JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Transfer of Aroma Compounds through the Lipidic−Aqueous Interface in a Complex System

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The transfer kinetics of aroma compounds from the aqueous phase to the lipidic phase (miglyol) and from miglyol to the aqueous phase have been studied in the presence or absence of a protein, β-lactoglobulin, and at different pH values. In the presence of β-lactoglobulin, the transfer at the interface from the aqueous to the lipidic phase decreases, with a greater effect of the presence of the protein at pH 3 than at pH 6. This barrier effect of the protein plays a role in the transfer of the aroma compounds between the different phases of the matrix.

KEYWORDS: Liquid−liquid equilibria; transfer; interface; aroma compounds; β-lactoglobulin; pH effect

INTRODUCTION

The interactions between aroma compounds and other food constituents are generally studied with the use of model systems, such as aqueous solutions containing only one aroma compound and a nonvolatile constituent. However, foods are multiphasic media, existing as gels or emulsions, or consisting of gellified emulsions. The distribution of volatiles at the equilibrium in the different phases is not enough to describe the phenomenon implicated in their release. Generally the aroma compounds are lipophilic, and before being released in the gaseous state, they must pass through several interfaces such as the lipidic−aqueous phase or aqueous−vapor phase interfaces.

Brossard et al. (*1*) have shown that in emulsions the effect of transfer rate of the aroma compounds at the oil−water interface played a primordial role in the aroma perception. Bakker and Mela (*2*), by studying the building of vapor phase above water solutions of diacetyl with instrumental approach, observed that the transfer rate of an aroma compound toward the vapor phase is higher in oil-in-water emulsions than in the water-in-oil emulsions stabilized with the same emulsifier. This result has been explained by the fact that the transfer rate from water toward air (oil-in-water emulsions) is more rapid than at the air−oil interface (water-in-oil emulsions) and, more importantly, because of the difference of viscosity between oil and water. In oil-in-water emulsions, the transfer rate of product−air thus depends on the transfer rate at the interface of the lipidic−aqueous phase.

Using a rotating diffusion cell to study the effect of protein on the retention of aroma compounds at the lipid−water interface, Harvey et al. (*3*) described the mechanisms that are implicated in the resistance to the transfer of aroma compounds at the oil−water interface; they also showed that the presence of sodium caseinate increased the resistance to the transfer of ethyl esters and 2,5-dimethylpyrazine at the triolein interface.

The characteristics of the oil−water interface may influence the transfer of the aroma molecule from one phase to another. The aim of this work is to study the transfer of aroma compounds at the miglyol−water interface, taking into account their hydrophobicity and their initial concentrations in the aqueous phase. The surface properties of proteins give them the ability to be adsorbed at the interfaces air−water or lipid−water, which decreases the surface tension. The role of a protein, β-lactoglobulin, on the aroma transfer at the interface lipid−aqueous solution is studied.

MATERIALS AND METHODS

Reagents. The seven studied aroma compounds were kindly provided by International Flavors and Fragrances (IFF, Longvic-lès-Dijon, France): acetaldehyde, 2,5-dimethylpyrazine, benzaldehyde, acetophenone, isoamyl acetate, *d*-linalool, and 2-nonanone. These compounds are classified into three groups according to their hydrophobicity (**Table 1**): acetaldehyde and 2,5-dimethylpyrazine, which show a hydrophilic character and have log P values equal to -0.8 and -1.6, respectively; benzaldehyde and acetophenone, having an intermediate hydrophobicity (log $P = 1.5$ and 1.8, respectively); and isoamyl acetate, 2-nonanone, and *d*-linalool, presenting a hydrophobic character (log $P = 2.2$, 2.9, and 3.5, respectively).

The initial concentrations are similar for compounds of the same group: ~500 ppm for acetaldehyde and 2,5-dimethylpyrazine and ~2000 ppm for isoamyl acetate and *d*-linalool. In the case of 2-nonanone, the maximum concentration possible is ~400 ppm because of its weak water solubility. The water solubilities of the studied aroma compounds are given in **Table 1**. For benzaldehyde and acetophenone the initial concentrations are very different: 1963 ppm for the aldehyde and 3630 ppm for the ketone; these concentrations are chosen with the aim of studying the effect of the initial concentration on the transfer of two compounds having the same affinity for the two phases.

β-Lactoglobulin was provided by Besnier-Bridel (Laval, France). This industrial preparation was purified by solubilization (20 g/100 g)

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10.1021/jf010939j CCC: $22.00 © 2002 American Chemical Society
Published on Web 01/31/2002

Table 1. Miglyol–Water Partition Coefficient, Hydrophobicity, Initial Concentration, and Aroma Flux at the Water–Miglyol Interface (Aromatization of the Aqueous Phase, Agitation)

aroma compound	$P_{(miglyol-water)}$	log P[a]	water solubility[b] (g L^{-1})	initial concn in the aqueous phase (ppm)	aroma flux at the miglyol–water interface (mg h^{-1} cm^{-2})
acetaldehyde	0.2	–0.8	∞[c]	452	0.03
2,5-dimethylpyrazine	1.4	–1.6	∞[c]	478	0.05
benzaldehyde	44.0	1.5	7.1 (9)[d]	1963	0.76
acetophenone	57.0	1.8	6.9 (4)	3630	1.20
Isoamyl acetate	139.0	2.2	2.4 (5)	1916	1.95
d-linalool	224.0	2.9	2.6 (6)	1622	1.96
2-nonanone	774.0	3.5	0.4 (9)	343	0.42

[a] Estimated values using Rekker's method (*4*). [b] Experimental values. [c] Reference *5*. [d] Variation coefficient in percent [(standard deviation/mean) × 100].

Table 2. Initial Aroma Compound Flux at the Miglyol–Aqueous Solution Interface and Aroma Compound Transferred at Equilibrium in the Presence or Absence of β-Lactoglobulin at 25 °C

aroma compound	aromatized phase	β-lactoglobulin (%)	initial aroma flux at the miglyol–aqueous solution interface (mg h^{-1} cm^{-2})	time to reach equilibrium (h)	aroma compound transferred at equilibrium (mg)	% variation[a]
d-linalool	aqueous	0	0.72	50	6.05	3.0 (NS)
		3 (pH3)	0.56	50	5.87	2.3 (NS)
		3 (pH 6)	0.59	50	5.91	
isoamyl acetate	aqueous	0	0.77	30	6.24	14.3 (S)
		3 (pH 3)	0.52	30	5.35	5.9 (S)
		3 (pH 6)	0.63	30	5.87	
	aqueous	0	0.19	>60	4.20 (at 60 h)	15.0 (S)
benzaldehyde		3 (pH 3)	0.17	>70	3.57 (at 60 h)	
	lipidic	0	0.007	10	0.093	4.3 (S)
		3 (pH 3)	0.008	10	0.089	

[a] Variation = (value in water – value in proteic solution)/(value in water) expressed in percent. NS, not significant; S, significant.

in an aqueous solution of 50 mM NaCl and dialyzed during 24 h with a regenerated cellulose membrane (Spectrapor, 6000−8000 Da) with NaCl (20 times its volume) The protein solution had a concentration of ∼120 g/L. This last solution was diluted to 3% with NaCl solution. Aqueous protein solutions were adjusted to pH values of 3 and 6 with HCl solution.

Miglyol is a triglyceride of caprylic (60%) and capric acid (40%).

Methods. The hydrophobicity (log *P*) was determined at 25 °C. The liquid−liquid partition coefficient *P* is, respectively, the ratio, at equilibrium, of the concentration (w/v) of the solute in *n*-octanol and water. A negative log *P* value indicates the hydrophilic character of the molecule; the most hydrophobic compound presents a positive value.

The transfers of the aroma compounds from the aqueous solutions to miglyol and from miglyol to the aqueous solutions, in the presence or absence of the protein, were measured at 25 °C in a small glass flask, equipped with a mounted Teflon stopper, containing 2 mL of aqueous phase and 2 mL of organic phase. The interfacial area was constant (1.29 cm^2). The aroma compounds were introduced in one phase, and the kinetics of transfer to the other phase was studied. A sample (2 μL) of the aqueous phase was taken at regular time intervals and analyzed by gas chromatography. Each sample constituted a new experiment. Each point of the curves in Figures 1−3 is an independent experiment. Each flask was used only one time. To draw one curve, 15 flasks were necessary. All of the presented results are the mean of a minimum of two repetitions of the whole curve. The numerous experiments explain the low experimental error (3%). To verify the equilibrium state, the experiments were conducted during ∼60 h.

The transfer from aqueous phase to miglyol was realized in standardized agitation conditions to reach equilibrium in a shorter time (results in **Table 1**). This agitation became impossible in the presence of protein because of the foam formation. All of the experiments presented in **Table 2** are realized without agitation (with and without protein, whatever the aromatized phase).

GC was performed on a Chrompack CP 9000 instrument (Chrompack Co., Middelburg, The Netherlands) equipped with a flame ionization detector and 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; detector temperature, 200 °C; column temperature (isothermal), between 80 and 160 °C upon the aroma compound; N_2 flow rate, 16 mL/min; H_2 flow rate, 25 mL/min; air flow rate, 250 mL/min.

RESULTS AND DISCUSSION

The partition coefficient (miglyol−water), the initial concentrations, and the flux of the aroma at the interface miglyol−water of these aroma compounds are given in **Table 1**.

The aroma flux at the interface varies as a function of the hydrophobicity of the volatile compounds and is in the same order for compounds of the same group. The nature of the volatile compound plays an important role toward the other constituents of the medium (water, lipid), as it shows the clear differences between the transfer rates and also between the values of the partition coefficient (miglyol−water) for the seven studied compounds. This phenomenon was soon observed by Harvey et al. (*3*). These authors have shown a difference of behavior between ethyl esters (relatively hydrophobic compounds) and 2,5-dimethylpyrazine (hydrophilic compound) during the transfer at the interfaces water−triolein and water−tributyrin.

The influence of the affinity of the receptor medium is illustrated, particularly in the case of 2-nonanone, where the transfer rate is ∼10 times higher than those of acetaldehyde and 2,5-dimethylpyrazine. These last compounds are hydrophilic, and initial concentrations are near those of 2-nonanone. The effect of the initial concentration is clearly observed for compounds belonging to the same group: the initial concentration of acetophenone is higher than those of benzaldehyde, and the flux of the first compound is significantly higher than those of the second. Otherwise, 2-nonanone presents a lower flux

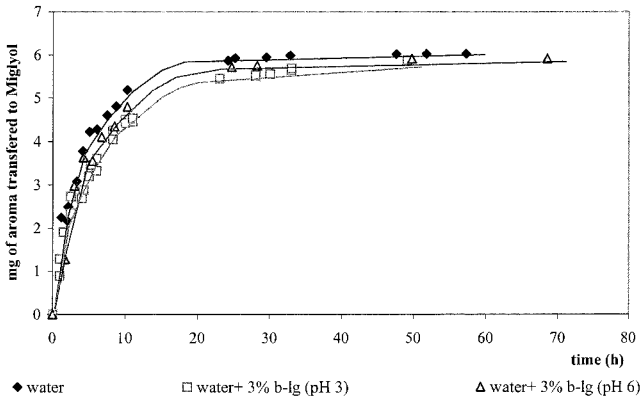

Figure 1. Transfer kinetics of *d*-linalool, with and without β-lactoglobulin, from the aqueous phase to miglyol, at 25 °C and at pH 3 and 6.

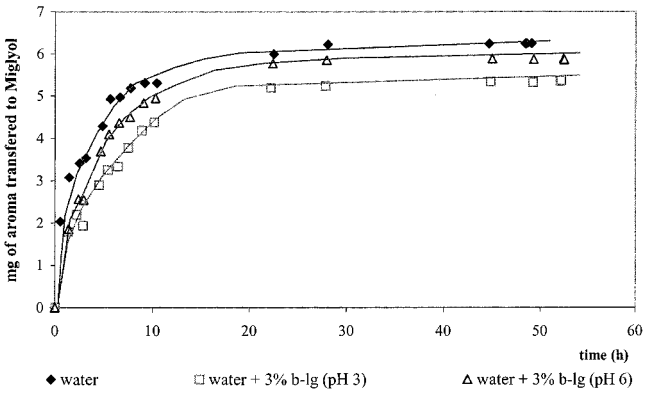

Figure 2. Transfer kinetics of isoamyl acetate, with and without β-lactoglobulin, from the aqueous phase to miglyol, at 25 °C and at pH 3 and 6.

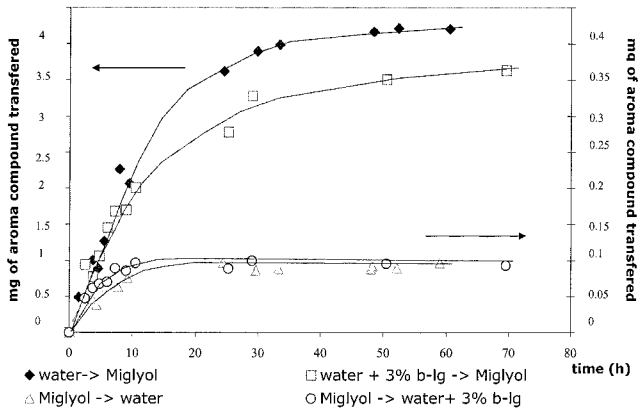

Figure 3. Miglyol–aqueous partition kinetics of benzaldehyde as a function of the aromatized phase, with and without β-lactoglobulin at 25 °C and pH 3. Transfers from aqueous solutions to miglyol are represented on the left *Y* scale and transfers from miglyol to aqueous solutions on the right *Y* scale.

compared to those of isoamyl acetate and *d*-linalool because of its low initial concentration and its initial pressure.

A protein, β-lactoglobulin, was introduced in the medium, to understand better the behavior of the aroma compounds in the food matrix and, particularly, the physicochemical interactions at the oil−water interface.

Figures 1−**3** present the kinetics of transfer from the aqueous phase, with or without protein, to miglyol of the aroma compounds (*d*-linalool, isoamyl acetate, and benzaldehyde, respectively).

The experimental system was not agitated. For *d*-linalool and isoamyl acetate, the effect of the pH was studied. For benzaldehyde, the transfer kinetics from water to miglyol and from miglyol to water were followed. In the presence of β-lactoglobulin, a decrease in transfer from the aqueous phase to miglyol of the three compounds is observed (**Figures 1**−**3**), with a more intense mode for isoamyl acetate and benzaldehyde. The values of the quantities of aroma compounds transferred at equilibrium (after 50 h in experiment) from aqueous solutions to miglyol are higher in the absence of protein. The protein effect varies upon the medium pH value: at pH 3 the decrease of the aroma transfer at the lipid−water interface is more important than at pH 6.

The physicochemical interactions between aroma and protein do not sufficiently to explain the differences of volatile compound transfer in the presence or absence of β-lactoglobulin in the aqueous solution. Indeed, the *d*-linalool retention by the protein is more important than those of benzaldehyde and isoamyl acetate (*6*). However, *d*-linalool is the compound least affected by the presence of the protein when it transfers from the aqueous phase to miglyol (**Figures 1**−**3**). The transfer decrease of the aroma compounds from the aqueous phase to the lipidic one in the presence of β-lactoglobulin may be explained by the affinity of the aroma compound for the protein that could have an influence toward diffusion. However, no significant difference is observed in diffusion coefficient measurements of benzaldehyde in aqueous solutions with or without 3% β-lactoglobulin (which are, respectively, equal to 10.9×10^{-10} and 10.6×10^{-10} m^2 s^{-1}).

The aroma transfer does not seem to be affected by the presence of the protein in the aqueous solution. However, when the protein is adsorbed at the water−lipid interface, a barrier effect could appear. After the migration to the interface, the protein becomes unravelled. Its structure opens and it stretches out to form a layer or a film (*7*).

The initial aroma flux at the interface in the different conditions is given in **Table 2**. For benzaldehyde, the initial flux from the aqueous phase to miglyol is not significantly different in the presence or in the absence of β-lactoglobulin. However, the protein effect is evident after some hours (**Figure 3**). The same phenomenon is observed for *d*-linalool (**Figure 1**). This evolution time can be explained by the adsorption kinetics of the protein at the water−lipid interface. The protein adsorption on a plane interface is a process controlled by diffusion, and it is slower under these conditions than in those used in the formation of an emulsion where the protein is very quickly adsorbed (*7*). The adsorption of a globular protein such as β-lactoglobulin at the water−lipid interface can be schematized by a model of a monolayer adsorption of more or less deformable particles (*8*). The surface pressure at the interface roughly increases in ∼5 min and continues to increase until it reaches an apparent equilibrium after 6 h (*9*). The time interval corresponds to the adsorption and to the protein rearrangement at the interface. The protein being completely adsorbed after 6 h, its barrier effect should be more important on the aroma compound transfer.

The pH influences the transfers of *d*-linalool and isoamyl acetate from the aqueous phase to miglyol in the presence of β-lactoglobulin: at pH 3, the transfer rate at the interface is lower than that observed at pH 6 (**Figures 1** and **2**; **Table 2**). These differences between the pH values of 3 and 6 should be due to the conformational changes of β-lactoglobulin as a function of intrinsic factors of the solution, which modify the behavior of the protein toward the interface (*10*). At pH values

near the isoelectric pH value ($pH_i = 5.2$), the conformation of the protein is more compact (global electric charge is null) and the quantity of sorbed protein and the thickness of the layer at the interface reach their maximal values (*11*). The adsorption characteristics of β-lactoglobulin at the oil−water interface have been reported by Das and Kinsella (*12*). The density of adsorption (milligrams of adsorbed protein per square meter of the interfacial surface) is maximum at pH 5 (9.8 mg/m^2) as the adsorbed quantity decreases when the pH is >5. Although Das and Kinsella (12) have not specifically studied the adsorption of β-lactoglobulin at pH 3 and 6, they have shown that at acid pH values the adsorbed protein quantity per surface unit is higher than that at pH values above pH_i. This may explain the more marked decrease of the transfer rate of *d*-linalool and isoamyl acetate in the presence of β-lactoglobulin at pH 3 compared to pH 6 as the physical barrier consisting of the protein at the interface is denser at acid pH.

The partition miglyol−aqueous solution kinetics have also been studied as a function of the aromatized phase and in the presence or absence of β-lactoglobulin at pH 3 (**Figure 3**, left *Y* scale used for the transfer from aqueous solutions to miglyol and right *Y* scale for the transfer from miglyol to aqueous solutions). The liquid−liquid equilibrium for benzaldehyde is reached 6−7 times more quickly when the aromatized phase is miglyol than when it is an aqueous phase, although the initial flux is ∼20 times weaker (**Table 2**). This can be explained by the aroma quantity which transfers from one phase to the other. The partition miglyol−water coefficient for benzaldehyde is equal to 44 (*6*). This value means that 44 times more aroma must transfer from water to the lipidic phase to reach the equilibrium than in the opposite way (from lipid to water). Castelain et al. (*13*) found the opposite effect when benzaldehyde transfers between lipidic and aqueous phases: the time to reach equilibrium was shorter when the aroma compound transferred from water to oil than from oil to water. However, these authors have introduced 6 times more aroma compound in oil than in water. Our results are obtained with the same concentrations, whatever the aromatized phase, and the same volumes of aqueous and lipidic phases.

The effect of the presence of the protein on the transfer of benzaldehyde from the aqueous to the lipidic phase has been shown. After 60 h in experiment, the quantity of aroma compound transferred is ∼15% lower in the presence of protein than in its absence. However, in the opposite mode (transfer from the lipidic to the aqueous phase), this effect was not observed, although the pH conditions, initial concentration, and volumes of aqueous and lipidic phases are the same: the results with and without are quite similar (3% within the experimental error) (**Figure 3**). This can be explained by the kinetics of adsorption of the protein at the interface, which is effective after 6 h (*9*). Indeed, because the partition equilibrium is reached after 10 h, the barrier effect of the protein is minimal. The presence of the protein plays a role in the transfer of aroma compounds between different phases, especially by decreasing the transfer of aroma compounds from the aqueous phase to the lipid phase.

CONCLUSION

The transfer kinetics of the aroma compounds at the lipidic−aqueous solution interface are influenced by different parameters, particularly the affinity of the aroma compounds for the medium. This affinity depends together on the nature of the aroma compound and of the aqueous and lipidic phases and also on the physicochemical characteristics of the medium (pH). The transfer at the interface from the aqueous to the lipidic phase decreases in the presence of β-lactoglobulin, because the protein is adsorbed at the interface and constitutes a barrier layer. This effect is greater at pH 3 than at pH 6, because of the higher density of the layer at the interface at more acid pH. The barrier effect on the transfer of aroma compound from the lipidic to the aqueous phase was not observed: a study using a higher interfacial surface may help to explain this.

The protein effect on the transfer from aqueous phase to lipidic phase seems to be dependent on time because the formation of the protein barrier at the aqueous−lipidic interface depends on the diffusion when the medium is not agitated. The absence of the protein effect in the opposite mode (from lipidic phase to aqueous phase) arises from the time dependence for the formation of the interfacial barrier. Espinosa (*6*) has shown that the time to reach the concentration equilibrium between the two liquid phases is shorter when benzaldehyde transfers from the lipidic phase to water than from water to the lipidic phase. Previous studies have also shown that, for the studied aroma compounds, the flux at the interface air−aqueous solution (containing β-lactoglobulin) is equal to or greater than that observed in pure water whatever the pH value. The transfer between the different phases in the food matrix may influence the transfer toward the gaseous phase and therefore modify its organoleptic perception. The study of these interactions between the aroma compounds and β-lactoglobulin must be continued to develop a better understanding of the implicated mechanisms, particularly to determine the role of the adsorbed protein at the lipidic−water interface. The use of the protein barrier effect to encapsulate aroma compounds in multiphasic (containing lipids) structures is considered to control more efficiently the transfer of aroma compounds in the different phases of the food matrix.

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Received for review July 19, 2001. Revised manuscript received November 16, 2001. Accepted November 29, 2001.

JF010939J