Application of Headspace Analysis to the Study of Aroma Compounds–Lipids Interactions

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ABSTRACT: Taking into account interactions between aroma compounds and food components is necessary to better manage the flavoring of food products. These interactions occur at a molecular level and reflect changes, at a macroscopic level, in thermodynamic equilibria, such as solubility or volatility. The rate of transfer of an aroma compound from the liquid to the vapor phase can be affected as well. The behavior of aroma compounds in water and lipid solutions was studied in two complementary ways, a thermodynamic and a kinetic approach (headspace analysis). The transfer rate of volatiles at the liquid–water interface does not only depend on the hydrophobicity of the aroma compounds. Vapor–liquid partition and activity coefficients show the presence of solute–solvent interactions. The Gibbs free energy values indicate their physicochemical nature. *JAOCS 75*, 127–130 (1998).

KEY WORDS: Activity coefficient, aroma compounds, headspace, lipid–aroma interactions, solubility, solute–solvent interactions, transfer rate, volatility.

The aroma and odor of foods are important parameters that affect the acceptability of such products to the consumer. Aroma is considered to be the second-most important individual sensory guide after that of sight in accepting a food before ingestion (1), and the human nose can discriminate between hundreds of different odor qualities over several log units of concentration (2). However, an aroma does not depend only on its concentration; for example, its potency as an aroma may be at the lowest concentration in a product, but it produces the strongest effect.

The aroma of a food is made up of many components. These compounds are organic molecules of low molar mass (<400) and, at atmospheric pressure and room temperature, the vapor pressure of these compounds is high enough to allow them contact with the nose receptors (3). These substances belong to different chemical classes; they include hydrocarbons, alcohols, ether-oxides, aldehydes, ketones, amines, esters, amides, and heterocyclics. They are generally present in foods at low concentrations (in the order of ppm).

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An aroma compound reacts with the food matrix to which it is added; the existence of weak interactions between the aroma and other constituents has been observed in numerous studies of simple systems (4,5).

The volatility of aroma compounds in a food system depends on the presence of nonvolatile components, such as carbohydrates, proteins, lipids, and salts. The binding behavior of aroma to food components and their rates of partitioning between different phases are of great practical importance in developing flavor cocktails and determining the relative retention of these flavorings during processing, storage, and mastication (6,7).

Knowledge of the nature of the binding of ligands to food components is also a significant factor in developing procedures for the removal of off-flavors from functional ingredients that are intended for use in food production (8). Indeed, quantitative data are needed to determine the binding of flavors to other molecules, to compare the binding affinities of different flavors, and to point out differences in the amount of binding of each aroma compound to macromolecules in complex mixtures. In the context of this study, the bonds that occur between a volatile and a nonvolatile compound are weak and reversible. These bonds are defined as physicochemical interactions and lead to modification of the thermodynamic equilibria that govern certain physicochemical properties, e.g., volatility.

Bonds between molecules can be divided into two groups. Chemical bonds involve the transfer of electrons between two atoms and can occur as covalent and ionic bonds. The ionic bond is approximately 10 times weaker than the covalent bond. The physicochemical bonds or physicochemical interactions include van der Waals forces, hydrogen bonds, and hydrophobic interactions.

Hydrophobic effects or hydrophobic interactions result in nonaffinity of the solute for water, which is polar. The apolar solute molecules, which are insoluble or only partially soluble in water but readily soluble in other solvents, regroup themselves and organize the polar solvent molecules between them. The solute interacts with water by London forces, while the water molecules establish hydrogen bonds between them (9–11).

The interactions between volatile compounds and substrate

may be considered at the macroscopic level for a thermodynamic point of view. Modification of the volatility of an aroma compound may be described by the laws of equilibria between phases. One of the main thermodynamic parameters is the chemical potential of constituent *i* in a given phase. This chemical potential (μ_i) varies with the molar fraction x_i:

$$d(\mu_i)(T) = RTd\ln(a_i) \quad [1]$$

where a_i is the activity of constituent *i*, which is proportional to the molar fraction x_i and to the activity coefficient:

$$a_i = \gamma_i \cdot x_i \quad [2]$$

where γ_i indicates the deviation of the system compared to an ideal solution. In the case of an ideal solution, the activity coefficient of each constituent is constant and equal to 1. The activity coefficient of a real solution differs from 1.

Two phenomena can explain the deviation in relation to ideality: if the solute–solvent interactions are negligible compared to the solute–solute interactions or solvent–solvent interactions, the partial pressure of a constituent in the vapor phase is higher than that in the ideal case ($\gamma_i > 1$). If the solute–solvent interactions are stronger than the solute–solute or the solvent–solvent forces, the partial pressure is lower than the ideal case ($\gamma_i < 1$).

The nature of intermolecular bonds can vary together with their energy, which is represented by the Gibbs free energy. Physicochemical interactions are reversible and weak. The contribution of each effect to van der Waals forces is not discussed much in the literature. Atkins (12) gave some examples for dipolar molecules (HCl, NH_3, H_2O): The energy of the dispersion effect is higher than that of the two other effects, except in very polar molecules, such as H_2O, where the orientation effect can reach 38 KJ $\cdot$ mol^{-1}. Nakai and Li-Chan (13) also pointed out the importance of the dispersion effect except for very polar molecules. The energy of hydrophobic interactions varies from 13 to 21 KJ $\cdot$ mol^{-1} (14). Karel (15) indicated that this type of interaction presented a higher energy than van der Waals bonds. The interaction can be characterized by considering the binding energy, even if it is not a sufficient parameter to determine the nature of the bond.

In this work, three aroma compounds, present in many food products, were selected because they display different physicochemical properties, particularly hydrophobicity and vapor pressure. The nature of the interactions can be established with lipids of different polarity by measuring kinetic and thermodynamic parameters. Transfer rates of the aroma compounds at the liquid–vapor interface were measured as well as their liquid–vapor partition coefficients, which allow the determination of their activity coefficients in the liquid solution.

MATERIALS AND METHODS

Reagents. The volatile compounds belong to different chemical classes: ethyl acetate (Prolabo, France), 2,5-dimethylpyrazine (Aldrich, France), 1-octen-3-ol (Fluka, France). All aroma compounds exhibit a purity at least equal to 99%. Ethyl acetate exhibits a fruity note, 1-octen-3-ol a mushroom note, and 2,5-dimethylpyrazine a toasted and nutty note. Their physicochemical characteristics are presented in Table 1.

Three lipid media were compared with water to determine the nature of the solute–solvent interactions. The purity of tetradecane and linoleic acid (Aldrich) was 85%.

Methods. Solubilities of the three aroma compounds were measured in the different solvents. The principle of the measurement is the liquid–liquid partition between two nonmiscible phases by passive equilibration. An excess of the liquid aroma compound under investigation was added to distilled water or another solvent in a test vial (45 mm long, 14 mm o.d., 11 mm i.d.) at ambient temperature. The excess is not crucial, but it should be so large that, at the end of the dissolution experiments, undissolved pure liquid can still be detected. The vials were closed with a cap formed by a septum whose inner surface is of PTFE. All setups were kept in a thermostatic room at 25.0 ± 0.5°C for at least 48 h to reach mutual equilibrium, which has been verified. The saturated solutions were quantitated by gas chromatography. This allowed measurements of organics in a wide solubility range.

The concentrations of aroma compounds in the vapor phase before and at equilibrium were determined. An inert gas (nitrogen) was passed through the liquid phase at a constant flow rate (3.0×10^{-5} L $\cdot$ min^{-1}) and carried the volatile molecules into the headspace. A sample of the vapor phase (10^{-6} L) was automatically injected into a gas chromatograph (GC) at regular intervals. The data allowed the determination of the vapor–liquid partition coefficient of aroma compounds at infinite dilution, representing their volatility:

$$K_i = Y_i/X_i \quad [3]$$

where Y_i and X_i are the molecular fractions of compound *i*, respectively, in the vapor and liquid phases.

K_i^∞ allows the calculation of the coefficient of compound *i*:

$$\gamma_i = K_i^\infty (P_T/P_i^s) \quad [4]$$

where P_T = total vapor pressure in the system (Pa) and P_i^s = saturated vapor pressure of aroma compound *i* (Pa). For each aroma compound in each system, the measurements were carried out at least three times.

TABLE 1
Characteristics of the Aroma Compounds

Aroma compounds	Formula	MM^a (g $\cdot$ mol^{-1})	Bb^b (°C)	Log P^c	P_i^{sd} (mm Hg)
Ethyl acetate	$C_4H_8O_2$	88	77	2.5	91.0
2.5-Dimethylpyrazine	$C_6H_8N_2$	108	155	–1.6	3.4
1-Octen-3-ol	$C_8H_{16}O$	128	17	2.5	0.5

[a]Molar mass.
[b]Boiling point.
[c]Determined from Rekker method (Ref. 16).
[d]Saturated vapor pressure at 25°C.

The chromatographic conditions were as follows: the GC was equipped with a 3-m stainless-steel column (inner diameter, 2.2 mm), packed with Chromosorb W-AW 100-120 mesh Carbowax 20 M 10%. The operating parameters of the chromatograph were as follows: injector temperature, 190°C; flame-ionization detector temperature, 200°C; column temperature, 100°C for ethyl acetate, and 130°C for 2,5-dimethylpyrazine and 1-octen-3-ol; N_2 flow rate, 2.5×10^{-5} L · min^{-1}; H_2 flow rate, 25.0×10^{-5} L · min^{-1}.

RESULTS AND DISCUSSION

Solubility of the aroma compounds in different liquid phases. Table 2 gives the solubility values of the three aroma compounds in the aqueous and organic phases at 25°C.

As expected from the value of log *P* for each aroma compound, the solubilities of ethyl acetate and 1-octen-3-ol are higher in organic solvents than in water. The opposite behavior is observed for 2,5-dimethylpyrazine. Whatever the nature of the aroma compound, the solubility increases from nonpolar (tetradecane) to polar (fatty acids) solvents. The solubility in ricinoleic acid is slightly lower than in linoleic acid. We suppose that the affinity of aroma compounds for the fatty acids is mainly due to hydrogen bonds. This is consistent with the fact that the three aroma compounds are all polar.

Transfer rate. The transfer kinetics of the aroma compounds from the liquid to the vapor phase have been measured. The initial slope of the curve represents the concentration of the aroma compound in the vapor phase as a function of time and allows the calculation of the transfer rate for the volatile (Table 3). The initial concentration of the aroma compounds in the liquid phase was 1 g · L^{-1}.

The transfer rates of 2,5-dimethylpyrazine and 1-octen-3-ol do not depend on their hydrophobicity. They are chemically different but they present the same value of log *P*. The transfer rate is also independent of the molar mass, the boiling point, or the saturated vapor pressure. The difference between the transfer rates of the three volatiles cannot be attributed to their different solubilities in water. Thus, neither the affinity for the liquid phase nor the ability of the aroma compounds to pass from liquid to vapor phase is directly responsible for the measured transfer rates.

Whatever the aroma compound, the transfer rate from the liquid to the vapor phase increases from water to organic solvents and is similar for the two fatty acids. For water, the affinity of the volatile for the liquid phase does not explain its kinetic behavior between liquid and vapor phases.

TABLE 3
Transfer Rate T_R (g · L^{-1} · min^{-1}) of Aroma Compounds from the Liquid to the Vapor Phase at 25°C

	Ethyl acetate	2,5-Dimethylpyrazine	1-Octen-3-ol
Water	0.20	1.5×10^{-3}	4.0×10^{-2}
Tetradecane	0.10	1.5×10^{-3}	1.4×10^{-2}
Linoleic acid	0.05	6.0×10^{-5}	2.0×10^{-4}
Ricinoleic acid	0.05	6.0×10^{-5}	2.0×10^{-4}

Vapor–liquid partition and activity coefficients. The vapor–liquid partition coefficients of aroma compounds have been determined at infinite dilution by headspace analysis. The activity coefficient of each aroma compound was determined from measurement of the vapor–liquid partition coefficient. The results of both thermodynamic data are presented in Table 4.

Whatever the nature of the aroma compound or of the lipid, the volatility of the aroma compound is lower in organic solvent than in water, which is consistent with the higher solubility in the latter. The value of the activity coefficient (γ_i) is always different from 1, which indicates the presence of solute–solvent interactions. In water, and to a lesser extent in tetradecane, the high value of γ_i is due to the existence of interactions between solute and solvent molecules. For 2,5-dimethylpyrazine in fatty acids, the value of γ_i is less than 1. Two hypotheses can be proposed: The molecule of solute is solvated by the fatty acid, or the size of solute molecules differs significantly from that of the solvent molecules. For 1-octen-3-ol and ethyl acetate in fatty acids, the values of γ_i are slightly higher than 1, which is representative of attraction forces between solute and solvent molecules, and it indicates that those interactions are of the same nature as those between some solute molecules.

Gibbs free energy. The Gibbs free energy (g^E) values of the aroma compounds in different media have been calculated from the equation:

$$g_i^E = RT \ln \gamma_i \qquad [5]$$

and are presented in Table 5.

The values of the Gibbs free energy of the aroma compounds in different solvents are always clearly lower than 40 KJ · mol^{-1}. Thus, the interactions between solvent and solute molecules are independent of the aroma compound or the

TABLE 2
Solubility of Aroma Compounds in Different Phases at 25°C (%, w/w)

	Ethyl acetate	2,5-Dimethylpyrazine	1-Octen 3-ol
Water[a]	8.6	∞	0.2
Tetradecane	10.6	12.5	2.3
Linoleic acid	26.0	76.7	27.7
Ricinoleic acid	24.0	76.1	22.4

[a]From Le Than *et al.* (Ref. 17).

TABLE 4
Vapor–Liquid Partition Coefficient (K_i^∞) and Activity (g_i) Coefficients of Aroma Compounds in Homogeneous Media at 25°C

	Ethyl acetate		2,5-Dimethylpyrazine		1-Octen-3-ol	
	K^∞	γ^∞	K^∞	γ^∞	K^∞	γ^∞
Water	8.22	68.2	0.1	22.6	2.16	3568
Tetradecane	0.39	3.2	0.015	3.4	0.013	21
Linoleic acid	0.13	1.3	0.002	0.45	0.001	1.7
Ricinoleic acid	0.14	1.4	0.002	0.45	0.001	1.8

TABLE 5
Gibbs Free Energy (KJ · mol^{-1}) of Aroma Compounds in Homogeneous Media at 25°C

	Ethyl acetate	2,5-Dimethylpyrazine	1-Octen 3-ol
Water	10.5	7.7	20.3
Tetradecane	2.9	3.0	7.5
Linoleic acid	0.6	–2.0	1.3
Ricinoleic acid	0.8	–2.0	1.5

medium, are weak, and are of physicochemical nature. The nature of the interactions could be further investigated by spectroscopic methods.

In conclusion, it appears that the behavior of the aroma compounds depends, from a thermodynamic and kinetic point of view, both on their nature and on that of the solvent. It shows the importance of the physicochemical properties of aroma compounds, particularly their hydrophobicity, for their partition between phases of a food products. The knowledge of these characteristics could help to better manage flavoring of lipid-containing as well as of fat-reduced products.

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