

Influence of the Food Matrix Structure on the Retention of Aroma Compounds

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The retention of the aroma compounds in a multicomponent medium like the food matrix is influenced by their affinity with the protein when lipid is present at a low level (0.5%). The effect of the structure of the media is also studied by using two media with the same composition; one was emulsified, and the other was not. Among the studied aroma compounds, 2-nonanone and isoamyl acetate present opposite behaviors: the volatility of isoamyl acetate is not affected by the change of the medium structure whereas that of 2-nonanone increases. The decrease of retention of 2-nonanone in an emulsified system would be due to a modification of the fixation site for this compound on the protein or to a competition between the lipid and the aroma compound while the protein is adsorbed at the lipid–water interface.

Keywords: *Aroma compounds; β -lactoglobulin; lipid; structure; retention*

Flavor release is complex, and several mechanisms can occur: mass transfer, matrix structural hindrance, flavor–matrix interactions, etc. Studies on interactions between aroma compounds and other nonvolatile constituents of food were generally carried out with very simple systems (Godshall, 1997; Sadafian and Crouzet, 1987; Farès, 1987; Le Thanh, 1992; Dubois-Barbier, 1994; Buttery, 1973). However, food is a multicomponent medium. The interactions between macromolecules in food change the affinity of the aroma compounds for the food matrix by modifying the nature and the number of free binding sites. To understand better the phenomena controlling aroma release in complex media, the knowledge of the food matrix structure must be considered. Several authors (Lundgren et al., 1986; Wilson and Brown, 1997) have observed the decrease of aroma release and perceived intensity with the increase of viscosity of the medium. Guinard and Marty (1995) have studied the release of three aroma compounds in three different gels. Their results showed an effect of gel firmness on maximal perceived intensity. Brossard et al. (1996) showed with emulsions containing Miglyol and water that the release of benzaldehyde, hence its perception, was governed by several factors such as the maximal aroma concentration in the lipidic phase, the transfer rate from the lipidic phase to the aqueous phase, and the ability of aroma release from the lipidic phase to the vapor phase. These sensory approaches informed of aroma perception in relation to the food structure; therefore, Taylor (1996) showed the limits of these measures, which need to be completed with physicochemical studies.

Dubois et al. (1996) have studied the effect of the structure of the medium on the aroma compound volatility in emulsions stabilized with a milk protein.

These authors showed that the interfacial surface area of a model cheese system containing calcium caseinate (11–22%) affected the volatility of diacetyl and allyl sulfide: their headspace concentrations decreased when the surface area of the oil droplets was increased. However, in a model system containing only 1% calcium caseinate, the surface area of the interface was shown to have no effect. This could be due to the poor coverage with proteins on the surfaces of the fat globules in model systems: the interactions between the aroma compounds and these proteins were less because the quantity of absorbed protein at the interface was reduced. Landy et al. (1996) showed that the volatility of ethyl esters in lipid-containing systems was not modified in the presence of sodium caseinate or sucrose stearate if the surface area of the liquid–liquid interface was increased from 1.6×10^{-3} to $10.0 \text{ m}^2/\text{mL}$ triolein. These authors concluded that the ethyl esters were not retained at the lipid–aqueous phase interface to any great extent. This can also be explained by only a very low quantity of proteins ($0.5 \text{ mg}/\text{m}^2$) being retained at the surface of the triolein droplets. Moreover, considering the high affinity of the aroma compound for triolein, and thus the low concentrations remaining in the aqueous phase, the quantity of the aroma compound bound to the interface was too low to be detected.

These works show that the microstructure of the medium changes on the release of the aroma compounds, particularly at the time of the changes of the medium viscosity or of the aroma molecule retention at the interfaces.

Our objective is to understand better the phenomena of retention and release of the aroma compounds in aqueous, lipidic, or multiphasic media. To study the behavior of the aroma compounds in a food matrix containing lipids we have to know the affinity between these compounds and each of the matrix constituents, particularly the lipids. Generally the aroma compounds are hydrophobic and highly soluble in the lipidic phase.

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That is why the affinity of the aroma compounds for Miglyol was determined using liquid–liquid and vapor–liquid partition techniques. The volatility was studied by taking into account composition and structure of the medium.

MATERIAL AND METHODS

Material. The aroma compounds, benzaldehyde, isoamyl acetate, *d*-linalool, and 2-nonanone, and the oil, Miglyol, were kindly provided by International Flavors and Fragrances (I.F.F., Longvic-lès-Dijon, France). Miglyol is a triglyceride of caprylic (60%) and capric (40%) acids. β -Lactoglobulin (β -lg) was from Besnier-Bridel (Laval, France). Aqueous protein solution (3%) was adjusted to a pH of 3 with HCl.

Methods. Vapor–Liquid Partition. The method used to measure vapor–liquid equilibrium was the headspace analysis or exponential dilution coupled with gas–liquid chromatography. An inert gas (nitrogen) passed through the liquid phase at a constant flow rate ($3 \times 10^{-5} \text{ m}^3/\text{min}$) and carried the volatiles into the headspace. A sample of the vapor phase (1 mL) was automatically injected into the gas chromatograph at regular intervals.

The chromatograph was equipped with a flame ionization detector (Chrompack CP 9000; Chrompack Co., Middelburg, The Netherlands) and with a 3 m stainless steel column (inner diameter 2.2 mm) packed with Chromosorb W-Aw 100–200 mesh Carbowax 20 M.10%. Gas flow rates were as follows: nitrogen, $1.6 \times 10^{-5} \text{ m}^3/\text{min}$; hydrogen, $2.5 \times 10^{-5} \text{ m}^3/\text{min}$; air $25 \times 10^{-5} \text{ m}^3/\text{min}$. The column temperature varied with the nature of the aroma compound (between 80 and 160 °C). The injector and detector temperatures were 190 and 200 °C, respectively.

The obtained data permitted the determination of the vapor–liquid partition coefficient of aroma compounds at infinite dilution. This coefficient can be expressed in molar fraction (K_{mol} , eq 1) or in mass fraction (K_{mass} , eq 2) of the aroma compound concentrations of the vapor and liquid phases, respectively.

$$K_{\text{mol}} = Y_i/X_i \quad (1)$$

where Y_i and X_i are the molar fractions of the vapor and liquid phases, respectively.

$$K_{\text{mass}} = C_v/C_L \quad (2)$$

where C_v and C_L are the mass concentrations of the vapor and liquid phases, respectively ($\text{g}\cdot\text{L}^{-1}$).

Exponential dilution consists of exhausting the liquid phase of aroma compounds in equilibrium with the vapor phase. The solute chromatographic peak area variation is an exponential function of time provided that the detector response is linear (Sorrentino et al., 1986). The vapor–liquid partition coefficient, K_{mol} , of the aroma compound in pure water or in a solution containing one or several nonvolatile constituents is given by the eq 3, where t is the time (min), S_{t_0} and S_t are the volatile

$$K_{\text{mol}} = \frac{1}{t} \frac{RTN}{DP_T} \ln \frac{S_t}{S_{t_0}} \quad (3)$$

peak areas at time $t = 0$ and t , respectively, T is the temperature (K), N is the number of moles of the liquid phase, D is the carrier gas flow rate (m^3/min), P_T is the total pressure (Pa), and R is the gas constant ($R = 8.314 \text{ J}/(\text{K mol})$).

Liquid–Liquid Partition. The liquid–liquid partition of the aroma compound was studied with an aqueous phase containing or not β -lactoglobulin at 3% (pH = 3.0). 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 at equilibrium the

Table 1. Aroma Compounds Partition Coefficients between Water and *n*-Octanol and between Miglyol and Water, with or without 3% β -lactoglobulin (25 °C)

aroma compd	$P_{\text{octanol-water}}^a$	$P_{\text{Miglyol-water}}$	$P_{\text{Miglyol-water}+\beta\text{-lactoglobulin}}$
benzaldehyde	32.4	44.0 (2) ^b	42.1 (1)
isoamylacetate	158.5	139.0 (2)	107.0 (2)
<i>d</i> -linalool	3467.4	224.0 (7)	202.0 (10)
2-nonanone	758.6	774.0 (5)	230.0 (6)

^a Calculated from the log P values with Rekker estimation (Rekker, 1977). ^b Variation coefficient in % in parentheses.

concentration of the solute in the organic and aqueous phases. Each system was repeated in triplicate.

RESULTS AND DISCUSSION

Partition Coefficients of the Aroma Compounds.

The behavior of the aroma compounds was studied in different systems: in one side, with a state change, involving the determination of the vapor–liquid partition coefficient (or volatility); in the other side, without a state change of the solute, involving the liquid–liquid partition. The knowledge of the liquid–liquid partition coefficients allows one to determine the affinity of the aroma compounds for the aqueous and lipidic phases.

Table 1 shows a comparison of the liquid–liquid partition coefficients in Miglyol and in aqueous solutions with or without β -lactoglobulin. This table gives also the values of the partition coefficient octanol–water ($P_{\text{octanol-water}}$) calculated from the values of log P obtained with the Rekker estimation (Rekker, 1977).

The values of the studied aroma compounds partition coefficients between Miglyol and water solutions decreased significantly in the presence of 3% β -lactoglobulin. This decrease was respectively of 7, 10, 23, and 70% for benzaldehyde, *d*-linalool, isoamyl acetate, and 2-nonanone.

The liquid–liquid partition coefficients of the aroma compounds between Miglyol and water solutions greatly depended on their physicochemical characteristics, particularly on their hydrophobicity. Indeed, this parameter represents the affinity of the aroma compounds for the lipidic and aqueous phases. The comparison of the predicted values ($P_{\text{Miglyol-water}}$) showed a good agreement except for *d*-linalool. The aroma compound liquid–liquid partition coefficients depend not only on their nature but also on that of the organic phase. Landy et al. (1998) observed similar results while studying ethyl butanoate and ethyl hexanoate. $P_{\text{Miglyol-water}}$ values were respectively 45 and 739. When the lipidic phase was triolein, the values increased to 113 and 1849, respectively.

For *d*-linalool the estimated value ($P_{\text{octanol-water}}$) varies from the experimental ($P_{\text{Miglyol-water}}$) because the applied method (Rekker, 1977) is not convenient with molecules containing a double bond near a chemical group.

Rousseau et al. (1996) had determined $P_{\text{Miglyol-water}}$ values for benzaldehyde and *d*-linalool via an indirect way by studying vapor–water and vapor–Miglyol equilibria. Our results varied in the same way but are higher.

The affinity of the aroma compounds for the aqueous and lipidic phases influences not only the liquid–liquid partition coefficient but also the volatility of the aroma compound (calculated from eqs 1–3). That is why we measured the vapor–liquid partition coefficient of the aroma compounds in Miglyol. The values are given in Table 2 and compared with those determined in water.

Table 2. Aroma Compound Vapor–Liquid Partition Coefficients (Molar Fraction) in Water and in Miglyol (25 °C)

Aroma compd	$K_{\text{mol(water)}}$	$10^3 K_{\text{mol(Miglyol)}}$
benzaldehyde	1.7	1.45 ^a
isoamylacetate	36.3	5.24
<i>d</i> -linalool	2.3	0.22
2-nonanone	33.6	0.64

^a Calculated from $P_{\text{Miglyol-water}}$ and $K_{\text{mass(water)}}$ values. $K_{\text{mass(Miglyol)}} = K_{\text{mass(water)}}/P_{\text{Miglyol-water}}$.

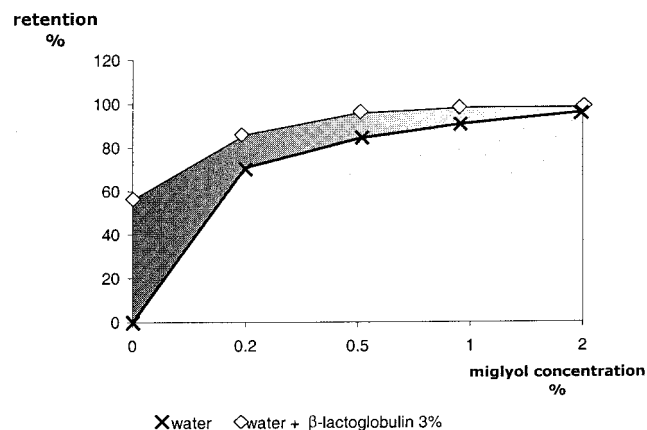


Figure 1. Influence of the Miglyol concentration on the retention of 2-nonanone with or without β -lactoglobulin at pH 3, 25 °C. (Percentage of retention = $[(K_{\text{mol(water)}} - K_{\text{mol(solution)}})/K_{\text{mol(water)}}] \times 100$.)

The vapor–liquid partition coefficients determined in Miglyol were lower than those in water. This effect became all the more important that the volatile compound affinity for Miglyol increased: thus, the volatilities in Miglyol compared to those in water decreased respectively by a factor of 50 000, 10 000, 7000, and 1000 for 2-nonanone, *d*-linalool isoamyl acetate, and benzaldehyde. Dubois et al. (1996) have shown that the volatility of allyl sulfide was 700 and 300 times lower respectively in tributyrine and in “anhydrous milk fat” than in water. In contrast, with an hydrophilic compound, diacetyl, the volatility was 3 times higher in “anhydrous milk fat” and the same in tributyrine as that in water.

In Table 2 are given the differences of the aroma compounds volatilities in Miglyol and in water, and Table 1 shows that 2-nonanone is the aroma compound having a higher affinity for Miglyol. Then the behavior of this compound is studied in a biphasic system with or without β -lactoglobulin, in the presence of a low lipid concentration (0.2–2% v/v). Figure 1 presents the influence of the Miglyol concentration on the retention of 2-nonanone with or without β -lactoglobulin (pH 3, 25 °C). This figure shows that the retention of 2-nonanone increases with the Miglyol in the presence or not of protein. When the Miglyol concentration was lower or equal to 0.5%, we observed retentions significantly different in the presence of protein compared to those in the absence of β -lactoglobulin. (The gray part between the two curves on Figure 1 represents the retention of 2-nonanone by the protein.) When the Miglyol concentration reached 1%, the retention of the aroma compound by the lipid was too important and the effect of protein was masked. This behavior has recently been observed by Jouenne (1997) for two aroma compounds, 2-nonanone and 2-octanone, under the same experimental conditions.

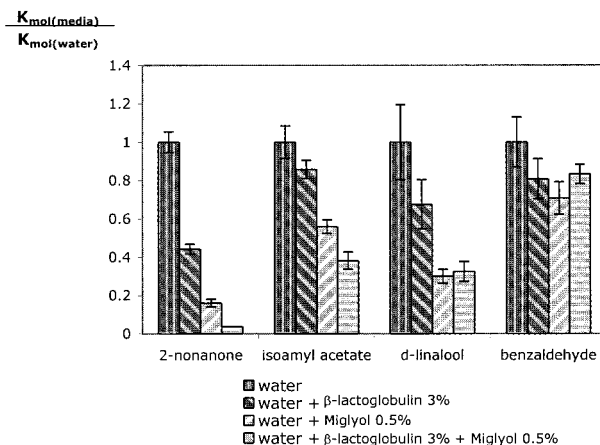


Figure 2. Influence of the composition of the medium on the aroma compounds volatility at pH 3, 25 °C.

The volatilities of the 4 aroma compounds studied (2-nonanone, isoamyl acetate, benzaldehyde, *d*-linalool) are determined in 4 different media: pure water, water with β -lactoglobulin (3%), water with Miglyol (0.5%), water with β -lactoglobulin (3%), and Miglyol (0.5%) (Figure 2). The volatility of the aroma compounds decreased with the presence of 3% β -lactoglobulin or 0.5% Miglyol. This decrease depended on the affinity of the aroma compounds for each constituent. In the protein solution the retention was in order 2-nonanone > *d*-linalool > benzaldehyde > isoamyl acetate. In the presence of the lipid the decrease was greater with a high partition coefficient Miglyol–water. Indeed, the retention of benzaldehyde, isoamyl acetate, *d*-linalool, and 2-nonanone in water with 0.5% Miglyol were respectively 29, 44, 70, and 84% and their partition coefficients in Miglyol–aqueous solution ($P_{\text{Miglyol-water}}$) were respectively 44, 139, 224, and 774.

Schirle-Keller et al. (1994) had also shown the influence of the hydrophobicity on the retention of the aroma compounds in media containing lipids. Small changes in oil content had a very significant effect on the vapor pressures of fat-soluble compounds (limonene and ethyl heptanoate). The amount of flavor compound in the headspace was strongly influenced by the amount of oil. The partial vapor pressure of water-soluble flavor compounds was less influenced than that of oil-soluble compounds by the presence of oil. Oil content had far less effect on vapor pressure of diacetyl than on that of either limonene or ethyl acetate (Schirle-Keller et al., 1994).

Our results showed the effect of β -lactoglobulin on the retention of 2-nonanone and isoamyl acetate in the presence of Miglyol. For *d*-linalool and benzaldehyde, a significant difference was observed between the vapor–liquid partition coefficients in the presence or not of protein when the medium contained 0.5% Miglyol. Then the hydrophobicity of the aroma compound and its affinity for the protein play an important role on their retention in multicomponent media. The retention is the result of a combined effect of the affinity of the aroma compounds for the lipidic and aqueous media and the capacity of the protein to fix these aroma compounds.

Influence of the Medium Structure on the Volatility of Aroma Compounds. The effect of the structure of the food matrix on the volatility of aroma compounds was studied by using two media with the same composition (water containing 3% β -lactoglobulin and 0.5% Miglyol pH 3) but with different structures to

Table 3. Physicochemical Characteristics of the Emulsion Containing 0.5% Miglyol and 3% β -Lactoglobulin before and after Headspace Analysis (pH 3, 25 °C)

characteristics	before headspace analysis	after headspace analysis
mean of fat droplet diameter (μm)	0.49	0.52
Specific interfacial area (m^2/mL)	12.3	11.5
interfacial area ($\text{m}^2/100 \text{ mL}$ of emulsion)	6.0	5.7
Interfacial protein concentration (mg/m^2)	17.0	ND ^a

^a ND: not determined.

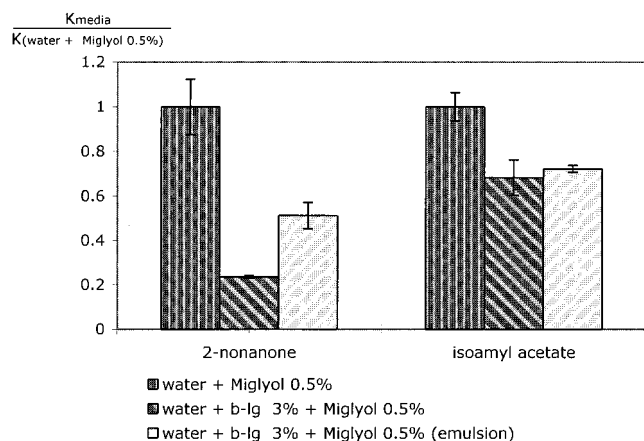


Figure 3. Influence of the composition and the structure of the medium on the relative volatilities of 2-nonanone and isoamyl acetate (pH 3, 25 °C).

constitute emulsified and nonemulsified systems. 2-Nonanone and isoamyl acetate were the chosen aroma compounds because the effect of β -lactoglobulin on their retention was clearly shown (Figure 2).

The stability of the emulsion was verified by measuring its characteristics, given in Table 3, before and after the headspace analysis (3 h).

The interfacial protein concentration (17 mg/m^2 with a mean of the fat droplet diameter of 0.5 μm) was high compared to the data given in the literature (Dubois et al., 1996). Under our experimental conditions, the protein concentration was in excess compared to those of Miglyol; Agboola and Dagleish (1995) observed that a high proteinic concentration favored protein-protein interactions. Then a high interfacial proteinic concentration could be due to the aggregation of the monomers of β -lactoglobulin. The volatilities of 2-nonanone and of isoamyl acetate in this emulsion have been compared to the vapor-liquid partition coefficient in a non emulsified medium with the same composition. Figure 3 shows the influence of the composition and of the medium structure on the relative volatilities of 2-nonanone and isoamyl acetate. The reference medium is composed of 0.5% Miglyol in water without β -lactoglobulin. The two aroma compounds did not present the same behavior in emulsified and nonemulsified media. Figure 3 shows that the volatility of isoamyl acetate was not affected by the change of structure of the medium whereas that of 2-nonanone increased. This difference of retention of 2-nonanone between the two media, emulsified or not, cannot be explained by the affinity between the lipid and the aroma compound because no modification was observed by the dispersion of the organic phase in the medium. Indeed, the interactions between lipids and aroma compounds are nonspecific

and generally due to a solubilization (Kinsella, 1990). As for β -lactoglobulin, its conformation has a great importance on the retention of the aroma compounds. This conformation could be modified by the adsorption of the protein at the Miglyol-water interface in the emulsified system. At the time of the adsorption of protein at the lipid-water interface, β -lactoglobulin becomes partially unwrinkled which allows the possibilities of interactions between apolar groups of the aroma molecule and the lipid to increase. When the protein becomes unwrinkled, the apolar groups are inserted into the lipidic phase (Phillips et al., 1994). This phenomenon would modify the protein conformation in biphasic medium, and these changes would be greater in an emulsified system because of the increase of interfacial area and therefore of the quantity of absorbed protein at the interface.

Hence, the decrease of retention of 2-nonanone in an emulsified system would be due to a modification of the steric environment of the fixation site of the protein for this compound: this modification occurs at the time of the β -lactoglobulin adsorption on the surface of the fat droplets and with the probable formation of proteic aggregates. Another way of explanation is the competition between the lipid and the aroma compound at the time of the protein adsorption at the lipid-water interface. Indeed, the interfacial adsorption of the proteins happens in two steps: electrostatic attraction between charged amino acid residues of the protein and polar heads of the lipid and after limited insertion of the apolar amino acid residues between the lipidic chains (Brown, 1984). Since aroma compound-protein interactions have a hydrophobic nature (O'Neill and Kinsella, 1987), the fixation sites of the protein adsorbed at the interface would be soon occupied by Miglyol.

Moreover, the volatility of 2-nonanone in the emulsified system does not increase enough to reach the volatility value corresponding to the biphasic medium without the protein. This fact shows that the effect of the presence of the protein in the medium, particularly in a continued phase, is not significant. Indeed, β -lactoglobulin was present with an excess in the medium and one fraction of this protein was modified neither by the adsorption at the Miglyol-water interface nor by the aggregate formation.

CONCLUSION

The behavior of the aroma compounds in a food matrix is influenced by the composition and the microstructure of the medium. In the presence of Miglyol at a concentration lower or equal to 0.5% the influence of β -lactoglobulin on the retention of 2-nonanone is observed. The contribution of β -lactoglobulin on the retention of the aroma compounds is also observed on *d*-linalool and to a lesser extent on isoamyl acetate and benzaldehyde. The study on the retention of 2-nonanone and isoamyl acetate in media with the same composition but with different structures (emulsified or not) had shown that the volatility of isoamyl acetate was not affected by the change of the medium structure whereas that of 2-nonanone increased. Therefore the microstructure of the medium can influence the behavior of the aroma compounds in the food matrix, particularly because of the modifications of the protein at the lipid-water interfaces. This protein, β -lactoglobulin, is affected by the conformational changes; since the fixation of the ligands is more or less specific, the latter can be

modified when these structural changes occur. The absorption of β -lactoglobulin at the interface modifies not only the thermodynamical equilibria but also the transfer of the aroma compounds at the interface.

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