Interactions Between Volatiles and Lipids in Complex Systems

M. Le Thanh[a], **I. Goubet**[a], **J.L. Le Quere**[b], **and A. Voilley**[a,*]

[a]Laboratoire de Génie des Procédés Alimentaires et Biotechnologiques, Ecole Nationale Supérieure de Biologie Appliquée à la Nutrition et à l'Alimentation, and [b]Laboratoire de Recherches sur les Arômes, Institut National de la Recherche Agronomique, 21000 Dijon, France

ABSTRACT: Partition equilibria of three aroma compounds in three different biphasic systems were studied by a liquid/liquid partition technique to investigate interactions that occur between volatiles and lipids in biphasic solutions. The lipids used were linoleic acid and ricinoleic acid and tetradecane as a model. The three aroma compounds were ethyl acetate, 2,5-dimethylpyrazine, and oct-1-en-3-ol. The results showed that the intermolecular forces involved were weak. Infrared spectroscopy was then performed on binary systems (tetradecane and linoleic acid or aroma compound) and on ternary systems (tetradecane, linoleic acid, and aroma compound) to determine the nature of the interactions. This method confirmed the presence of hydrogen bonds between linoleic acid and 2,5-dimethylpyrazine or oct-1-en-3-ol and also between molecules of oct-1-en-3-ol when the latter are present at high concentrations in lipids. The observations provided sufficient information to explain the behavior of oct-1-en-3-ol in biphasic solutions, but further work is needed to fully elucidate the nature of interactions between ethyl acetate or 2,5-dimethylpyrazine and lipids and also to explain the behavior of these compounds in biphasic solutions.

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KEY WORDS: Aroma compound, fatty acids, Fourier transform infrared spectroscopy, interaction, liquid/liquid partition coefficient, tetradecane.

Release and retention of aroma compounds are governed by the same physicochemical properties, and they determine the sensory qualities of foodstuffs and extraction ease of aroma compounds. Indeed, these characteristics are linked to the partition of volatiles in the different phases of food or biological media and also depend on the interactions that occur between aroma compounds and constituents of each phase. Therefore, determining the kind of interactions that occur in each phase is of great interest. It would help us to understand the phenomena involved in the perception of aroma compounds in emulsions or to explain the difficulties encountered in the extraction of volatiles from a biphasic system. Much has been done to reach this goal by measurement of thermodynamic equilibria in simple model solutions (1–3), but little research has been done on complex systems, and moreover, the nature of binding involved has not been clearly demonstrated. Knowledge of molecular-level phenomena is now required to understand and predict interactions.

Our aim was to show the type of interactions that occur between volatiles and lipids to explain the behavior of aroma compounds in biphasic solutions. To reach this goal, we studied the interactions between aroma compounds and constituents of biphasic solutions. The results concerning the factors that can affect the volatility of the aroma compounds have already been published (4). They have shown the importance of the presence of fatty acids in the medium for the vapor/liquid partition coefficient and also for the interactions between volatiles and substrates. The vapor/liquid partition coefficient measurements showed solute/solvent interactions but were inadequate to explain their nature and intensity. In the present study, we tried to understand which kinds of phenomena were involved in partition of volatiles between two phases. We first measured liquid/liquid partition coefficients of each volatile in biphasic solutions. The interactions were then studied at molecular level by Fourier transform infrared spectroscopy (FTIR) with solutions of lipid in tetradecane and several concentrations of volatiles.

MATERIALS AND METHODS

Chemicals. Ethyl acetate, ricinoleic acid, oct-1-en-3-ol, methyl linoleate, tetradecane, linoleic acid, and 2,5-dimethylpyrazine were used. Their physicochemical characteristics are given in Table 1.

Thermodynamic measurements. The three volatiles studied were oct-1-en-3-ol, ethyl acetate, and 2,5-dimethylpyrazine. Three biphasic systems were used to study liquid/liquid partition: tetradecane/water, linoleic acid/water, and ricinoleic acid/water.

The effect of concentration of each aroma compound on its liquid/liquid partition coefficient, P, was first studied to determine at which concentrations solute/solute interactions were appearing. The liquid/liquid partition coefficient, which is the ratio of the mass fractions of the aroma compound, re-

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*To whom correspondence should be addressed at Laboratoire de Génie des Procédés Alimentaires et Biotechnologiques, Ecole Nationale Supérieure de Biologie Appliquée à la Nutrition et à l'Alimentation, 1 Esplanade Erasme, 21000 Dijon, France.

TABLE 1
Physicochemical Characteristics of the Compounds Used

	Compound (manufacturer)	Chemical formula	Molecular weight	Boiling point (K)	Density	Purity (%)
Aroma compounds	2,5-Dimethylpyrazine (Aldrich)	$C_6H_8N_2$	108	428	0.990	99
	Ethyl acetate (Prolabo)	$C_4H_8O_2$	88	350	0.901	99
	Oct-1-en-3-ol (Fluka)	$C_8H_{16}O$	128	448	0.836	99
Lipids	Linoleic acid (Aldrich)	$C_{18}H_{32}O_2$	280		0.90	99
	Methyl linoleate (Sigma)	$C_{19}H_{34}O_2$	294		0.95	99
	Ricinoleic acid (Fluka)	$C_{18}H_{34}O_3$	298		0.94	85
	Tetradecane (Aldrich)	$C_{14}H_{30}$	198		0.76	99

spectively, in the organic and the aqueous phases, was determined by measuring the equilibrium concentration of the aroma compound between aqueous and organic phases. These analyses were performed by gas chromatography. A Packard 437 gas chromatograph with a flame-ionization detector and a Hewlett-Packard 3380A integrator were used. The stainless-steel column (3 m × 2.2 mm i.d.) was packed with 100/120 mesh Carbowax 20M-10%. The injector and detector temperatures were maintained, respectively, at 190 and 200°C. The column was operated at 100°C for ethyl acetate and 130°C for 2,5-dimethylpyrazine and oct-1-en-3-ol. The nitrogen flow rate was 20 mL min^{-1}, the hydrogen flow was 25 mL min^{-1}, and the air flow rate was 150 mL min^{-1}.

Measurements of the liquid/liquid partition coefficient of each aroma compound were performed at infinite dilution, in three different lipid/water systems at 25°C. Each value was determined by a triplicate.

Finally, the evolution of the liquid/liquid partition coefficient was studied according to variation of temperature from 298 to 353 K. Indeed the variation of ln P with temperature can provide information on the nature of the bonds between solute and solvent.

Spectroscopic measurements. The nature of the interactions between linoleic acid and aroma compounds was investigated at the molecular level by FTIR. The spectra were recorded at room temperature with a Bruker IFS 85 spectrometer (Wissembourg, France), equipped with a deuterated triglycine sulfate infrared detector and exhibiting 2 cm^{-1} resolution (1 cm^{-1} precision by zero filling before Fourier transform). Tetradecane was used as solvent. The spectra were recorded with the spectrum of tetradecane as background spectrum. Linoleic acid and methyl linoleate were used as lipids. Their infrared spectra were recorded from 0.1 M solutions of each in tetradecane. Solutions of ethyl acetate, 2,5-dimethylpyrazine, and oct-1-en-3-ol in tetradecane were used to determine the spectrum of each compound. Increasing the concentration from 10^{-3} to 1 M allowed observation of the type of interactions involved in solute/solute interactions.

Solute/solvent interactions were observed from solutions of aroma compound and linoleic acid or methyl linoleate in tetradecane. Concentration of either linoleic acid or methyl linoleate was set at 0.1 M, whereas aroma compound concentration was increased from 0.001 M to 0.1 M. Comparison between the spectra obtained with linoleic acid and methyl linoleate allowed investigation of the role of the carbonyl and the hydroxyl groups of the acid in bondings.

RESULTS AND DISCUSSION

Liquid/liquid partition coefficients. For all lipid/water systems examined, the liquid/liquid partition coefficient was constant for a concentration ratio up to 0.01, expressed as the molar fraction for any of the three volatiles in the organic phase. Above this concentration, the liquid/liquid partition coefficient of 2,5-dimethylpyrazine decreased, whereas those of ethyl acetate and oct-1-en-3-ol increased. The aroma compounds were no longer at infinite dilution in the organic phase. There were solute/solute interactions but these observations are insufficient to explain the behavior of 2,5-dimethylpyrazine. Therefore, the variation of the liquid/liquid partition coefficient for the three volatiles in different lipid/water systems was studied.

The liquid/liquid partition coefficients at infinite dilution are listed in Table 2. They differed according to the solvent and the solute tested. For the fatty acid/water systems, the liquid/liquid partition coefficient of ethyl acetate was less than those of 2,5-dimethylpyrazine and of oct-1-en-3-ol. Conversely, with the tetradecane/water system, the liquid/liquid partition coefficient of the 2,5-dimethylpyrazine was the least. But whatever the aroma compound, the liquid/liquid partition coefficient obtained in the tetradecane/water system was less than that obtained with the two fatty acids/water systems. These latter two were not significantly different. The liquid/liquid partition coefficient measurements also confirmed vapor/liquid partition measurements previously completed (4): The attraction between aroma compounds and fatty acids was greater than between aroma compounds and tetradecane. This can be explained by the greater hydropho-

TABLE 2
Liquid/Liquid Partition Coefficients (P^{∞}) of Aroma Compounds in Biphasic Systems at 25°C[a]

Biphasic system	Ethyl acetate	2,5-Dimethylpyrazine	Oct-1-en-3-ol
Tetradecane/water	18.9^a(1.6)	6.6^a(0.4)	180^a(8)
Linoleic acid/water	52^b(5)	81^b(8)	1847^b(145)
Ricinoleic acid/water	55^b(5)	87^b(8)	1931^b(135)

[a] Standard deviation, calculated from three replicates, is in parentheses. In columns, the values with the same superscript are not significantly different ($P > 0.05$).

bicity of tetradecane compared to fatty acids. The lack of significant difference between the results of the systems containing the two fatty acids could be due to the similar chemical properties of the fatty acids.

When the temperature was increased, ln P^{∞} increased linearly for ethyl acetate, independent of the nature of the lipid ($0.966 < r^2 < 0.99$; r^2 = determination coefficient). The same phenomenon could be noticed for 2,5-dimethylpyrazine ($r^2 = 0.97$) or oct-1-en-3-ol ($r^2 = 0.95$) in a tetradecane/water system and for 2,5-dimethylpyrazine in fatty acid/water systems, except that the relation was not exactly linear ($r^2 = 0.85$). Conversely, ln P of oct-1-en-3-ol increased and then decreased when temperature was raised in the linoleic acid/water and ricinoleic acid/water systems. These results suggested that interactions between oct-1-en-3-ol and fatty acids could be due to hydrogen bonds, whereas for the two other aroma compounds another type of binding, hydrophobic interaction, was predicted. These hypothesis were checked by spectroscopic measurements.

Spectroscopic measurements. The spectra of each binary system (lipid in tetradecane or volatile in tetradecane) were first observed to determine the characteristic absorption bands of each compound. The results are shown in Table 3.

For both ethyl acetate and 2,5-dimethylpyrazine, no change in the spectra was observed when volatile concentration was increased. This can be explained by either the absence of solute/solute interactions or because FTIR is not suitable to detect them.

Conversely, there was an increase in absorption at 3360 cm^{-1} and a decrease in absorption at 3623 cm^{-1} in the spectra of oct-1-en-3-ol when its concentration was increased (Fig. 1). The absorption bands at 3623 cm^{-1} could be assigned to the free OH groups, whereas those at 3360 cm^{-1} were due to bound OH groups of oct-1-en-3-ol. The lack of an absorption band at 3360 cm^{-1} when oct-1-en-3-ol concentration was lower than 0.01 M confirmed that, below 0.01 M, no solute/solute interactions exist. Similar phenomena already have been observed with concentrations of *n*-alcohols greater than 0.1 M in *n*-alkane/water systems (7). The increase of liquid/liquid partition coefficients of oct-1-en-3-ol, when the concentration was increased, is probably due to the auto-associations of alcohol molecules, and this hypothesis has been confirmed by infrared spectroscopy. Auto-association of alcohols has been suggested in undecane (6) and cyclohexane (7). The negative shift observed when oct-1-en-3-ol concentration was increased is typical of an intermolecular binding between oct-1-en-3-ol molecules by hydrogen bonds. The in-

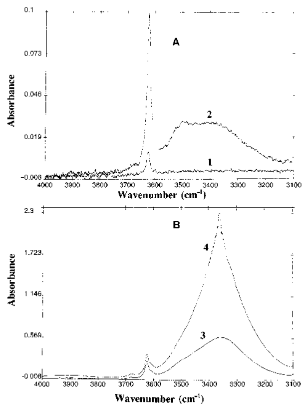


FIG. 1. Fourier transform infrared spectrum of oct-1-en-3-ol: (A) in tetradecane at: 1: 0.01 mol/L; 2: 0.1 mol/L: (B) in tetradecane at: 3: 0.5 mol/L; 4: 1.0 mol/L.

TABLE 3
Characteristic Infrared Absorption Bands of Lipids and Volatiles Studied

Compound	Functional group	Absorption band (cm^{-1})
Linoleic acid	C=O	1713
Methyl linoleate	C=O	1748
Ethyl acetate	C=O	1748
	C-O-C	1236
2,5-Dimethylpyrazine	C=N	1487
	Aromatic ring	1034
Oct-1-en-3-ol	Free OH	3623
	Bound OH	3360

crease in the liquid/liquid partition coefficient with an increase in oct-1-en-3-ol concentration could be explained by the association between molecules through dimerization or polymerization (8).

To detect solvent/solute interactions, the spectra of ternary systems were investigated. Increasing concentration of ethyl acetate in solutions of linoleic acid and tetradecane led to the observation of an absorption band shoulder at 1264 cm^{-1}, which may be due to a positive shift of the C-O-C band (Fig. 2). The type of interaction involved in linoleic acid/ethyl acetate interactions could not be deduced from these observations.

Mixing 2,5-dimethylpyrazine with linoleic acid in tetradecane led to the observation of a vibration band at 1042 cm^{-1} and a shoulder at 1730 cm^{-1}, with intensity increasing when 2,5-dimethylpyrazine concentration was increased (Fig. 3). They are due to the positive shifts of the C=O and of the aromatic ring bands and can be explained by interactions between 2,5-dimethylpyrazine and linoleic acid. Two hypotheses have been put forward. The interactions may be due either to the formation of a complex (Scheme 1) between the linoleate ion and the pyrazinium ion or to hydrogen bonds (Scheme 2) between the acid and the aromatic ring. The 1730 cm^{-1} band cannot be explained by the monomer form of the acid or by the formation of a salt or ester. The 1042 cm^{-1} band cannot be due to the pyrazinium salt. We suggest that a complex between these two molecules can be formed through a hydrogen bond. This hypothesis has already been proposed for phenol and heterocyclic compounds, such as pyridine, pyrimidine, and pyrazine (9). In the latter study, a 5- to 10-cm^{-1} shift due to bindings between OH groups and the aromatic ring was observed. In the present work, an 8-cm^{-1} shift was observed. The interactions observed between 2,5-dimethylpyrazine and linoleic acid in tetradecane are not sufficient to explain the variation of the liquid/liquid partition coefficient observed when the 2,5-dimethylpyrazine concentration was increased in biphasic systems. Indeed, we noticed that the liquid/liquid partition coefficient decreased when the 2,5-dimethylpyrazine concentration was increased. The behavior of the compound in water must also be taken into account. It can be assumed that 2,5-dimethylpyrazine is a proton acceptor and that the pyrazinium ion formed has a greater affinity for water than for lipids. That could result in a decrease of the liquid/liquid partition coefficient when the volatile concentration is increased. The results are in agreement with previous studies (10,11).

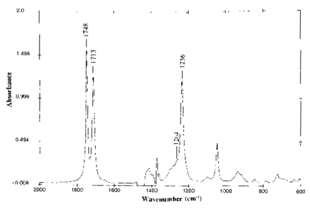


FIG. 2. Fourier transform infrared spectrum of ethyl acetate at 0.1 mol/L and linoleic acid at 0.1 mol/L in tetradecane.

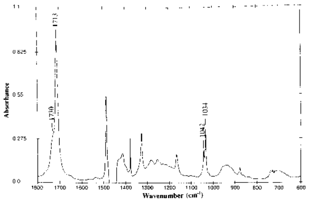
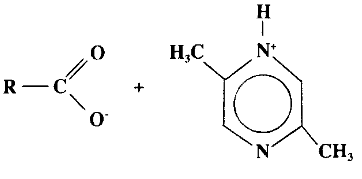
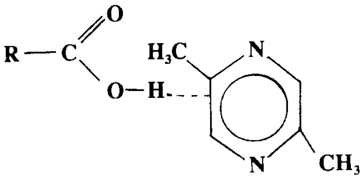


FIG. 3. Fourier transform infrared spectrum of 2,5-dimethylpyrazine at 0.1 mol/L and linoleic acid at 0.1 mol/L in tetradecane.

The spectrum of oct-1-en-3-ol with linoleic acid in tetradecane showed a typical vibration band of free -OH of the alcohol at 3623 cm^{-1} and a broad band at 3406 cm^{-1} typical of bonded -OH (Fig. 4). The vibration band at 3406 cm^{-1}, of which the intensity increased with an increase in the solute concentration (Fig. 4), could be due to the formation of hydrogen bonds between the OH group of oct-1-en-3-ol and either the C=O or the OH of the acid. The spectrum obtained with the ternary mixture of oct-1-en-3-ol, methyl linoleate, and tetradecane showed a 3520 cm^{-1} band that is significantly different from the band at 3406 cm^{-1} obtained with linoleic acid (Fig. 4B). These two spectra allowed us to deduce that



SCHEME 1



SCHEME 2

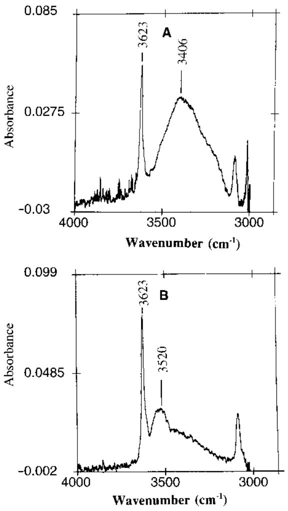


FIG. 4. Fourier transform infrared spectroscopy spectrum of oct-1-en-3-ol: (A) at 0.1 mol/L and linoleic acid at 0.1 mol/L in tetradecane; (B) at 0.1 mol/L and methyl linoleate at 0.1 mol/L in tetradecane.

the bands at 3406 and 3520 $\text{cm}^{-1}$are typical of the binding of an OH group to the C=O of the lipid.

Moreover, the stronger the attraction between the functional group and the OH involved in a hydrogen bond, the smaller is the wavenumber of the O-H binding (12). Variations of the wavenumber of the OH bound group can therefore provide information on the strength of the bonds formed. The measured variations are reported in Table 4. The attraction strengths between the oct-1-en-3-ol molecules were stronger than those between oct-1-en-3-ol and linoleic acid, which were stronger than those between oct-1-en-3-ol and methyl linoleate. The observations made on the behavior of oct-1-en-3-ol in homogeneous or biphasic solutions could be explained by the formation of hydrogen bonds when its concentration was increased.

In the first part of this study, thermodynamic measurements were used to show that attraction between lipids and aroma compounds depends on their nature. Hydrophobicity plays an important role, although differences in the nature of interactions with lipids were observed according to the aroma compound considered.

To confirm these results, the nature of interactions was studied at a molecular level by FTIR. The existence of hydrogen bonds between oct-1-en-3-ol and linoleic acid was also confirmed. The interaction between 2,5-dimethylpyrazine and linoleic acid was explained by the formation of a hydrogen bond between the acid and the aromatic ring of the pyrazine.

TABLE 4
Shifts of Absorption Band of OH Bound Groups Observed by FTIR[a]

Compounds	Δν (cm^{-1})
Oct-1-en-3-ol–oct-1-en-3-ol	263
Oct-1-en-3-ol–linoleic acid	217
Oct-1-en-3-ol–methyl linoleate	103

[a] FTIR, Fourier transform infrared.

FTIR can provide interesting information at the molecular level to explain the phenomena observed, but further work using other methods is needed to fully understand the solute behavior shown by thermodynamic measurements.

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