

Influence of Ingredients on the Self-Diffusion of Aroma Compounds in a Model Fruit Preparation: An Nuclear Magnetic Resonance–Diffusion-Ordered Spectroscopy Investigation

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Diffusion-ordered spectroscopy (DOSY)–pulsed field gradient (PGF) nuclear magnetic resonance (NMR) spectroscopy was used to measure self-diffusion coefficients of aroma molecules in model fruit preparations. The impact of the sucrose content on aroma diffusion was specifically investigated, and the relationship with viscosity, water activity, and dry matter parameters was evidenced. DOSY-PGF NMR spectroscopy was found to be a relevant and accurate technique to follow self-diffusion of aroma compounds at low concentrations in a complex food matrix and to obtain information on diffusion of the sucrose and of the water molecules. We showed that aroma self-diffusion was strongly decreased in fruit preparation because of the high sucrose content, which induces the formation of a network through hydrogen bonds with water. Self-diffusion coefficients were determined for aroma molecules of different natures, and values are related to the physicochemical properties of the molecule.

KEYWORDS: DOSY NMR spectroscopy; aroma compound self-diffusion; fruit preparation; sucrose content; matrix structure

INTRODUCTION

The control of flavoring of a food product as well as the flavor perception during consumption is a great challenge (1). The availability of volatiles is connected to transport phenomena such as diffusion into the food product or mass transfer through the interface with air (2). However, because of the complexity of the transport mechanisms and of the composition of food matrices, factors that govern the release of flavor during consumption are not yet well-understood. In fact, many parameters can influence the transport of flavor molecules. For instance, the physicochemical characteristics of the food components, the aroma molecules, and the interactions between aroma molecules and food components have to be taken into account (3).

Moreover, liquid, semisolid, or solid food products contain components of different natures, which lead to different textures, and thus can also influence aroma release. Several studies have shown the impact of viscosity on flavor release in model media. For example, the release of diacetyl in the headspace decreased with increasing viscosity in the presence of xanthan gum, carrageenan, or guar gum (4). Similarly, the headspace con-

centration and the perceived flavor intensity of benzaldehyde and limonene were significantly reduced with increasing firmness of gels prepared from carrageenan, gelatin, or starch (5).

The release of flavor molecules from model food systems such as desserts containing sugar and/or hydrocolloids has been the subject of various studies. These studies used headspace techniques for measuring mass transfer and/or partition coefficients (6–12), to obtain information on flavor diffusivity, which is described as the diffusive mass transfer. However, headspace techniques give indirect macroscopic information both on diffusion in food matrices and on interactions between flavor molecules and food matrix ingredients.

To obtain additional information on the diffusion process of flavor molecules, other methods have been developed to measure diffusivity in gelled matrices: the concentration–profile method (11, 13–15), the capillary method, or the diaphragm cell method (16, 17). Voilley et al. (14) and Rega et al. (11) used the concentration–profile method to investigate the diffusivity of flavor molecules in water solution and in model systems containing 60% of sucrose. They showed that for all flavor molecules, diffusion coefficients are about 10 times lower in sucrose solution than in water solution. However, it must be pointed out that these techniques for measuring diffusivity are time-consuming and not always well-adapted to complex gel matrices. Actually, the concentration–profile method needs to have a matrix firm enough to be cut into identical slices for

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Table 1. Physicochemical Characteristics of Aroma Compounds

aroma compound	flavor note ^a	MW (g mol ⁻¹)	vapor pressure at 25 °C (mm Hg)	hydrophobicity log <i>P</i> ^b	solubility in water at 25 °C (g L ⁻¹)	accessible surface (×10 ¹⁰ m ²) ^c
ethyl acetate	fruity, pear	88	101	0.73	82.00	265.80
ethyl butyrate	apricot, kiwi	116	15.60	1.80	5.65	328.93
ethyl hexanoate	fruity, strawberry	144	1.97	2.80	0.49	391.37
linalool	flower of orange	154	0.09	2.91	2.60	396.27
hexanal	freshly cut grass	100	10.30	1.80	1.78	305.89

^a Ref 31. ^b Calculated by the method of Rekker (32). ^c Theoretical values obtained from ChemOffice software (Chem3D).

each repetition. For that purpose, Voilley and co-workers added agar-agar as a gelling agent (13, 14), which perhaps changed the diffusion medium. In addition, an extraction step or the use of a fluorescent solute or probe is necessary to quantify diffusing molecules.

On the other hand, spectroscopic techniques have also been developed for measuring translational diffusion of solutes in liquid or gel matrices (18–23). Translational motion is the most fundamental form of transport and is closely related to molecular size (Stokes–Einstein equation). This diffusive process is called self-diffusion, or sometimes tracer diffusivity, and reflects the random translational motion of molecules driven by internal kinetic energy. Thus, the spectroscopic techniques used describe microscopic displacements, which range from 10⁻¹² to 10⁻⁴ m, covered by a solute per second. With the fluorescence recovery after photobleaching technique, Contreras-Lopez et al. (20) showed that different polysaccharides added at 1% w/w to sucrose solutions had no significant effect on the translational diffusion of small solutes, whatever the nature of the polysaccharide. However, this technique requires the use of fluorescent molecules, which are not suitable for the great majority of aroma compounds.

Thanks to the large number of methodological, instrumental, and data-processing improvements allowing applications to various domains, diffusion-ordered nuclear magnetic resonance spectroscopy (DOSY NMR), with the use of pulsed field gradient (PFG) is a convenient, accurate, and noninvasive method to measure translational diffusion constants or self-diffusion coefficients (24–26). PFG NMR combines NMR information about components of a mixture with their diffusion coefficients. Self-diffusion measurements are based on the fact that the NMR signal attenuation of a component is directly linked to its diffusion coefficient. The NMR diffusion time scale is defined by a diffusion delay Δ , which generally ranges from milliseconds to seconds, so that translational motion of some hundred micrometers is probed. The data-processing approach called DOSY generates a two-dimensional plot with a chemical shift scale in one dimension and diffusion coefficient values in the second dimension (21, 26, 27). The self-diffusion coefficient is thus directly estimated using the DOSY method. Moreover, at the same time, the various components of a complex mixture can be separated and identified in a single experiment (28). This technique has been widely used for measuring self-diffusion coefficients of molecules in synthetic polymers (29, 30). Recently, we have shown that DOSY NMR is well-suited to the measurement of the diffusion of aroma molecules at low concentrations in model gel matrices (21).

The present work is focused on a complex food matrix consisting of a model fruit preparation frequently used in dairy products to flavor plain yogurt. This gelled matrix contains a high content of sucrose, a mix of starch and carrageenan, and aroma compounds at low concentrations. The aim of this study was 2-fold: first, to show that DOSY-PGF NMR spectroscopy

is the technique of choice to investigate self-diffusion of small molecules at low concentrations in complex food matrices, and second, using this technique, to understand how the gel structure and the composition of a food matrix influence the self-diffusion of aroma molecules of different natures.

MATERIALS AND METHODS

Materials. Commercial sucrose (Erstein, France), a stabilized and cross-linked waxy corn starch (Roquette Frères, France), a 50/50 mixture of κ - and κ 2-carrageenans (Danisco, Denmark), calcium sulfate, potassium chloride, and a lactate buffer (pH 3.8) were used for this study. Ethyl acetate, ethyl butyrate, ethyl hexanoate, linalool, hexanal (purity higher than 97%, food quality), and D₂O (99.9% purity) were obtained from Sigma Aldrich (France). Physicochemical parameters of aroma compounds are given in Table 1 (31, 32).

Model Fruit Preparations and Sucrose Solutions. The model fruit preparation contained 35% (w/w) of sucrose, 1.4% of starch, 0.8% of carrageenans, 0.16% of KCl, and 0.03% of CaSO₄. Sucrose, starch, and carrageenans mixed together were dissolved in lactate buffer (pH 3.8) in D₂O. The preparation was stirred at 200 rpm during the heating at 85 °C for 10 min and during the cooling to 35 °C over 12 min. A model fruit preparation without sucrose was also prepared (sucrose was replaced by lactate buffer). Sucrose solutions were prepared at three concentrations: 35, 20, and 5% (w/w) in D₂O. The preparations and solutions were then flavored at room temperature and introduced into a liquid NMR tube (5 mm of diameter). For each aroma compound, the final concentration was 100 μ L L⁻¹ for the measurement in D₂O and 300 μ L L⁻¹ in fruit preparations or in sucrose solutions. All samples were stored at 10 °C for 24 h, corresponding to the parameters required to reach texture and flavor equilibrium.

Rheology, Water Activity, and Dry Matter Measurements. The viscosity (η) of the sucrose solutions in D₂O was obtained with a coaxial cylinder high sensitivity viscometer (Low Shear 40, Contraves) at 30 °C to mimic the temperature in the mouth during mastication. For gel matrices in D₂O, the G' and G'' moduli were measured with a SR5 rheometer (Rheometric Scientific, France) using cone and plate geometry (4 cm, 0.02 rad). Mechanical spectra with frequency varying between 100 and 0.1 rad s⁻¹ were established at 30 °C and at a constant strain of 1%. Values measured at 1 rad s⁻¹ were recorded.

The water activity of the sucrose solutions and matrices in H₂O was measured at room temperature with a water activity instrument (AquaLab series 3TE, Decagon Devices, Inc., Washington, DC). Dry matter values (% w/w) were obtained by drying the samples at 103 °C for 24 h.

NMR Analyses. All ¹H NMR spectra were recorded at 500 MHz using a Bruker Avance 500 spectrometer with a 5 mm z -gradient Bruker inverse probe at 30 °C. The temperature was calibrated using the Bruker sample temperature calibration tube (80% Glycol/DMSO). Because it has similar physicochemical characteristics, D₂O was chosen as the solvent rather than H₂O in order to avoid the presence of the broad NMR peak of water protons. However, H₂O traces were present in D₂O solution. This small weight percentage of H₂O is called “residual water”, and its NMR signal is observed at 4.7 ppm.

¹H one-dimensional (1D) and DOSY NMR spectra were recorded with a 90° flip angle impulsion length of 6.5 μ s. For DOSY spectra, STE bipolar pulses with or without longitudinal eddy current delay (STE-BPP and STE-BPP-LED) experiments were used with a pulse

delay between 450 and 1500 μs after each gradient, a PFG pulse length (δ) between 1.2 and 2.4 ms, and a diffusion delay (Δ) between 150 and 600 ms. Application of STE-BPP with multipresaturation sequence was also used for the complete fruit preparation. All sequence parameters were adapted for each sample in order to observe that the aroma NMR signal intensity completely disappeared at 95% of the full gradient strength. Thirty-eight experiments were recorded with gradient intensity linearly sampled from 5 to 95%. The gradient system had been calibrated to 47.5 G cm^{-1} at maximum intensity.

During the NMR diffusion delay Δ , the signal intensities of diffusing components decrease exponentially according to $I/I_0 \propto \exp(-Dq^2\Delta)$ where I/I_0 is the signal attenuation, D is the self-diffusion coefficient in $\text{m}^2 \text{s}^{-1}$, q^2 is the gradient amplitude with $q = \gamma \cdot g \cdot \delta$ where γ is the gyromagnetic ratio of the observed nucleus ($\text{rad S}^{-1} \text{T}^{-1}$) and g and δ are the gradient strength (T) and length (s), respectively. The decay rates of the exponential curves are thus proportional to their respective diffusion coefficients.

All data were processed using Gifa 5.2 software with the ILT method using the Maximum Entropy algorithm (MaxEnt) as already described (21). The ILT was computed only on the columns presenting a signal 32 times greater than the noise level of the experiment. DOSY spectra are presented with chemical shift on the horizontal axis and diffusion on the vertical axis expressed in $\mu\text{m}^2 \text{s}^{-1}$.

Data Analysis. For aroma compounds and sucrose, several peaks were present on the DOSY spectra corresponding to each proton group in the molecule. For one molecule, proton peaks were picked up on the DOSY spectrum and an average self-diffusion coefficient was calculated from diffusion values of each peak. Measurement of aroma diffusion in D_2O was repeated two or three times. In the other media, measurements were performed once except for ethyl acetate in the 35% sucrose solution, for which two repetitions were performed as only two peaks were observed.

Mean aroma diffusion coefficients and variation coefficients (CV) were calculated from all of the values obtained (r repetitions \times p peaks). The 95% confidence intervals (STATGRAPHICS software) are indicated in the figures. For the measurements in D_2O , we obtained CVs smaller than 5%, and for the other media, they were smaller than 15%.

Analysis of variance (ANOVA) and least significant difference tests (LSD) were used to assess significant differences between coefficients in the different matrices and for the different aroma compounds (STATGRAPHICS software). Significance was established at $p < 0.05$.

RESULTS

In the first step, the influence of ingredients on the diffusion in the model fruit preparation of ethyl butyrate, a frequently used aroma compound, was investigated. In the second step, the impact of the nature of the aroma compounds was studied with four other molecules.

Self-Diffusion of Ethyl Butyrate in D_2O and in the Model Fruit Preparation. The diffusion coefficient of ethyl butyrate was measured in D_2O and in the model fruit preparation at 30 $^\circ\text{C}$. The DOSY spectrum of ethyl butyrate in D_2O is presented in **Figure 1A** with the corresponding ^1H 1D spectrum in the insert. In the DOSY spectrum, five peaks corresponding to the five proton groups of the molecule, $\text{CH}_3(1)$ at 0.81 ppm, $\text{CH}_3(5)$ at 1.16 ppm, $\text{CH}_2(2)$ at 1.52 ppm, $\text{CH}_2(3)$ at 2.26 ppm, and $\text{CH}_2(4)$ at 4.07 ppm, were well-aligned. The value of the self-diffusion coefficient was recorded for each of the five peaks, and an average self-diffusion coefficient of $881 \mu\text{m}^2 \text{s}^{-1}$ was found. The measurement was repeated, and a CV of 3% was found. The peak at 4.7 ppm corresponds to the residual water peak giving a diffusion coefficient of $2191 \mu\text{m}^2 \text{s}^{-1}$ at this temperature.

The same NMR experiment was performed for this aroma compound in the model fruit preparation. A concentration of aroma compound of $300 \mu\text{L L}^{-1}$ was added to the fruit preparation in order to observe aroma NMR peaks with a good signal-to-noise ratio. Moreover, because of the presence of

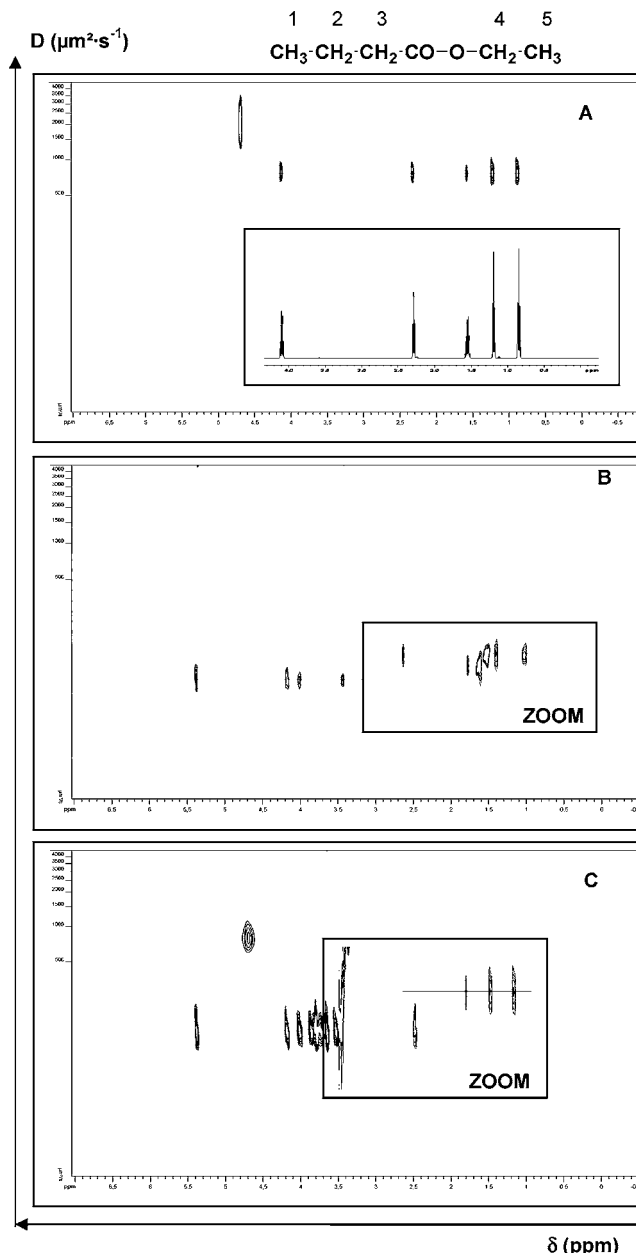


Figure 1. ^1H NMR DOSY spectra of ethyl butyrate in D_2O (A), in the fruit preparation (B) and in 35% sucrose solution (C) at 30 $^\circ\text{C}$. The ^1H 1D NMR spectrum of ethyl butyrate in D_2O is shown in the insert of panel A.

numerous peaks of the matrix components, saturation of three intense peaks at 4.7 ppm (residual water peak) and at 3.6 and 3.7 ppm (sucrose peaks) was applied. From the DOSY spectrum (**Figure 1B**), three peaks at 0.81, 1.51, and 2.24 ppm were distinctly observed for ethyl butyrate, while the two other peaks were perturbed or hindered by the presence of the NMR peaks of the matrix components. The average self-diffusion coefficient and the confidence interval were calculated from these three peaks; a value of $141 \mu\text{m}^2 \text{s}^{-1}$ was obtained (**Figure 2**). The self-diffusion coefficient was thus dramatically decreased in the model fruit preparation by 84%, as compared to the value in D_2O , with a confidence interval smaller than 10%. The mobility of ethyl butyrate was therefore strongly reduced in this complex medium. The DOSY spectrum without saturation gave the same results although it was less resolved. For the next experiments, no peak saturation was applied in order to evaluate the self-diffusion of residual water.

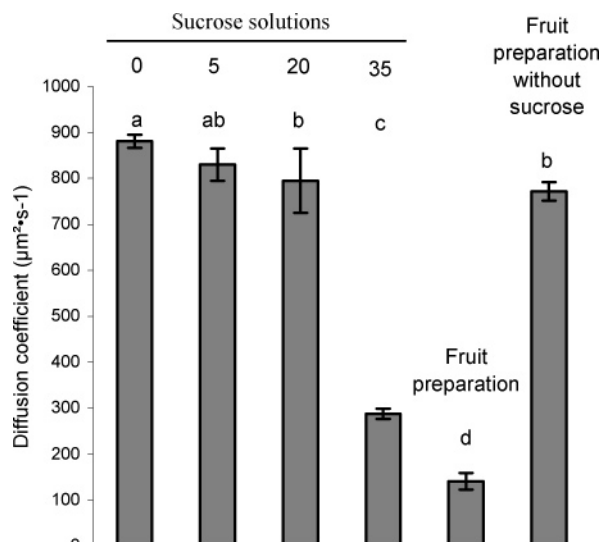


Figure 2. Mean self-diffusion coefficients ($\mu\text{m}^2 \text{s}^{-1}$) of ethyl butyrate in matrices and sucrose solutions at 30 °C. The significant differences are indicated with different letters (ANOVA and LSD tests, $P < 0.05$), and the 95% confidence intervals are represented on the bars.

Some components in the fruit preparation are known to have an influence on diffusion and on the release of aroma compounds. Starch and carrageenan, which are known to be the major components involved in the gelled network, have been shown to reduce significantly the headspace concentration and the perceived flavor intensity of aroma compounds (5). On the other hand, it has been reported that the release of aroma compounds is significantly influenced by the presence of a high sucrose content (6–9). To investigate the impact of sucrose and starch–carrageenan in the fruit preparation separately, self-diffusion coefficients of ethyl butyrate were measured in a D_2O solution containing 35% of sucrose and in the model fruit preparation without sucrose. **Figure 1C** shows the DOSY spectrum of ethyl butyrate in 35% sucrose solution. Three peaks of ethyl butyrate, 1, 2, and 3, are visible and well-aligned; the peak 4 is perturbed by the presence of the sucrose peaks between 3.4 and 5.5 ppm.

Results are summarized in **Figure 2**. The diffusion coefficients were significantly different in the 35% sucrose solution and in the matrix without sucrose as compared to the values obtained in D_2O and in the complex matrix. When compared to the value in D_2O , the self-diffusion coefficient decreased by 67% in the 35% sucrose solution while the reduction was of 12% in the fruit preparation without sucrose. Thus, the observed decrease in the model fruit preparation (85%) seems to be mainly due to high sucrose content. Starch and carrageenans seem to have a significant but much less important effect on the diffusion of ethyl butyrate.

The NMR technique has the great advantage of also giving direct information on molecular interactions, by comparing chemical shifts of solutes alone and in the presence of macromolecules. Therefore, we could investigate the existence of interactions between ethyl butyrate and sucrose, starch, or carrageenan. This has been done by comparing ^1H 1D NMR spectra of ethyl butyrate in D_2O solution, in the 35% sucrose solution, and in the fruit preparations with and without sucrose. The scalar coupling of peaks had a lower resolution in complex media than in D_2O solution due to the decrease of the mobility of the aroma molecule. However, no significant difference between the chemical shifts of aroma peaks in the different media was experienced. Consequently, there was no direct

molecular interaction between the ethyl butyrate and the components of the fruit preparation.

To understand the role of the different factors involved in the diffusion phenomenon, the different media were characterized (**Table 2**). The model fruit preparations with or without sucrose were characterized as gelled structures with a relatively high G' modulus, G' constant as a function of frequency (not showed) and $\tan(\delta) = G''/G'$ lower than 0.1; the viscosity of the 35% sucrose solution at 30 °C was slightly higher than that in water. As stated by the Stokes–Einstein equation, the 3-fold decrease in the self-diffusion coefficient from D_2O solution to 35% sucrose solution could be partly explained by the increase in medium viscosity from 0.98 to 4.12 mPa s^{-1} . In addition, the self-diffusion coefficient in the fruit preparation without sucrose was just slightly lower than that in D_2O solution (–12%), which suggests that despite the presence of starch and carrageenan, the gelled structure is not the main factor responsible for the decrease in the self-diffusion coefficient. Thus, by comparing the self-diffusion values in model fruit preparations with and without sucrose (**Figure 2**), we observed that the presence of a high sucrose content had a more prominent effect on aroma self-diffusion than did the structuration of the medium.

The model fruit preparation without sucrose had a much lower dry matter content (2.60%) than the two other media: the complete fruit preparation (37.6%) and the 35% sucrose solution. It may be postulated that the sucrose content was mostly responsible for the decrease in the self-diffusion coefficient. This is in agreement with the observation of Voilley et al. (14) who found that the diffusion of volatile compounds decreased as the dry matter content increased. Moreover, this may be related to the decrease in water activity. This parameter is also dependent on the sucrose content. The fact that the mobility of ethyl butyrate is strongly reduced in the fruit preparation is therefore mainly to be ascribed to the high sucrose content, resulting in a high dry matter content and in low water activity.

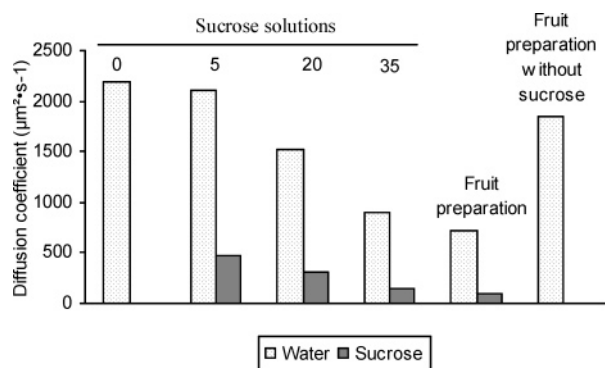
Self-Diffusion of Ethyl Butyrate in Sucrose Solutions. To better understand the relationship between sucrose content and the diffusion of aroma compounds, the self-diffusion coefficient of ethyl butyrate was also measured in 20 and 5% (w/w) sucrose solutions (**Figure 2**). Although the self-diffusion coefficient of ethyl butyrate decreased by a factor of 3.1 as the sucrose concentration increased from 0 to 35%, there was no significant difference either between diffusion in D_2O and in the 5% sucrose solution or between 5 and 20% sucrose solutions. However, diffusion in the 35% sucrose solution was substantially lower than that in the 20% solution. The viscosity of sucrose solutions in D_2O (**Table 2**) showed a slight increase up to a content of 20% and then a greater increase. This can be matched with the decrease in water activity between 20 and 35%, while this parameter was almost constant for lower sucrose concentrations (**Table 2**). As suggested by other authors (7, 33), a critical concentration of sucrose exists around 35%, which corresponds to the formation of a network through hydrogen bonds between water and sucrose (34, 35).

Self-Diffusion of Water and Sucrose Molecules. The formation of a network between water and sucrose molecules through hydrogen bonds can be assessed from the self-diffusion coefficients of sucrose and residual water. This information is directly available via the DOSY spectra (**Figure 3**). The self-diffusion coefficient of the sucrose molecules decreased linearly while the sucrose content increased, reflecting the overall medium viscosity variations. In parallel, the self-diffusion coefficients of residual water (peak at 4.7 ppm) also decreased progressively as the percentage of sucrose increased. It fell by

Table 2. Rheological Parameters, Water Activity, and Dry Matter Values of the Diffusion Media

	sucrose solutions (%)				fruit preparation	fruit preparation without sucrose
	0	5	20	35		
rheological parameters	$\eta = 0.98^a$	$\eta = 1.12^a$	$\eta = 1.90^a$	$\eta = 4.12^a$	$G' = 781$	$G' = 533$
water activity		0.997	0.989	0.976	$\tan(\delta) = 0.11^b$	$\tan(\delta) = 0.077^b$
dry matter (%w/w)		4.99	19.99	34.99	37.59	2.60

^aViscosity η (mPa s⁻¹) at 30 °C. ^b G' (storage modulus; in Pa) and $\tan(\delta)$ ($= G''/G'$). G' and G'' were determined at a frequency of 1 rad s⁻¹ and a strain of 1%.

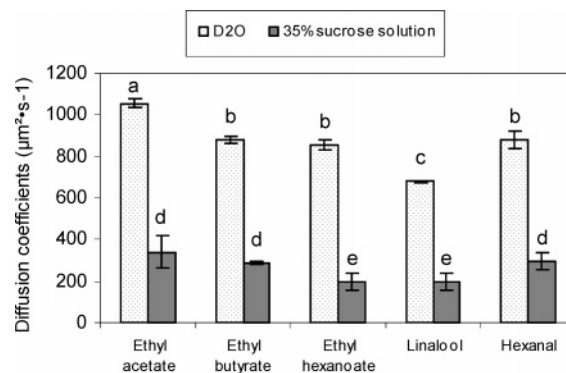
**Figure 3.** Mean self-diffusion coefficients ($\mu\text{m}^2 \text{s}^{-1}$) of residual water in matrices and sucrose solutions at 30 °C.

4% in the 5% sucrose solution, by 31% in the 20% sucrose solution, and by 59% in the 35% sucrose solution. These diffusion coefficients of residual water correspond to a diffusion coefficient averaged between free and bound water molecules in fast exchange on NMR scale and can be paralleled with water activity variations.

In conclusion, the self-diffusion coefficients of sucrose and water decrease almost linearly as the sucrose content increases, which is the direct consequence of the formation of hydrogen bonds. Clearly, as has already been suggested, the self-diffusion coefficients of sucrose and residual water are strongly decreased in fruit preparations due to the presence of high sucrose content.

Impact of the Nature of the Aroma Compounds on Self-Diffusion in Water and in Sucrose Solution. The self-diffusion coefficients of different aroma compounds in D₂O and in 35% sucrose solution were also measured. Four aroma molecules that are frequently used in food products were chosen (ethyl acetate, ethyl hexanoate, linalool, and hexanal) and were compared with ethyl butyrate. Diffusion measurements were performed at 100 $\mu\text{L L}^{-1}$ in D₂O and at 300 $\mu\text{L L}^{-1}$ in the sucrose solution. We had previously checked that the values of self-diffusion coefficients were independent of the concentration of the aroma compounds within the range investigated.

The overall results are presented in **Figure 4**. In D₂O, the self-diffusion coefficients of the five aroma molecules ranged from 679 to 1053 $\mu\text{m}^2 \text{s}^{-1}$ (CV smaller than 5% for each aroma molecule). These values are in the range of published data (around 1000 $\mu\text{m}^2 \text{s}^{-1}$) for small molecules such as aroma compounds (36). However, quite significant differences between the aroma compounds were observed. These differences can be discussed on the basis of the physicochemical parameters of the aroma compounds (**Table 1**). For these five linear molecules, molecules diffuse more slowly as the molecular weight (or accessible surface) and the hydrophobicity character ($\log P$) increase: $D_{\text{ethylbutyrate}} > D_{\text{ethylbutyrate}} \approx D_{\text{hexanal}} \approx D_{\text{ethylbutyrate}} > D_{\text{linalool}}$. As regards the three esters, ethyl acetate, which was the smallest molecule of those tested, diffused faster than ethyl butyrate and ethyl hexanoate, which are larger and more

**Figure 4.** Mean self-diffusion coefficients ($\mu\text{m}^2 \text{s}^{-1}$) of aroma compounds in D₂O and in the 35% sucrose solution at 30 °C. The significant differences are indicated with different letters (ANOVA and LSD tests, $P < 0.05$), and the 95% confidence intervals are represented on the bars.

hydrophobic molecules. The self-diffusion coefficient of hexanal was similar to that of ethyl butyrate and ethyl hexanoate. This is not surprising since they have similar molecular weights and hydrophobicities (**Table 1**). Linalool was the aroma compound that diffused the most slowly, which seems to contradict the results of Voilley et al. who found for some compounds that the most soluble in water displayed the greatest diffusivity (14). As a matter of fact, other parameters can play a role: For instance, linalool is more soluble than hexanal and ethyl hexanoate but has a very low vapor pressure, which makes it less volatile. Thus, hydrophilic and small molecules diffuse faster in D₂O than do others. Actually, there is no direct relationship between self-diffusion and a single parameter; several physicochemical characteristics must be taken into account.

In the 35% sucrose solution, the self-diffusion coefficients of all five aroma compounds were significantly lower (by about 70%) than those in D₂O solution (**Figure 4**). There is no specific diffusion process dependent on the nature of the aroma compound in the presence of sucrose molecules, confirming that no specific molecular interaction occurs. It can be inferred that the nature of the bulk medium had a higher impact than the nature of the diffusing molecule.

DISCUSSION

In this work, we showed that DOSY NMR spectroscopy is the technique of choice to investigate self-diffusion of flavor molecules at low concentrations in complex matrices. Although NMR spectrometer is not available in all laboratories, we must point out that, with recent NMR improvements, DOSY experiments are now easy to carry out. In contrast with other techniques, neither specific preparation nor fluorescent probe is needed to follow the translational motion of small molecules. Moreover, DOSY NMR gives accurate measurements of self-diffusion coefficients. Self-diffusion values obtained in this study

lead to CVs lower than 15% in complex matrices, while some CVs obtained with the concentration–profile technique are up to 50%. This technique needs a quantification step, which could induce additional variability. Dispersion of diffusion coefficient values found in the literature could be explained by the technique used. Moreover, because measurements have been performed at different temperatures, it is more difficult to make comparisons (11, 14, 16). Additionally, it appears clearly that the great advantage of the DOSY NMR method is that direct information on the self-diffusion of aroma molecules, residual water, and sucrose can be obtained in one step.

In this study, we showed that the decrease in the self-diffusion of aroma molecules is principally dependent on the sucrose content: The self-diffusion decreased as the medium viscosity increased. However, the relationship between viscosity and aroma self-diffusion parameters is quite complex since there was no linear relationship. Several studies have shown that the volatility or the diffusivity of flavor molecules decreased with viscosity when increasing sugar concentration (16). Voilley and Bettenfeld (13) showed that, as the concentration of dextrose increased from 0 to 40%, the diffusion coefficients of ethyl acetate, acetone, and 2-propanol decreased by a factor of about 3.6. In the present study, with sucrose, as the concentration increased from 0 to 35%, the self-diffusion of ethyl butyrate decreased by a factor of 3.1. This comparison suggests that the diffusion of aroma compounds in sugar solutions decreases as the viscosity increases but that the extent of this decrease does not depend on the nature of the sugar.

In fact, we clearly showed that aroma self-diffusion is highly related to the mobility of water molecules. As sucrose is added to water solution, the self-diffusion of sucrose and water molecules decreases, reflecting the fast exchange between sucrose and water molecules through hydrogen bonds. This leads to a proportional decrease of sucrose and water self-diffusion coefficients and to a more hydrophobic character of the bulk solvent. On the other hand, the decreased self-diffusion of aroma compounds is not directly related to the increasing sucrose content. For sucrose concentrations below 20%, the effect is low, whereas beyond a critical sucrose concentration, between 20 and 35%, a drastic decrease in the self-diffusion coefficient is observed. At this critical sucrose concentration, water molecules are not available enough for the hydration of aroma compounds, which explains their slower diffusion. Self-diffusion of these molecules is therefore largely linked to the diffusion of water, which induces mobility of surrounding molecules, and this effect seems to be independent of the nature of the aroma compound.

In fruit preparations, two parameters influence the self-diffusion of aroma molecules: the structure of the matrix and the composition of the microenvironment. In the fruit preparation without sucrose, interactions between the carrageenan chains and the swollen starch granules determine the structure, while the microenvironment is defined by very large pores of free water. As there are enough available water molecules, small aroma molecules are able to move freely in this microenvironment, so that their self-diffusion in this water phase is not directly related to the gel structure. It is therefore the local viscosity of the water phase that has to be taken into account. For fruit preparations, the gel structure is quite similar, but the microenvironment is modified by the large quantity of sucrose linked to water molecules. As a result, diffusion of small molecules is slowed due to the increase in local viscosity. In conclusion, it is not possible to correlate directly the diffusion process with the structure of this complex food product. The

composition of the microenvironment plays a major role: the more viscous the microenvironment, the smaller the diffusion of aroma molecules. The diffusion of aroma compounds in model fruit preparations is therefore strongly dependent on the characteristics of the microenvironment, and the availability of water seems to be a key factor. However, although the sucrose content is the predominant factor to explain the decrease in the self-diffusion coefficient, carrageenan and starch components probably have an additional effect. Other experiments should be conducted to understand how the formation of the network acts on diffusion phenomena.

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