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Flavor Release and Rheology Behavior of Strawberry Fatfree Stirred Yogurt during Storage

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The influence of storage on the aroma release in headspace and rheological properties in strawberry-flavored fatfree stirred yogurts was determined. Three periods of storage at 10 °C were chosen for analysis: 7, 14, and 28 days. The headspace composition was assessed in a flask in static mode. The SPME fiber was carefully chosen, and results are presented in detail (choice and degradation). The flow properties of the final product were measured in order to follow n (flow behavior index) and K (consistency index), and the apparent viscosity was determined (η in Pa·s). The quantity of flavors in the headspace of products at the 28 days of aging was significantly weaker for methyl 2-methyl butanoate, ethyl hexanoate, and hexyl acetate. The decrease was half of that in comparison with the seventh day. It was supposed that modification in rheological parameters can partly explain these results. Indeed, the apparent viscosity of the products significantly increased during the three times of storage. The composition of the flavored yogurt, proteins, exopolysaccharides, and fruit preparation, seemed to have a great impact on the release of aroma compounds. The aroma compound amount in the headspace decreased when the matrix changed from water to yogurt. With the fruit preparation, the headspace amounts for esters were significantly lower than in water alone, respectively, 23, 27, 29, and 17% less for methyl 2-methylbutanoate, ethyl hexanoate, hexyl acetate, and benzyl acetate. In flavored yogurt, the amount of aroma compounds in the headspace decreased again in comparison with the result obtained with the fruit preparation. Ethyl hexanoate and hexyl acetate presented the higher decreases of 48 and 53%, respectively.

KEYWORDS: Yogurt; SPME; strawberry; thickeners; flavor release; rheology

INTRODUCTION

Removing the fat content of yogurt modifies its properties (1, 2). Thus, maintaining yogurt texture can be a problem. In the food industry, some polysaccharides are introduced to tend to restore the yogurt's original characteristics (3). However, even if fat substitutes yield a product with characteristics similar to those of the original product, studies are necessary to understand the effect of the introduction of new agents in yogurt (4). Modifying fat content, replacing or removing fat, could modify the texture (5) and the aroma perception (6).

Rheological properties of stirred yogurt have been well studied. Stirred yogurts have flow properties that are characteristic of a non-Newtonian and weakly viscoelastic fluid (7–10). Keogh et al. (4) studied the role of milk fat, protein, gelatin, and hydrocolloids (starch, locust bean gum/xanthan mixture) on the rheology of stirred yogurt. These authors showed that the consistency index (K) and syneresis were more frequently

influenced by the composition than the behavior index (n) and the critical strain (γ_c). Hess (11) concluded, thanks to a rheological approach in nonfat yogurt, that polymer associated with the casein network prevents disruption of a portion of the network. Moreover, thickeners are generally incorporated in yogurt as part of a fruit preparation, and then a decrease in the viscosity of yogurt with fruit preparation in comparison to the control was observed (12–14).

As yogurt is a live product, structural changes to the milk gel can occur at various stages between the incubation and the storage (14–16). Bacterial content in yogurt leads to an increase in the strength of the protein network, on the one hand by an increase in lactic acid amount and on the other hand by exopolysaccharide production (EPS). The highest production of EPS occurred in the first hours of fermentation during the growth phase (17, 18).

Flavors are also important factors for food product acceptability by consumers. The influence of the product's structure and composition on the flavor behavior has been the subject of many works (19–21). The structure of a product will influence the transport of aroma compounds into the headspace of the products, whereas the composition will influence the interactions

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Table 1. Composition of the Blend Aroma in Propylene Glycol (PG)

aroma compound	concn ($\mu\text{g}\cdot\text{g}^{-1}$ in PG)	log <i>P</i>	CAS Registry No. ^a
methyl-2-methyl butanoate	15.0	1.22	868-57-5
ethyl hexanoate	10.0	2.79	123-66-0
hexyl acetate	8.6	2.79	142-92-7
hexan-1-ol	13.0	1.88	111-27-3
benzyl acetate	4.6	1.71	140-11-4

^a Provided by the author.**Table 2.** Composition of the Model Fruit Preparation

ingredient	percentage
modified waxy cornstarch	1.20
citrus pectin	0.60
guar gum	0.19
aspartame and acesulfame	0.17
fructo-oligosaccharides	12.30
fructose	12.30
calcium citrate	0.06
sodium citrate	0.15
strawberry pulp	67.00
water	6.03

between aroma compounds and nonvolatile ingredients. In low-calorie yogurt, the thickeners would play an important role in the flavor behavior. Indeed, thickeners have been shown to reduce flavor release in various matrices (19, 22–24). However, little information has been obtained on the impact of thickeners in aroma release from fatfree yogurt; for example, the effects of pectin, xanthan, and gelatin, with or without fat, on the perception of polar and nonpolar aroma compounds were studied in an acidified milk (1). The authors showed that each thickener has its proper role in aroma release. The smell and flavor of maltol were unaffected by thickeners, whereas the smell and flavor of ethyl 2-methylbutyrate were affected. Brauss et al. (25) showed that low-fat yogurt did not show the same aroma release as the full-fat yogurt. Fat substitutes modify both texture and aroma release.

To study the aroma release from stirred yogurt during storage, a blend of five aroma compounds in food concentration was chosen and thickeners were incorporated in industrial yogurt via a model fruit preparation. Flavor release from yogurt was determined by using a solid-phase microextraction (SPME). A rheological characterization was performed to try to link the release of aroma compound and the macroscopic properties of yogurt. Flavor release and rheological properties of the product were followed during a 28 day storage period.

MATERIALS AND METHODS

Blend Aroma. Aroma compounds were purchased from Sigma-Aldrich (l'Île d'Abeau, France). The stock solution of aroma compounds was prepared by dissolution in propylene glycol (PG) and stored in brown glass bottles at 4 °C (Table 1). According to the literature, this blend aroma would not influence lactic bacteria growth (26).

Model Fruit Preparation. One batch of the model fruit preparation used was supplied by the FAE Co. (Fruit Alliance Europe). Table 2 shows its composition.

Flavored Yogurt Sample. Stirred yogurt delivered by the Danone Co. (France) was obtained by natural lactic fermentation of reconstituted milk at 5% proteins content (fatfree milk powder + mineral water). Yogurt was mixed with the model fruit preparation (13% w/w) and flavored with the blend aroma at different amounts (0.01–0.1% w/w).

Headspace Analysis. Static headspace analysis was conducted in triplicate using 40 mL flasks with a valve cap (Supelco, Bellefonte,

PA). Before sampling, the yogurts were gently homogenized with a spoon to mix the small amount of syneresis that appeared during the aging period. Then an aliquot of 20 g was introduced in a 40 mL flask. An equilibration time of 15 min at 21 °C was used before syringe sampling (1 mL gastight syringe) or SPME sampling. SPME was performed using a commercially available polydimethylsiloxane fiber (PDMS) with a 100 μm film thickness and a polydimethylsiloxane–divinylbenzene fiber (PDMS/DVB) with a 65 μm film thickness. Both fibers were housed in a manual holder. Fibers were introduced in the headspace vessel during 2 min and desorbed in the GPC injector at 250 °C for 2 min. The fiber was held at 250 °C for 8 min to clean it. The SPME fiber is fragile, and degradation occurs during its use. Thus, to prevent faulty results due to fiber degradation, we selected two fibers with the same responses of nine fibers from the same lot for the two coatings used. Indeed, a great variation was observed in the amount of aroma compounds trapped between fibers of the same manufacturing lot. The selection of fibers was an important step to obtain an acceptable variability.

Flavoring Content during Aging. The aroma compound concentrations in yogurt were determined by simultaneous steam distillation–solvent extraction with a Likens–Nickerson apparatus to control the flavoring content during aging. One hundred grams of the product was introduced in a 250 mL vessel. Thirty-six grams of NaCl and 0.5 mL of antifoam (Clerol FBA 3107, Cognis St Fargeau Ponthierry, France) were then added. The addition of NaCl improves the extraction output, by a “salting-out” effect. Two hundred microliters of octanol solution as the extraction standard was added to the preparation. The sample was extracted by dichloromethane, during 30 min after the boiling point was reached. These extractions were made in triplicate.

GC Conditions. GC analysis was performed on an HP 5890 apparatus (Hewlett-Packard, Palo Alto, CA) equipped with a split/splitless injector and an HP 5970 mass selective detector. The detection was realized by full-scan mode in the mass range from 29 to 330. A fused-silica capillary column, DB-Wax, 30 m, 0.32 mm i.d., 0.5 μm film thickness (J&W Scientific, Folsom, CA), was used with helium carrier gas (velocity = 42 $\text{cm}\cdot\text{s}^{-1}$ at 40 °C). The column was held at 40 °C and the temperature increased at 3 °C $\cdot\text{min}^{-1}$ to 120 °C and at 7 °C $\cdot\text{min}^{-1}$ to 220 °C.

Rheology Parameters. The flow behavior index *n* and the consistency factor *K* were assessed according to the technique suggested by Benezech and Maingonnat (27) and Schmitt et al. (28), using a coaxial cylinder viscometer (RM180 DIN 2:2, Rheometric Scientific France) at 21 °C. Parameters *n* and *K* were obtained by use of the Ostwald law: $\tau = KD^n$, where τ is the shear stress and *D* the shear rate (s^{-1}). The apparent viscosity (η) at a shear rate of 64 s^{-1} was obtained with the same apparatus.

Statistical Analysis. All statistical tests (ANOVA) were performed using Statgraphics plus software, version 4.0 (Manugistics, Rockville, MD).

RESULTS AND DISCUSSION

Choice of the Fiber. The two types of fiber described above were tested to optimize the extraction of flavor compounds from blend aroma and endogenous aroma compounds from yogurt in the headspace of the product. The compounds analyzed as endogenous flavors of yogurt were selected as the most representative: acetaldehyde, butane-2,3-dione, and pentane-2,3-dione (15, 29, 30).

The variation coefficients obtained with fibers PDMS and PDMS/DVB on the headspace analysis of the aroma compounds in water are presented in Table 3. As expected, the higher the flavor concentrations were, the lower the variation coefficients were. In all cases, the mean variation coefficient was lower than 7%, and it was possible to validate the sampling method by manual injection for the whole experiment.

The comparison between two SPME fibers and a classical headspace extraction with a gas syringe was tested. In agreement with Fabre et al. (31), gas syringe sampling (1 mL) was not

Table 3. Variation Coefficients (Percent) of the Extracted Amounts of Aroma Compounds in Water by SPME Analysis with PDMS and PDMS/DVB Fibers as a Function of the Blend Aroma Content

aroma concn in water	PDMS	PDMS/DVB		
	0.1%	0.1%	0.05%	0.01%
methyl-2-methyl butanoate	3.4	0.7	12.6	3.2
ethyl hexanoate	4.8	0.2	7.8	8.2
hexyl acetate	4.3	0.1	7.5	8.2
hexan-1-ol	5.3	1.2	0.8	3.1
benzyl acetate	7.2	1.1	3.2	8.3

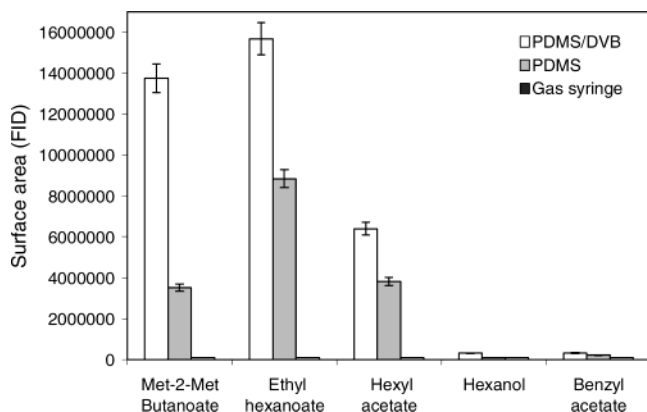


Figure 1. Comparison of extractions of aroma compounds from water by headspace SMPE (PDMS and PDMS/DVB) and static headspace analysis (gastight syringe) with GC-FID.

sensitive enough to quantify release of aroma compounds from an aqueous solution (**Figure 1**). Kataoka et al. (32) showed that the PDMS/DVB fiber is suitable for headspace analysis of strawberry fruit and that the PDMS fiber is suitable for headspace analysis of strawberry juice. However, in the present work the mix coating fiber PDMS/DVB was better to extract our components than the PDMS fiber with regard to extracted quantities. Hexanol and benzyl acetate were less extracted than the other compounds due to a good solubility in media and a poor volatility, respectively. As reviewed by Kataoka et al. (32), PDMS and PDMS/DVB fibers can be used to analyze strawberry aroma type, but we thought that the polarity and low molecular weight of methyl methyl-2-butyrate, ethyl hexanoate, and hexyl acetate were responsible for greater peaks with the PDMS/DVB fiber than with the PDMS fiber.

The surface areas obtained from headspace analysis of unflavored yogurt with flame ionization detector (FID) are presented in **Figure 2**. The PDMS fiber trapped fewer endogenous aroma compounds than the PDMS/DVB fiber. Acetaldehyde, butane-2,3-dione, and pentane-2,3-dione were not detected with the PDMS fiber. The most polar compounds were less trapped with PDMS than the mix coating fiber PDMS/DVB. For hydroxy-3-butanone, no significant difference was obtained between the two fibers. The PDMS/DVB fiber was the best to analyze aroma compounds from blend aroma and endogenous flavors from yogurt. Our results are in accordance with the Kataoka et al. review (32). We observed that endogenous polar aroma molecules analyzed are low molecular weight and low log *P* and were better extracted with the PDMS/DVB coating than with the PDMS coating. The variability measured on the peak area of hydroxy-3-butanone could be due to the endogenous origin of molecules.

Influence of Blend Aroma Amount on SPME Efficiency. The effect of aroma content on the SPME efficiency was studied in water solution and in yogurt. Three amounts of blend aroma

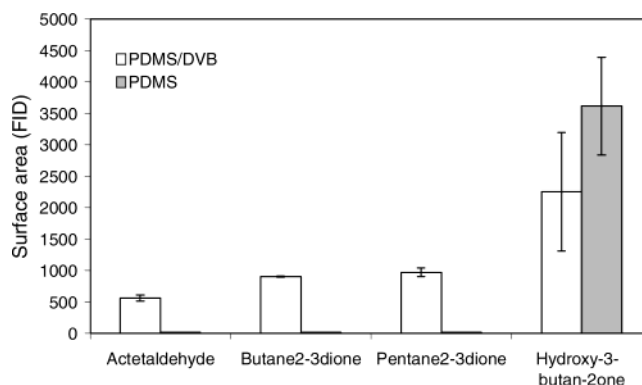


Figure 2. Comparison of extractions of endogenous aroma compounds from unflavored yogurt by headspace SPME (PDMS and PDMS/DVB) with GC-FID.

Table 4. Ratio of the Compound Peak Areas Obtained by Headspace SPME Analysis as a Function of the Blend Aroma Content in Water (Grams per 100 g)

aroma compound	blend aroma ratio	
	0.05/0.01	0.1/0.01
methyl-2-methyl butanoate	4.2	3.5
ethyl hexanoate	4.8	10.0
hexyl acetate	5.0	10.0
hexan-1-ol	4.5	8.0
benzyl acetate	4.7	10.0

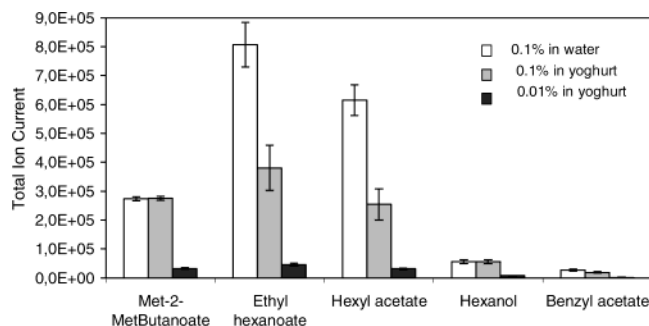


Figure 3. Analysis by GC-MS of aroma compounds extracted by headspace SPME (PDMS/DVB fiber) from flavored water and yogurt as a function of the blend aroma amount.

were tested: 0.01, 0.05, and 0.1% (w/w) with the PDMS/DVB fiber. The ratios between the surface area from the headspace of samples as a function of aroma amount in water were calculated (**Table 4**). The increase in the compound surface areas was proportional to the increase of aroma compound concentrations from 0.01 to 0.1% in samples except for methyl-2-methyl butanoate. The extraction of the latter was strongly affected with a high content of the blend aroma in water. The ratio between the peak surface for headspace analysis from aqueous solution at 0.01 and 0.1% of methyl-2-methyl butanoate was 3.5 in place of 10. A saturation effect of the SPME fiber was assumed to explain the nonlinearity in the fiber response for methyl-2-methyl butanoate.

The same experiment was conducted in yogurt with a direct flavoring. Two amounts of aroma were studied. It was expected that, as the flavor release from yogurt would be lower than in water, the fiber would not be saturated with the highest amount of 0.1%. Results are presented in **Figure 3**. In yogurt with 0.1% of blend aroma, a saturation effect appeared with methyl-2-methyl butanoate as previously shown in water. Indeed, no significant difference was observed in headspace quantity of

Table 5. Rheological Parameters and pH in Flavored Yogurt at Three Aging Times

aging time	consistency index K^a	flow behavior index n^a	apparent viscosity ^a (Pa·s)	pH ^a
day 7	4.05 ± 0.39	0.55 ± 0.05	0.67 ± 0.01	4.32 ± 0.10
day 14	5.14 ± 0.47	0.49 ± 0.02	0.71 ± 0.02	4.20 ± 0.09
day 28	5.23 ± 0.57	0.50 ± 0.03	0.78 ± 0.10	4.14 ± 0.10

^a ± standard deviation.

this compound as a function of the medium (water or yogurt) at 0.1% blend aroma. For the other aroma compounds, flavor release was lower in yogurt than in water. A higher affinity of those aroma compounds for the yogurt ingredients than for water can explain the decrease of flavor release.

The extracted quantities of endogenous aroma compounds from yogurt were also affected by high amounts of blend aroma (results not shown). In the presence of 0.1% blend aroma, the surfaces of endogenous aroma compounds from yogurt were lower than with lower concentrations: 0.01 and 0.006% of blend aroma. The endogenous aroma compounds produced by bacterial strains are present in very weak amount in yogurt and thus in the headspace. Therefore, they could be sensitive to a competitive effect to the sorption on the fiber with the volatile compounds used to flavor yogurt. It was also supposed that saturation effects, as observed with methyl-2-methyl butanoate, could change the sorption properties of SPME fibers. Thus, many precautions must be taken before using SPME in the analysis of flavor release: linearity/saturation check, stability along extractions, and reproducibility between fibers. The amounts of acetaldehyde, butane-2,3-dione, and pentane-2,3-dione analyzed in the nonflavored yogurt and in the 0.01 and 0.006% flavored yogurts were not significantly different. Thus, at 0.01% aroma blend in yogurt, the analysis of both endogenous aroma compounds and flavoring compounds was possible without any saturation effect in our time and temperature conditions.

Influence of Aging on Rheological Parameters and Flavor Release in a Flavored Yogurt. The model fruit preparation was introduced at a rate of 13% in the fatfree stirred yogurt. The final product was flavored with the blend aroma at 0.01%. The products were stocked during 7, 14, and 28 days at 10 °C. The aging periods were selected in agreement with the practices of the dairy industry.

Rheological Parameters. The evolution of the consistency index (K) and the flow behavior index (n) of the product during aging is presented in **Table 5**. The consistency index of the yogurt increased from 4.05 to 5.14 during the first 7 days of storage. No significant difference was observed for longer storage periods. The flow behavior index of yogurt decreased weakly, but results were not significant, despite a good variation coefficient obtained in the flow curve measurements. We observed also that apparent viscosity showed a significant increase during storage. These results were in agreement with the literature (14–16).

The increase in the consistency index and the apparent viscosity could be due, on the one hand, to an acidification of the product, which reinforces the protein network (15). Indeed, the pH of yogurt decreased from 4.32 to 4.14 in 28 days at 10 °C (**Table 5**). The decrease in pH of the product was confirmed by the increase in the lactic acid concentration determined with a Bioringer enzymatic kit (results not shown). On the other hand, the residual microbial activity in yogurt would generate exopolysaccharides (EPS) in the medium, which take part in

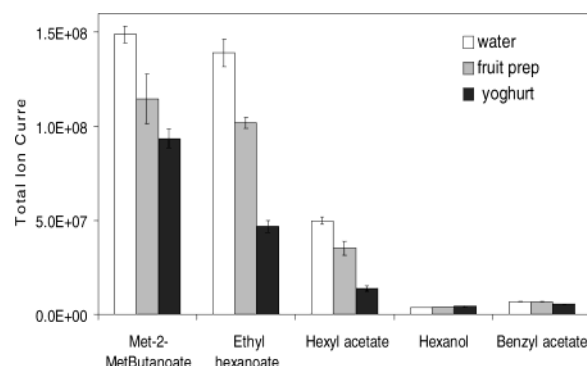


Figure 4. Flavor release of aroma compounds in water, water + fruit preparation, and yogurt + fruit preparation by headspace SPME (PDMS/DVB fiber) and GC-MS detection. The data were obtained with 0.01% blend aroma at 7 days of aging.

the protein network and could reinforce the textural properties of yogurt (15, 26, 27). Hess et al. (11), Marshall and Rawson (33), and Laws and Marshall (17) observed that modifications might be due to EPS production and pH decreases. However, the authors agreed that the highest EPS production generally occurred in the beginning of the lactic fermentation. Moreover, Shah et al. (27) and Birollo et al. (34) studied the viability of lactic microflora and pH during storage and found not only that pH decreased but also that the survival activity of lactic bacteria can decrease dramatically around the 25th day of storage depending on the bacteria strain. However, no data are available on those parameters for a longer period of fermentation.

Flavor Release. The flavor release was measured from flavored water, flavored water + 13% fruit preparation, and flavored yogurt (blend aroma, 13% fruit preparation, fatfree yogurt). All of the samples were flavored with 0.01% of the blend aroma. The amount of aroma compound in the headspace decreased when the matrix changed from water to yogurt (**Figure 4**). In the water + 13% fruit preparation, the headspace amounts for esters were significantly lower than in water alone, respectively, 23, 27, 29, and 17% less for methyl-2-methyl butanoate, ethyl hexanoate, hexyl acetate, and benzyl acetate. For hexanol no significant difference was found.

The presence of starch, pectin, and guar in yogurt was supposed to affect the flavor release of esters. These data are in good agreement with numerous works about the release of flavor compounds in the presence of hydrocolloids (19, 23, 24, 35). As a function of hydrocolloid nature, the release of aroma compounds is reduced in comparison to water. For the other ingredients in the fruit preparation, Decourcelle et al. (36) showed that the presence of strawberry pulp and sweeteners did not affect the flavor release in fatfree yogurts.

When the fruit preparation is added in yogurt, the amount of aroma compounds in the headspace decreased again in comparison with the result obtained when fruit preparation is added in water. Ethyl hexanoate and hexyl acetate presented the higher decreases, 48 and 53%, respectively. We supposed that protein material could be implied in the decrease of esters released from our yogurt. Specific interactions between milk proteins and some aroma compounds have been studied by many authors. Proteins from milk, for example, caseins and whey proteins, are known to bind aroma compounds as a function of their chemical nature, which leads to a decrease in flavor release (37–40). Moreover, starch, pectin, and guar gum could develop molecular interactions with proteins, and a complex network is obtained. No data are available in the literature describing the polysaccharide–protein association in a complex system such as stirred yogurt.

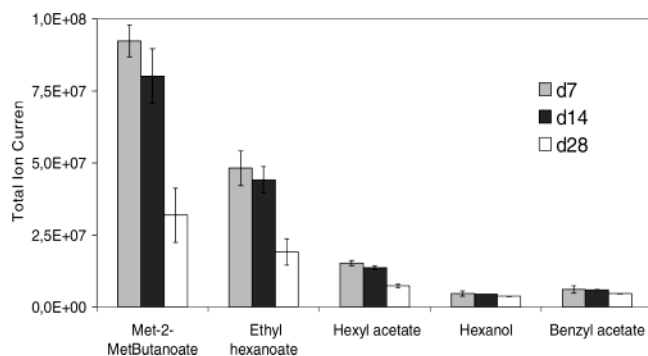


Figure 5. Flavor release in fatfree stirred yogurt with fruit preparation at three aging times by headspace SPME (PDMS/DVB fiber) and GC-MS detection. The data were obtained with 0.01% blend aroma.

At a macroscopic scale the result for this polysaccharide–protein interaction is an increase in the apparent viscosity of the yogurt. Thus, the stirred yogurt is always a gel, which can play a role in flavor release. It was shown in some works that the texture of a product has an impact on the flavor release (41, 42). The present result obtained in an industrial yogurt was in good agreement with the works of Brennan et al. (43), Wendin et al. (1), and Penna et al. (44). These authors agree that the flavor release behavior not only is governed by the viscosity or the matrix network but also results from the occurrence of specific interactions.

The flavored yogurt was stored during 28 days at 10 °C. The flavor release was measured at 7, 14, and 28 days. No measurement was made at 0 days because it is known that the structure of the protein network in yogurt is not well established in the first days of aging. The total amount of aroma compounds in the samples was determined by a simultaneous steam distillation–solvent extraction. Thus, we checked that no loss in aroma compounds occurred during this period. During the aging of the products between 7 and 28 days, a decrease of some aroma compounds in the headspace was observed (Figure 5). No significant difference was noted between the two first times (7 and 14 days). At the third period of aging, the quantity of flavors in the headspace of yogurts was significantly weaker for methyl 2-methylbutanoate, ethyl hexanoate, and hexyl acetate. The decrease was half of that in comparison with the seventh day. For endogenous flavors, no significant difference was observed (data not shown). SPME seemed to be not sensitive enough in our conditions to quantify these aroma compounds. Moreover, the behavior of endogenous flavors in yogurt appeared to depend of many factors such as bacteria strain and temperature of fermentation (45).

As previously described, flavor release was strongly modified in the presence of yogurt in comparison with water. Putting aside the presence of proteins and polysaccharides, which can interact with aroma compounds, yogurt is a product having rheological characteristics that change during aging (14–16). The main effect observed on a plain yogurt is an increase of viscosity due to the bacterial activity, which decreases the pH during aging (14–16). Indeed, the strength of the protein network increases along with the increase of lactic acid amount and exopolysaccharides production, from live bacteria in yogurt (17, 18). Thus, the changes in rheological properties observed during aging could partly explain the results in flavor release. As described in the literature, in model systems, both the texture of the matrix and the aroma–matrix interactions have to be taken into account, even at the very low polymer and aroma concentrations that were used in the present study. Thus, we can suppose that the odor and the texture of our yogurts at

28 days of aging would be different in comparison with the product at 7 days. Indeed, the fruity note due to esters may be dramatically reduced. Modifications in flavor and in acceptability of yogurts during storage were described in some works (1, 43, 45, 46). Laye et al. (45) observed modifications of flavor during storage of yogurts, and panels showed differences for flavor, aroma, and acceptability of yogurt. In a recent work, Ünal et al. (46) observed that defects of syneresis in relation to the appearance and mouthfeel in set yogurts could be eliminated by increasing the dry matter content and amount of thickeners such as locust bean gum.

Fatfree yogurt is a complex matrix with proteins and polysaccharides (EPS, thickeners). Both macromolecules can interact with aroma compounds, and they also develop a complex network that could play a role in the behavior of flavor. To elucidate the influence of each ingredient against viscosity and flavor behavior, more works will be necessary, but we emphasize that the present study was performed using ingredients in food concentrations.

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