample temperature to equilibriate to 10 °C prior to each analysis. The sample of yogurt was changed for each type of test.

Release Measurement Using Headspace (HS) Analysis. All of the release measurements were performed at 4 °C, corresponding to the storage temperature of yogurt. No evolution of the yogurt matrix was observed at 4 °C.

Determination of Partition Coefficients by Phase Ratio Variation (PRV) Method. Gas-to-matrix partition coefficients were determined at 4 °C by the PRV method (*21*). A partition coefficient was the ratio of the equilibrium concentrations of the aroma compounds between the gas phase and the matrix. The PRV method made it possible to determine the partition coefficient without any external calibration. Comparison of the performances of different static HS methods showed that the PRV method was the most suitable method to measure absolute volatilities in multicomponent systems (*22*).

The vials of 22.4 mL (Chromacol, France) were filled with different amounts of yogurt matrices: 0.05, 0.1, 0.5, 1, and 2 mL. The corresponding volume ratios (β) were 223.0, 111.0, 43.8, 21.4, and 10.2. All experiments were performed in triplicate to validate the repeatability of the measurements. When the equilibrium state was reached (after 12 h at 4 °C), the vials were placed on a thermostated support at 4 °C. A syringe was used to take 2 mL of HS gas that was injected with an automatic HS sampler CombiPal (CTC Analytics, Switzerland) into a gas chromatograph with a flame ionization detector (GC-FID HP6890, Germany), heated at 250 °C. Aroma compounds were transferred in a semicapillary column of 30 m in length, with an internal diameter of 0.53 mm and a film thickness of 1 μm (BP20 Carbowax, Interchim, France). The carrier gas was helium at a flow rate of 8.6 mL/min. For the FID detector, H_2 and air flow rates were 40 and 450 mL/min, respectively. The oven program was 37.5 min long: starting at 50 °C, 4 °C/min up to 70 °C; 5 °C/min up to 170 °C; 8 °C/min up to 220 °C; and 6 min at 220 °C. A nonlinear regression was applied in order to accurately determine the partition coefficients (*23*).

Determination of Aroma Compound Release by Solid Phase Microextraction (SPME) Method. The analysis of the aroma compound release in the vapor phase above the yogurts was performed by SPME. The same GC-FID used to determine partition coefficients was used. HS vials filled with 5 g/vial of product and previously stored overnight at 4 °C were placed in the HS sample tray maintained at 4 °C. The time to reach equilibrium between the SPME fiber [poly(dimethylsiloxane) (PDMS) with a 100 μm film thickness] and the sampler HS ranged from 1 min to 4 h. During this equilibrium time, the vial was maintained under stirring at 4 °C. After this time, aroma compounds were desorbed by inserting the fiber into the GC injector set at 250 °C for 1 min. The operating conditions for GC-FID were similar to those of the PRV method. Results obtained from the SPME method were expressed as area units. Each HS analysis was done in triplicate.

Data Analyses. All data analyses were performed using the SAS software package, version 9.1 (*24*). The influence of protein composition on yogurt complex viscosity, partition coefficient, and aroma compounds release was assessed by one-way analysis of variance (ANOVA) per product. When significant product differences were observed ($p < 0.05$), product mean intensities were compared using the Student−Newman−Keuls (SNK) multiple comparison test.

RESULTS AND DISCUSSION

Protein Composition Influenced Physical Properties. *Rheological Properties.* The viscoelastic properties of the yogurts were determined by frequency sweep tests. **Figure 1** shows the variations of the storage modulus G' and of the loss modulus G'' as a function of sweep frequency for the three yogurts. The G' value was higher than the G'' value over the frequency range and regardless of the yogurt. Moreover, the G' and G'' values

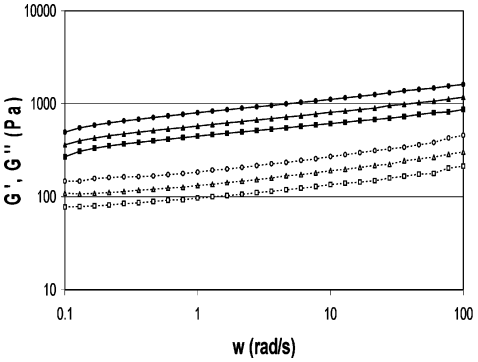

Figure 1. Storage modulus G' and loss modulus G'' of the three yogurts as a function of the angular speed. G' of CAS yogurt, ●; G' of MPO yogurt, ■; G' of WP yogurt, ▲; G'' of CAS yogurt, ○; G'' of MPO yogurt, □; and G'' of WP yogurt, △.

Table 3. Complex Viscosity (Pa s) and Confidence Intervals at a Low Shear Stress of 0.1 Pa (η_0^*) and at 50 Pa (η_{50}^*) Shear Stress of Yogurts at 10 °C[a]

yogurt	η_0^* (Pa s)[b]	η_{50}^* (Pa s)
CAS	79.2 ± 9.7 a	2 ± 0.4 a
MPO	52.7 ± 17 b	1.6 ± 0.7 a
WP	38.1 ± 4.4 c	0.7 ± 0.4 b

[a] The letters a–c indicate means that significantly differ at $p < 0.05$ (SNK test). [b] Data already published in ref *1*.

increased slightly with the frequency. These results suggest that the products display the typical behavior of gelled structures (*25*). Moreover, CAS yogurt gave the highest G' values while WP yogurt had the lowest G' values. Varying the time scale of the applied deformation provides information about the nature of the bonds in the gel network (*26*). Regardless of the yogurt, the slopes of the curves were similar, suggesting that the overall nature of the bond in the gels did not vary greatly between the three yogurts.

Protein composition had a strong effect on the complex viscosity of yogurt. One-way ANOVA showed a significant influence of the protein composition on the complex viscosity at 0.1 and 50 Pa. These results are presented in **Table 3**. At low shear stress (0.1 Pa), the yogurt fortified with caseinate (CAS yogurt) had the highest complex viscosity ($\eta^* = 80$ Pa s). The WP yogurt was the least viscous ($\eta^* = 40$ Pa s), and the MPO yogurt was intermediate ($\eta^* = 60$ Pa s). These results at a shear stress of 0.1 Pa correspond to visual thickness of the yogurt with a spoon. At a value of 50 Pa, corresponding to yogurt under mastication conditions (*20*), the WP yogurt had a lower value for complex viscosity than MPO and CAS yogurts. However, these two products were not significantly discriminated.

These results were in agreement with numerous studies (*5*, *8*−*10*). For Guzman-Gonzales et al. (*10*), an increase of the whey protein/casein ratio contributes to a decrease of yogurt viscosity. Cho et al. (*9*) reported higher viscosities when enriching yogurts with skim milk powder rather than with whey protein. Moreover, at similar protein levels, the addition of caseinate instead of skim milk powder in the dairy mix strongly enhanced the yogurt viscosity. Modler and Kalab (*27*) supposed that yogurts enriched with caseinate exhibit a high degree of fusion of casein micelles, as compared to yogurts stabilized with whey proteins. This micelle fusion effect could contribute to greater firmness.

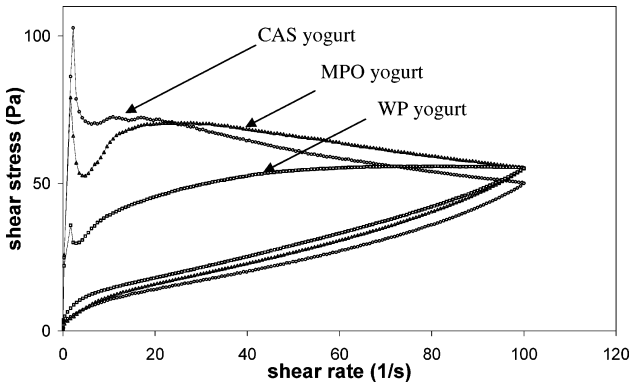

Figure 2. Shear stress as a function of increasing and decreasing shear rate for the three yogurts at 10 °C: CAS yogurt, ○; MPO yogurt, □; and WP yogurt, △.

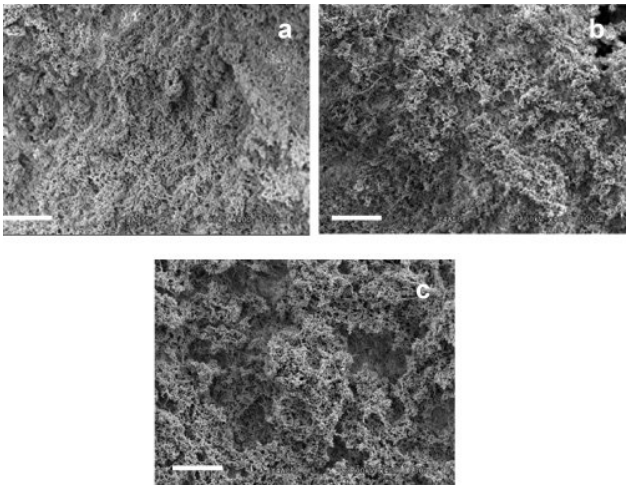

Figure 3. Microstructure of the three yogurts obtained from milk bases enriched with WP yogurt (**a**), MPO yogurt (**b**), and CAS yogurt (**c**) (white bar is 50 μm).

However, Remeuf et al. (*6*) and Kailasapathy et al. (*7*) observed that yogurt enriched with whey protein had a higher viscosity than yogurt made with skim milk powder. This discrepancy between studies could be explained by variations in the whey protein preparation, which strongly influences the protein functionalities (*5*).

The flow curves of the different stirred yogurts are shown in **Figure 2**. For low shear rate values, the stirred yogurts enriched with caseinate had a higher shear stress (corresponding to the upward curve) than yogurts enriched with whey protein or milk powder. On the contrary, at high shear rate values, the shear stress of CAS yogurt was lower than the other two yogurts. Moreover, the area of the hysteresis loop of CAS yogurts between the up and down shear rate vs the shear rate curve was higher than those of MPO and WP yogurts. The area of the hysteresis loop represents the structural breakdown of stirred yogurt during shearing. The results can be interpreted as a greater increase in structure damage when yogurt was enriched with caseinates. Moreover, the WP yogurt was less resistant to low shear rate than the CAS yogurt but was more stable when the shear rate increased.

Microstructure. SEMs of the yogurts showed that protein composition led to differences in the organization of the gel network. As can be seen in **Figure 3**, a heterogeneous structure composed of large globular aggregates in a network forming large pores (sink zones) characterized the yogurt fortified with caseinates (CAS yogurt). When yogurts were enriched with whey protein, the protein network presented a more uniform distribution of the gel and smaller pores than CAS yogurt. Micrographs of MPO yogurt appeared to be between those of the two others.

These observations can be compared with those of several authors (*4*−*6*, *27*). Remeuf et al. (*6*) observed irregular gel organization in yogurt fortified with MPO yogurt and CAS yogurt and a very fine network containing very small pores in WP yogurts. The protein network in the yogurt containing only skim milk powder induced a coarse texture with large globular aggregates. When yogurts were enriched with whey protein, the network became finer, the size of the aggregates became smaller, the network of cross-links became denser, and the pores became smaller (*5*); these results corroborated those of the present study. Puvanenthiran et al. (*5*) reported that the addition of whey protein led to a structure where casein micelles appeared in the form of individual entities surrounded by finely flocculated protein and linked to very small whey protein aggregates. This structure might increase the number of bonds between particles and thus explain the dense and finely branched network in yogurt from whey protein-enriched milk base (*27*). We can suppose that when yogurts were enriched with caseinate, interactions between casein micelles were more likely. Yogurts fortified with casein preparations exhibited fusion of casein micelles. This would be indicative of a lower degree of interaction between whey protein and casein micelles, possibly leading to the coarse network of the CAS yogurt, contrary to WP yogurt.

Some relationship between yogurt microstructure and complex viscosity can be deduced. As reported by Modler and Kalab (*27*) and Remeuf et al. (*6*), micelle fusion is influenced by the whey protein/casein ratio. Our results suggest that the homogeneous microstructure observed in WP yogurt was characterized by a low complex viscosity and a heterogeneous structure due to an enrichment in caseinate by a high viscosity. These results are in agreement with the texture perception (thickness and granuleous intensities) of the three yogurts (*1*). Yogurts differing in their protein composition presented different texture characteristics: The enrichment of yogurt with caseinate led to a thicker and more granular texture. This observation is in agreement with the rheological measurements carried out on studied yogurts, and the heterogeneity of the microstructure could explain the granular properties of the CAS yogurt. Thus, SEM and rheological measurements can provide a better assessment of structure and texture of milk products.

Protein Composition Influenced Aroma Release. *Air/Yogurt Partition Coefficients of Aroma Compounds.* In the present study, the PRV method made it possible to detect and quantify eight partition coefficients of aroma compounds among the 17 of the strawberry aroma at 4 °C in the WP yogurt and six in MPO yogurt and CAS yogurt (**Table 4**). Only a limited number of molecules could be studied by the PRV method given that aroma compounds were studied at low concentration (sensory concentration), low temperature (4 °C), and low volatility for several of them (for example, vanillin or maltol). Thus, many aroma compounds could not be detected and quantified by this method. Nevertheless, the eight quantified aroma compounds presented a wide range of hydrophobicity (log P varying from −1.34 for diacetyl to 4.57 for limonene). They were mainly hydrophobic and were among the most volatile of the strawberry aroma (data in water at 25 °C). Thus, the presence of fat in yogurts reduced their aroma volatility (*30*).

Table 4. Partition Coefficients of Aroma Compounds for the Three Yogurts at 4°C[a]

	$K_{i,gas/matrix}$ (×10³)		
	CAS yogurt[b]	MPO yogurt[b]	WP yogurt[b]
decanoic acid			1.14 (0.25–5.22)
diacetyl	4.18 (1.16–15.13)	9.97 (6.12–16.25)	11.88 (6.97–20.25)
ethyl acetate	20.7 (18.9–22.7)	20.7 (16.4–26.2)	22.9 (20.9–25.1)
ethyl butanoate	20.7 (19.3–22.2) b	21.8 (19.5–24.3) b	25.7 (24.1–27.4) a
ethyl hexanoate	4.29 (3.26–5.65) b	5.42 (4.38–6.71) ab	6.67 (6.23–7.14) a
hexanal	4.28 (1.97–9.28)	9.28 (5.06–17.04)	8.79 (6.86–11.26)
limonene			4.85 (2.86–8.21)
Z-3-hexenol	1.82 (1.09–3.02)	1.51 (0.58–3.95)	1.02 (0.37–2.81)

[a] The letters a and b indicate means that significantly differ at $p < 0.05$ (SNK test). [b] Confidence interval is in parentheses.

Measured partition coefficients over yogurt at 4 °C ranged from 1.02×10^{-4} for *Z*-3-hexenol to 2.57×10^{-3} for ethyl butanoate (**Table 4**) and were on the same order of magnitude for the three yogurts. The volatility of the aroma compounds in the dairy matrix is influenced by their physicochemical properties and by their interactions with product ingredients (fat, proteins, and carbohydrates) (*12*). Hydrophilic compounds (diacetyl and ethyl acetate), which presented low log *P* values, were more volatile in yogurt at 4 °C than in water at 25 °C (**Tables 2** and **4**). Even if the temperature was lower in yogurt than in water, the highest partition coefficient was obtained in yogurt and could be explained by the low affinity of diacetyl and ethyl acetate for anhydrous milk fat, leading to an increase of volatility. Ethyl hexanoate, which is one of the most hydrophobic aroma compounds of the strawberry flavor, showed a reverse behavior with a lower volatility in yogurt at 4 °C than in water at 25 °C due to the additional effect of fat and temperature (**Table 4**).

Two aroma compounds showed a significant product effect by one-way ANOVA, used to discriminate the three yogurts by their protein composition (**Table 4**): ethyl butanoate and ethyl hexanoate ($p < 0.05$). These two aroma compounds were among the most volatile of the series studied. The retention of these two aroma compounds in WP yogurt was lower than in CAS and MPO yogurts. Moreover, whereas retention of these two esters was higher in CAS yogurt, the CAS and MPO yogurts were not significantly discriminated. The partition coefficients were approximately 20 and 35% higher in WP yogurt than in MPO and CAS yogurts, respectively.

Concerning the other aroma compounds, a similar trend was observed but was not significant, probably due to the relative high variability of the measurements as a result of unfavorable experimental conditions (low temperature, complex matrices, and low concentrations). Meanwhile, the release of diacetyl, ethyl acetate, and hexanal was higher in WP yogurts than in CAS yogurts. The release over MPO yogurt was intermediate.

A reverse trend can be observed for *Z*-3-hexenol alcohol. Its partition coefficient was higher in CAS yogurt and lower in WP yogurt. Except for *Z*-3-hexenol, partition coefficient results suggested that the majority of aroma compounds detected by the static HS method had a greater affinity for sodium caseinate than for whey protein.

Little data are available concerning the comparison of aroma compound retention by mixed caseinate and whey protein. The large majority of studies concern the effect of the protein alone. Jouenne and Crouzet (*31*) have shown a strong interaction of limonene with β-lactoglobulin by apparent binding constants and interaction between β-lactoglobulin and aldehydes. Ketones are reversibly bound to whey protein through hydrophobic

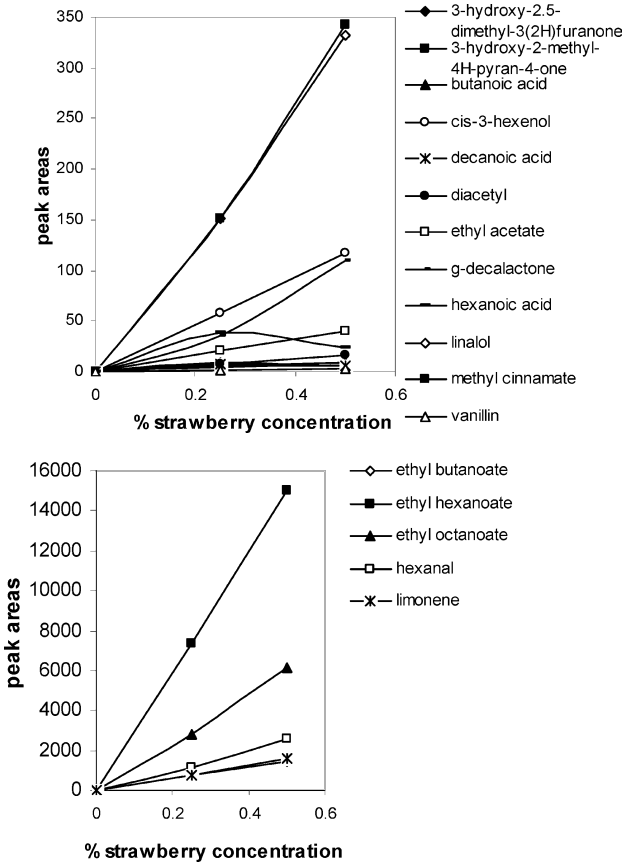

Figure 4. Proportionality between the aroma compound concentration and the FID response with a PDMS fiber at 37 °C during 1 h of stabilization time between the PDMS fiber and the HS phase in the MPO yogurt.

interactions and hydrogen binding (*32*). Esters (terpenyl acetate, ethyl hexanoate) also bind the hydrophobic core of β-lactoglobulin (*31*, *33*). In the present study, no effect of protein composition was found for ethyl acetate. This result was already observed by Pelletier et al. (*14*), who found that most of the esters studied interact with β-lactoglobulin, except ethyl acetate. Moreover, concerning the binding of aroma compounds with caseinate, Landy et al. (*34*) showed that ethyl acetate does not interact with sodium caseinate in an aqueous medium, confirming our results. On the other hand, Farès et al. (*35*) reported the formation of a strong bond between diacetyl and sodium caseinate, contrary to ethyl acetate, which interacts with the protein through weak (hydrogen) binding.

The PRV method did not make it possible to detect all of the aroma compounds of the strawberry flavor under the experimental conditions of the present study. To investigate the impact of the protein composition on the release of all of the aroma compounds, it was necessary to use and develop another HS methodology.

Quantification of Aroma Compound Release in the HS by the SPME Method. The SPME method was used to detect and quantify all of the aroma compounds of the strawberry aroma in the vapor phase above the yogurts. The extraction abilities of the SPME fibers were much greater than the traditional static HS method: Seventeen aroma compounds were detected and quantified.

Many precautions were taken before using SPME for the analysis of aroma release. Reproducibility between fibers was tested. The linear range of detection of the aroma compounds

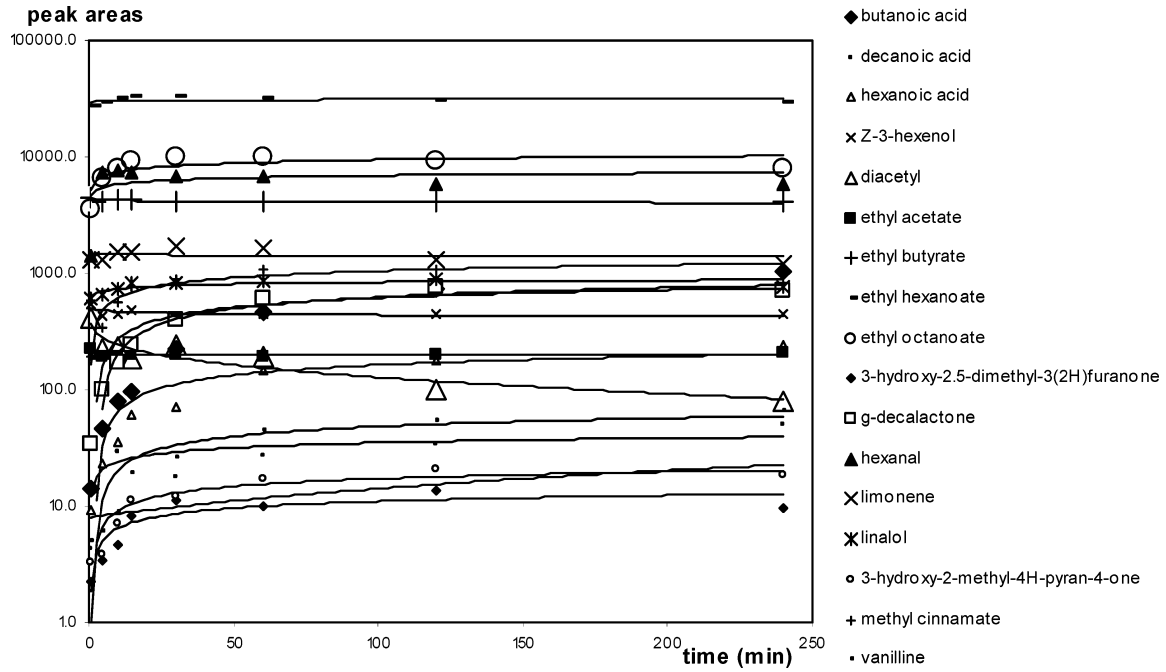

Figure 5. Stabilization time at 37 °C of aroma compounds between the PDMS fiber and the HS phase in yogurt MPO.

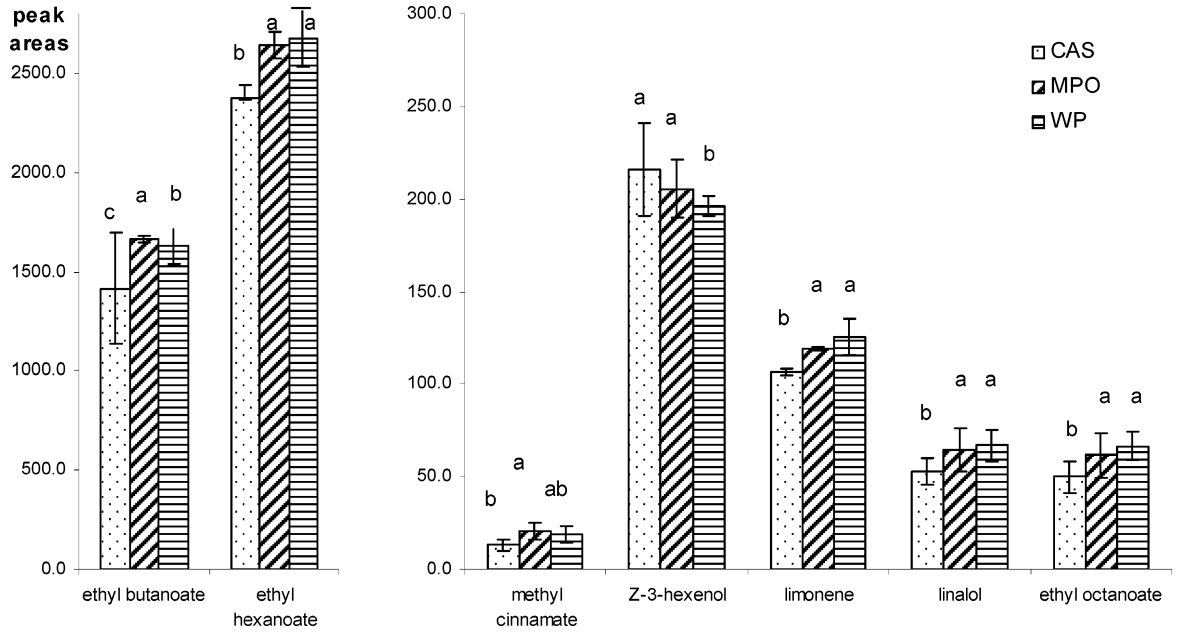

Figure 6. Flavor release (peak areas) from the three yogurts (CAS, MPO, and WP yogurts) for the significant aroma compounds (seven on 17 of the studied strawberry flavor) determined by the HS SPME method. The letters a–c indicate means that significantly differ at $p < 0.05$ (SNK test).

was controlled except for hexanoic acid (**Figure 4**). For a large majority of aroma compounds (16/17), no competition effect on the fiber was detected. Only the hexanoic acid release quantity was not linear when the concentration increased. Different equilibrium times (from 1 min to 4 h) were investigated (**Figure 5**) in order to quantify the release of aroma compounds but not the kinetic parameter of mass transfer. The time to reach the equilibrium was determined for each aroma compound: 10 min for ethyl octanoate and up to 2 h for hexanoic acid. Nevertheless, peak areas of a majority of the aroma compounds remain stable for 1−2 h, except for some of the most hydrophilic aroma compounds of the flavor used in this study (ethyl acetate, butanoic acid). Only diacetyl decreased slightly over time, which could have been due to an instability or a weak competition effect. Thus, 2 h was the chosen equilibration time.

Seven aroma compounds could significantly discriminate the three products by one-way ANOVA ($p < 0.05$): ethyl hexanoate, hexanal, ethyl octanoate, methyl cinnamate, ethyl acetate, *Z*-3-hexenol, and ethyl butyrate (**Figure 6**). CAS yogurt was characterized by a low release of aroma compounds, except for *Z*-3-hexenol. The aroma compound release of WP and MPO yogurts was higher than for CAS yogurt. Differences between WP and MPO yogurts were observed as follows: The release of ethyl octanoate was higher for WP yogurt than for MPO yogurt, and the contrary was observed for methyl cinnamate. However, variations of aroma release were quite low between

yogurts: about 10−30% of variations between CAS and MPO yogurts and less than 10% between WP and MPO yogurts.

Results similar to those obtained using the PRV method were observed, but the results were significantly confirmed on a greater number of aroma compounds. As was previously shown, only *Z*-3-hexenol presented a different behavior. The alcoholic function and the low hydrophobicity of this compound might explain a more specific interaction with whey protein than with caseinate.

Both methods (PRV and SPME) were complementary. The PRV method gave absolute values of partition coefficients, but only two aroma compounds could significantly discriminate the products studied under our conditions. The SPME method only provided a comparison between the yogurts by relative variations, but the extraction by a fiber resulted in a greater degree of sensitivity than conventional static HS analysis. Indeed, seven aroma compounds could significantly discriminate the yogurts by their protein composition variation.

Moreover, it was shown in a previous study that the type of protein influenced the olfactory properties of the yogurts (*1*). Yogurts enriched with caseinates were perceived as being less intense for a majority of olfactory notes, contrary to yogurts enriched with whey protein. Thus, physicochemical bindings between the aroma compounds and the matrices were in agreement with the olfactory differences between the yogurts.

This work demonstrates that the protein composition influenced the physicochemical properties of flavored stirred yogurts. Slight differences in protein composition induced large differences of microstructure and rheological behavior (yogurt enriched with caseinate had a complex viscosity 100% higher than yogurt enriched with whey protein), and smaller differences in aroma release (yogurt enriched with caseinate had a aroma release in static conditions 30% lower than yogurt enriched with whey protein). Thus, the physicochemical characteristics (structural organization and flavor release) can contribute to explain the variation in yogurt texture and olfactory perception. From a methodological point of view, the SPME method was a more sensitive method for a relative quantification of aroma compound release from a real food matrix, even at low concentrations.

This study contributes to a better understanding of the impact of formulation of a complex dairy product by focusing on the structure properties and the flavor release characteristics at the same time. Different hypotheses can explain the relationship between the microstructure of the product and the behavior of the aroma compounds. It was observed that the release of the aroma compound was lower in CAS yogurt than in WP yogurt. On one hand, the physicochemical interactions between aroma compounds and proteins can determine the flavor release in yogurt. On the other hand, the heterogeneous network with large pores of CAS yogurt might constitute a more effective barrier for the aroma compounds transfer in static conditions than the homogeneous network of WP yogurt.

These physicochemical results (aroma release and physical properties) are in agreement with the sensory properties (texture and flavor) shown in an earlier study (*1*). Further experiments are in progress to study the impact of protein composition and mechanical treatment on the dynamic aroma release parameters (diffusion coefficients in the yogurts) and to investigate the real time release of flavor and sensory perception during yogurt consumption.

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