Transfer of Aroma Compounds in Water−Lipid Systems: Binding Tendency of β-Lactoglobulin

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Interactions of volatile aroma compounds with protein in aqueous solutions, especially whey proteins, have received significant attention in recent years. This work attempts to improve our understanding of the mass transfer in multiphasic systems, such as emulsions at the lipid−water interface, and to reveal the role of β-lactoglobulin in the release rate of solutes. For this purpose the rotating diffusion cell has been used. From a practical point of view it enables evaluation of the transfer through the aqueous phase, through the oil and the interfacial transfer. The effect of β-lactoglobulin, medium pH, and solute concentration has been investigated. Benzaldehyde and 2-nonanone have been studied, and miglyol has been chosen as an oil phase. It has been demonstrated that mass transfer has a rate-limiting step, which depends on physicochemical parameters such as hydrophobicity of the volatile, diffusion and partition coefficients, and rheological properties of the aqueous phase.

Keywords: *Aroma compounds; emulsion; structure; transfer at interfaces; β-lactoglobulin; miglyol*

INTRODUCTION

The increase of low-fat foods in the market has stimulated the interest in flavor binding. New food products incorporate novel proteins but lack flavor. This is especially true if whey proteins are used as fat replacers since they have different binding affinities for flavor compounds. Interactions of volatile aroma compounds with the protein macromolecules undoubtedly has an effect on the flavor perception of food. Improving flavor quality needs a better understanding of the binding process and release of flavor compounds from different food products (dairy products, low-fat frozen dairy desserts, emulsions containing protein). O'Neill (1994) overviewed flavor binding to food proteins emphasizing the role of β-lactoglobulin, a hydrophobic ligand-carrier protein. It has emulsifying properties and is often used as a model protein for studying ligand−protein interaction because its structure and properties have been well-studied. β-Lactoglobulin demonstrates a high affinity for a wide range of organic ligands, especially small hydrophobic molecules, i.e., retinol (Futterman and Heller, 1972), β-ionone (Dufour and Haertle, 1990), ketones, and esters (Jouenne and Crouzet, 1996). Binding of flavor compounds by proteins results in the suppression of the primary flavoring impact. In oil-in-water emulsions flavor compounds have first to be released from fat to the aqueous phase before they are released from the aqueous phase to the headspace (McNulty and Karel, 1973a−c; McNulty, 1987; Druaux and Voilley, 1997).

To investigate the rate of transfer of solutes from an aqueous phase to another through a lipid layer, as a model of water−oil−water emulsions, the rotating diffusion cell was developed (Albery et al., 1976). Such technique allowed us to study the resistance to the mass transfer within the aqueous boundary layers, across the oil−water interface and within the oil layer (Harvey et al., 1995). This work attempts to improve our understanding of the transfer phenomena of aroma compounds in an emulsion at the lipid−water interface in the absence or presence of β-lactoglobulin.

MATERIALS AND METHODS

Reagents. The aroma compounds, 2-nonanone and benzaldehyde, and the oil, miglyol, were kindly provided by International Fragrances and Flavors (IFF, Longvic-lès-Dijon, France). Miglyol is a triglyceride of caprylic (60%) and capric acid (40%). β-Lactoglobulin was from Besnier (France). Membrane filters were of poly(tetrafluoroethylene) (Sartorius, 118 07 47N, Palaiseau, France). The thickness of the membrane was 3.05×10^{-5} m, and its porosity was 0.8. NaN_3 (Merck, Darmstadt, Germany) was added to avoid bacterial growth. Aqueous protein solutions were adjusted to pH values of 3 and 9 with HCl and NaOH, respectively.

The diffusion coefficients of the aroma compounds in the oil phase, D_o, were experimentally obtained by the Stokes method (Voilley, 1986). The viscosity of miglyol and β-lactoglobulin solutions was determined experimentally on a viscometer Rheomat 30 at 25 °C.

The liquid−liquid partition coefficient P is respectively the ratio of the concentration (v/v) of the solute in the liquid and aqueous phases; it was determined at 25 °C by measuring the equilibrium concentration of the solute between miglyol and aqueous phases.

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 100−200 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), 160 °C for benzaldehyde and 150 °C for 2-nonanone; N_2 flow rate, 16 mL/min; H_2 flow rate, 25 mL/min; air flow rate, 250 mL/min.

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Table 1. Diffusion Coefficients of the Aroma Compounds in an Aqueous Phase and in Oil at 25 °C and Their Liquid−Liquid Partition Coefficients between Oil and Aqueous Phase

		diffusion coefficients ($\times 10^{10}$ m^2/s) in:				liquid−liquid partition coefficients (miglyol−aqueous phase)		
			β-lactoglobulin (3%)				β-lactoglobulin (3%)	
aroma compd	hydrophobicity (log P)[a]	water	pH 3	pH 9	miglyol	water	pH 3	pH 9
2-nonanone	2.9	9.35	7.10	5.52	0.78	794	218	190
benzaldehyde	1.5	10.79	10.79		1.25	44	44	

[a] Calculated with Rekker estimation (1977).

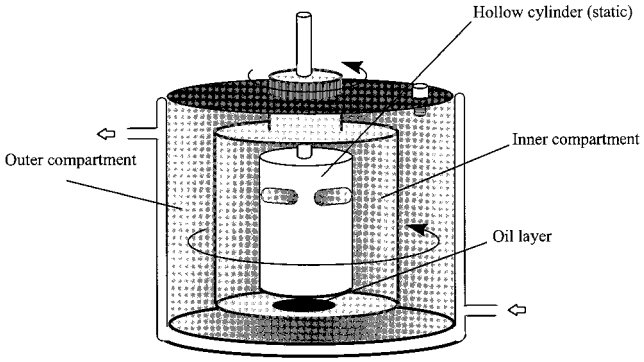

Figure 1. Rotating diffusion cell.

The rotating diffusion cell is designed hydrodynamically so that stationary diffusion layers of known thickness are created on each side of the oil layer (Figure 1). The flux of aroma compound from the inner compartment across the oil layer was measured by periodically sampling with a microsyringe from the solution in the outer compartment. The filter was filled with oil under vacuum for better saturation of the membrane filter pores. Miglyol was presaturated with water to ensure conditions similar to those of real food emulsions. In experiments with β-lactoglobulin, both compartments were filled with the aqueous protein solution.

The rotating diffusion cell method enables us to carry out a study on the mass transfer of solutes from aqueous phases to oil and from oil to aqueous phases. The fundamental theory of the Levich model (Levich, 1962) provides the means of aroma compound transfer from the inner to the outer compartment by the overall permeability coefficient k (m/s):

$$\frac{1}{k} = k_Z\omega^{-1/2} + R_{oil} + R_I \qquad (1)$$

where ω is the rotation speed of the filter (s^{-1}) R_{oil} is related to the diffusion through the lipid in the filter, calculated from $b/\alpha D_o P$ (where b is the thickness of the membrane (m), α is the porosity of the membrane, D_o is the diffusion coefficient of the solute in the oil phase (m^2/s), and P is the liquid−liquid partition coefficient); R_I describes the resistance due to the solute transfer across the two aqueous phase−oil interfaces, defined as $2/\alpha v_i$ (where v_i is the rate constant of the solute through the oil−water interface (m/s)); and k_Z is the slope of the Levich plot and represents the resistance to diffusion through the two stagnant aqueous diffusion layers, R_{aq}.

The theoretical Levich slope is calculated from the kinematic viscosity of the aqueous phase, η (m^2/s), and the diffusion coefficient of the solute in the aqueous phase, D_{aq} (m^2/s):

$$k_Z = 1.286\eta^{1/6}D_{aq}^{-2/3} \qquad (2)$$

A plot of $1/k$ against $\omega^{-1/2}$ enables us to obtain the sum of the $R_I + R_{oil}$ (the intercept is estimated from the experimental points). The independent assessment of R_{oil} allows the interfacial resistance R_I to be determined, and the resistance to the stagnant aqueous diffusion layer R_{aq} is calculated from the total resistance (Landy et al., 1998).

RESULTS AND DISCUSSION

Preferential retention and release of flavor compounds during processing treatments (e.g., heating, high-shear mixing) and storage are factors affecting the perceived flavor of the product and its acceptability. Hence, the ability of protein to bind aroma compounds has to be considered to get a view of the kinetic distribution of solutes during eating as a function of the interface structure. Some other factors are the nature and concentration of flavor and other components of the food, pH, temperature, time, etc.

Physicochemical Characteristics of the Aroma Compounds. Benzaldehyde and 2-nonanone belong to different hydrophobic groups that allows a better understanding of the difference in their behavior. The diffusion and liquid−liquid partition coefficients between water and miglyol for benzaldehyde were experimentally found not to differ significantly in the presence of β-lactoglobulin or without protein (Table 1). The decrease of the diffusion coefficient of 2-nonanone in protein solution in comparison to water is affected by the second solute—β-lactoglobulin. The binding interaction between 2-nonanone and β-lactoglobulin, primarily hydrophobic in nature, is pH-dependent. It is possible that the globule size is related to the thiol reactivity (cysteine), which depends on the pH, concentration, ionic strength, and temperature (Aymard et al., 1996). β-Lactoglobulin is in the monomeric form at pH 3, and the free thiol group is not reactive, but the hydrophobic residues of the folded state may come into contact with aqueous medium and the aroma compound, respectively. O'Neill and Kinsella (1987) and Pelletier et al. (1998) assumed that 2-nonanone is bound in the hydrophobic pocket of β-lactoglobulin. A lower diffusion coefficient at pH 9 can be explained by a better binding of the aroma compound.

In the case of liquid−liquid partition coefficients, a reduction of the affinity to miglyol for 2-nonanone was observed in the presence of β-lactoglobulin and with an increase of pH of the aqueous phase. This is explained by a higher retention of 2-nonanone by β-lactoglobulin. There is a higher affinity between the aroma compound and the protein at pH 9. A similar effect of basic pH on the retention of 2-nonanone in β-lactoglobulin solution has been observed by Jouenne and Crouzet (1996) and explained due to changes in the monomer−dimmer equilibrium at room temperature and to the partial denaturation of the protein. Different viscosity of the system is another fact that has to be considered.

Transfer of Aroma Compounds in Water−Lipid Systems. The effect of a ligand concentration was examined for benzaldehyde in aqueous phase with or without protein. The initial aroma compound concentration in the inner compartment was for benzaldehyde: 0.984×10^{-3} M (100 ppm) and 2.42×10^{-3} M (245 ppm). Each experiment was carried out at least three times, and the variation coefficient was 2.5−6.1% (for 100 ppm) and 5.3−18.8% (for 245 ppm). The Levich plots for the transfer of benzaldehyde in the absence of β-lactoglobulin are shown in Figure 2. The intercept for a

Table 2. Values of the Intercept 1/*k* and Resistances to Mass Transfer through the Lipid Phase (R_{oil}) and the Lipid−Aqueous Phase Interface (R_I) for Benzaldehyde and 2-Nonanone in Different Aqueous Phases and pH

aroma compd	aqueous phase	$1/k \times 10^{-5}$ (s/m)	$R_{oil} \times 10^{-5}$ (s/m)	$R_I \times 10^{-5}$ (s/m)
benzaldehyde, 100 ppm	water	0.073	0.069	0.004
245 ppm	water	0.126	0.069	0.057
245 ppm	3% β-lactoglobulin (pH 3)	0.315	0.069	0.245
2-nonanone	water	0.913	0.006	0.907
	3% β-lactoglobulin (pH 3)	2.356	0.022	2.334
	3% β-lactoglobulin (pH 9)	0.996	0.026	0.970

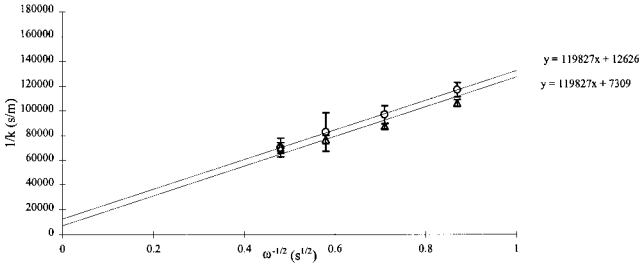

Figure 2. Levich plots of benzaldehyde at 100 ppm (Δ) and 245 ppm (○) in water through miglyol at 25 °C.

Table 3. Relative Percentages[a] of the Resistances R_{aq}, R_{oil}, and R_I to the Overall Mass Transfer of the Aroma Compounds through a Miglyol Layer at Different Conditions

aroma compd	β-lactoglobulin (%, m/m)	R_{aq} (%)	R_{oil} (%)	R_I (%)
benzaldehyde, 100 ppm	0	93.5	6.2	0.3
245 ppm	0	89.2	5.9	4.9
245 ppm	3	78.3	4.8	16.9
2-nonanone	0	55.7	0.3	44.0
pH 3	3	38.9	0.6	60.5
pH 9	3	64.1	0.9	35.0

[a] Calculated by considering that at $\omega^{-1/2} = 0.87$ $s^{1/2}$, 1/*k* corresponded to 100% of the resistances.

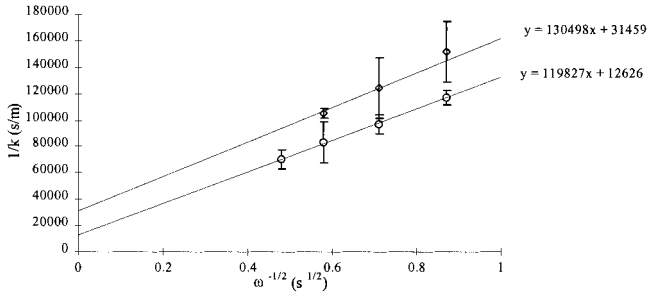

Figure 3. Levich plots of benzaldehyde (245 ppm) in water (○) and in 3% β-lactoglobulin solution (◇) through miglyol at 25 °C.

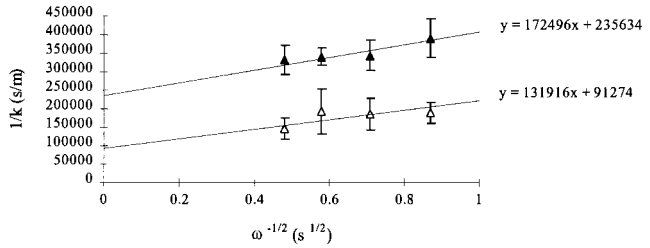

Figure 4. Levich plots of 2-nonanone in water (Δ) and in 3% β-lactoglobulin solution at pH 3 (▲) through miglyol at 25 °C.

higher aroma concentration is greater; i.e., the resistance to the interface R_I has increased by a factor higher than 10 (Table 2). The comparison of the relative percentages of the resistances to the mass transfer allows a more accurate estimate of the rate-limiting step of the transfer (Table 3). The resistance to the stagnant aqueous diffusion layer R_{aq} has not changed significantly (93.4% and 89.2%), and it is the rate-limiting step of the transfer of benzaldehyde. The observed tendency in changing overall mass transfer due to the interfacial transfer, i.e., on the ligand concentration, has to be taken into account especially when there is a mixture of aroma compounds and, hence, a competition.

Effect of *β*-Lactoglobulin in Aqueous Solution. The Levich plots of benzaldehyde (245 ppm) in aqueous solutions (pH 3) with and without protein are shown in Figure 3. The intercept for the protein solution is higher which means that the transfer of benzaldehyde through the interface and the oil layer has been affected by β-lactoglobulin. The overall resistance (1/*k*) increase (from 0.126×10^5 up to 0.315×10^5 s/m) is due to the large resistance to the transfer through the oil−water interface R_I (Table 2) and the resistance to the transfer through the aqueous layer R_{aq}, i.e., the slope, being similar. The comparison of values of the relative percentages (Table 3) allows to reveal the rate-limiting step, which is R_{aq} in both of the aqueous phases. Kinetic models have shown that benzaldehyde total flux varies between 30% and 38% in both models.

These data show that the transport of benzaldehyde through the interface in β-lactoglobulin solution is affected by the presence of protein molecules. Some studies on β-lactoglobulin/benzaldehyde complex formation have shown strong binding of the ligand, but at pH 6 (Relkin and Marin, 1996). Some of our own research has shown that the retention of benzaldehyde by β-lactoglobulin is relatively weak as miglyol is a good solvent for it (Espinosa-Diaz et al., 1996). It is possible that the retention of benzaldehyde by the protein may be masked by miglyol (competition at the interface). It can be a reason for uneven distribution.

The Levich plots for the transfer of 2-nonanone in different aqueous phases are shown in Figure 4. A notable difference is observed in the interface. The presence of β-lactoglobulin induced an increase of the interfacial resistance R_I, i.e., the overall resistance 1/*k* (Table 2). The value of R_{oil} is also higher due to the lower liquid−liquid partition coefficient of 2-nonanone in β-lactoglobulin (pH 3) compared to the one in water. In the water solution the transfer through the aqueous layer is lower (a higher resistance) than the transfer at the interface, while in β-lactoglobulin solution the resistance to the interface controls the flux (Table 3). The loss of 2-nonanone from the inner compartment was in the range of 68−76% in the water phase and 28−40% in the protein phase (pH 3).

Our results are in agreement with those obtained from a mathematical model by Harrison and Hills (1997) who assumed that the main effect of adding binding polymers to an aqueous solution is to reduce the free flavor available for release and to alter the interfacial mass-transfer coefficient. Investigating the interactions of β-lactoglobulin with flavor compounds (among them benzaldehyde and 2-nonanone), Jouenne

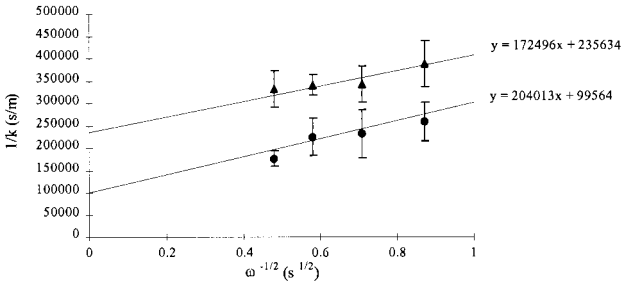

Figure 5. Levich plots of 2-nonanone in β-lactoglobulin solution at pH 3 (▲) and pH 9 (●) through miglyol at 25 °C.

and Crouzet (1996) found weak interactions with the first one but more significant binding for the latter at the same pH value of the medium. A higher retention of 2-nonanone has been also observed by measurements of vapor−liquid partitioning (Espinosa-Diaz et al., 1996).

All these results show that proteins have an important role in flavor retention and in the kinetics of flavor release. In emulsions, the presence of protein at the water−oil interface may increase the resistance to transfer of aroma compound. This can lead to a later diffusion and delay ligand elution. From a practical standpoint, this could result in a persistence of the flavor note during eating.

Effect of Medium pH. The effect of pH is particularly important since many different processing conditions are used in food formulations especially in the presence of protein.

A pH 3 value affects the intercept of the Levich curve significantly and the slope as well (Figure 5). It is observed that at pH 9 the interface does not represent the limiting step as was found at pH 3 (Table 3). The effect of medium pH was revealed also by the diffusion and partition coefficients of 2-nonanone (Table 1). The loss of 2-nonanone from the inner compartment adjusted to pH 9 is in the range of 22−33%, comparable to the loss at pH 3 (28−40%).

In view of the fact that β-lactoglobulin possesses different binding sites at pH 3 and 9, the rate of the transfer of small ligands depends on the pH of the aqueous phase. On one hand, β-lactoglobulin can act as a barrier between oil and water, thus preventing the diffusion of aroma compounds in the oily phase. On the other hand, the protein may act as a carrier of them, as at basic pH the unfolded structure allows a larger contact surface with the oil and the hydrophobic ligand may come into contact with miglyol. Hence, it presumes better exchange of the aroma compound at pH 9. A balance between association of the volatile with the whey protein and partitioning into the lipid phase may occur. Another point of view is that the binding is not strong and it can be reversible (Fischer and Widder, 1997). Binding of 2-nonanone was found completely reversible, but in the range of pH 4−7 (Relkin and Marin, 1996). All this suggests that the binding of 2-nonanone may be achieved by the choice of processing conditions to have a desirable level.

CONCLUSIONS

It is a challenge to produce a low-fat food with a natural aroma profile considering the quick disappearance of the flavor in the mouth. The results of this work support the view that using the flavor-binding capacity of the protein might help in generating a prolonged and sequential flavor release.

It has to be considered that the resistances to transfer at interfaces depend on the nature of the aroma compound. The overall resistance to transfer at the interface miglyol−water is 12.5 times higher for 2-nonanone than for benzaldehyde at the same concentration (100 ppm). On the other hand, for both aroma compounds, the presence of β-lactoglobulin at the interface entails an increase of the resistance to transfer.

Investigations of aroma transfer phenomena have relied on model systems to obtain reliable results. But the question remains, how do food ingredients of the flavor cocktail compete with each other? Further research is required to study the capacity of protein to either mask undesirable flavors or simulate the desired product flavor (mixture of compounds). Moreover, β-lactoglobulin tends to unfold (pH changes, heating) and to bind a greater amount of flavor compounds. Since heating is often required at some stage of the product processing, a study of the temperature effect is essential, as at pasteurization temperature the amount of binding increases and less flavor is available.

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