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Retention of aroma compounds by β-lactoglobulin in different conditions

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Abstract

This contribution to the study of the retention and the release of aroma compounds was conducted on model systems in the presence or absence of β-lactoglobulin. To know the interactions between the aroma compounds and this protein we studied the hydration of the protein and the effect of water activity on the sorption of the volatiles. It has been shown that water is necessary in the formation of aroma–protein interactions. The presence of β-lactoglobulin in aqueous solution increased the retention of the four aroma compounds considered. They modify the flux at the interface air–aqueous solution. These results could be applied in the technology of foods such as emulsions which could be stabilized with proteins, and particularly in dairy products which are generally flavored. © 2002 Published by Elsevier Science Ltd.

Keywords: Aroma compounds; Transfer; Interface; β-lactoglobulin; Hydration; Sorption

1. Introduction

The knowledge of the distribution of aroma compounds between the different phases of the food product at equilibrium is not enough to describe the phenomena involved either during the process of their release or during mastication in the mouth. The volatile compounds are generally lipophilic and, before being released in the vapor state, they have to transfer through several interfaces such as the interface between the lipidic and aqueous phases or between the liquid and the vapor phases. The mass transfer at the different interfaces in a multicomponent system must be taken into account. In biphasic media two steps are necessary to consider for the perception of the aroma: the transfer from the lipidic phase to the aqueous one and those from the aqueous phase to the vapor one. The product–air partition coefficient determines the quantity of the aroma compounds which can be released (De Roos, 1997). In foods, such as emulsion or foams, the interfacial area is generally very important and is stabilized with the help of an emulsifier which decreases the interfacial tension and prevents coalescence (Cayot & Lorient, 1998). The surface properties of proteins allow them to be absorbed at the air–water or water–lipid interfaces, thus decreasing the surface tension. This study deals with a protein, β-lactoglobulin, widely studied in different conditions and largely used in flavored dairy products. The hydration state of this protein modifies its ternary and quaternary structures. To appreciate the effect of β-lactoglobulin on the retention of aroma compounds, the water sorption isotherm was determined and the effect of water activity on the sorption of aroma compounds was studied. The role of β-lactoglobulin for the transfer of aroma compounds at the interfaces was also considered. In fact, the perception of food aroma is mainly determined by the concentration of volatile compounds released in the vapor phase more than the concentration in the food. That is why all the factors affecting this release may also modify the perception. Morris (1987) has observed a decrease of the aroma release in foods containing thickening agents and has attributed this phenomenon to the barrier effect of these macromolecules. The predominant factors playing a role in the release are the composition and the structure of the media. The use of macromolecules such as protein, having functional properties and aroma retention ability, allows us to study the release of volatile compounds in systems with the same composition but different structure. This is why the transfers and the retention of aroma compounds at the interface air–aqueous solutions in the presence or absence of β-lactoglobulin were determined at different pH values.

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2. Material and methods

2.1. Reagents

The aroma compounds, benzaldehyde, isoamyl acetate, *d*-linalool, 2-nonanone, were kindly given by International Flavors and Fragrances (I.F.F., Longvic-lès-Dijon, France). β-lactoglobulin was provided by Besnier-Bridel (Laval, France). Aqueous protein solutions (3%) were adjusted to pH values of 2, 3, 6 with HCl and to a pH value of 9 with NaOH.

2.2. Methods

2.2.1. Sorption

Using sorption measurements, the study of the protein–aroma compounds interactions can become evident as function of the state of protein hydration. In the first step, the water sorption isotherm of the protein was determined and secondly the sorption of the aroma compounds at low and high protein hydration was conducted at ambient temperature (25 °C). The measurements were repeated in duplicate. The sorption kinetics were controlled regularly at short intervals in order to confirm equilibrium.

2.2.1.1. Isotherm of water sorption of the protein (β-lactoglobulin). This isotherm was obtained using the method described by Jowitt and Wagstaffe (1989). The water content is measured in a sample placed in different relative humidity atmospheres and at a constant temperature. The relative humidities were fixed by saturated salt solutions (Table 1). The water content of the sample was determined by a gravimetric method: when the water sorption equilibrium was reached (constant mass of the sample), the sample is dried (104.0±0.5 °C during 24 h). After cooling at room temperature in dry atmosphere, the sample was weighed to determine its final mass, this is dry matter. The initial water content was calculated by difference.

2.2.1.2. Sorption of the aroma compounds by the protein. The sorption of the aroma compounds by β-lactoglobulin was determined at different water contents using a method derived from those described above (Jowitt and Wagstaffe, 1989). Depending on the water content, the technique used was different. For low water content, the measurements were directly measured on the protein powder. As for the high water content, 3% β-lactoglobulin aqueous solutions were used.

Table 1
Water activity of saturated salt solutions and of pure water at 25 °C

Saturated salt solutions	Water activity
LiCl–6 H_2O	0.111
CH_3COOK–1.5 H_2O	0.226
$MgCl_2$–6 H_2O	0.330
K_2CO_3–2 H_2O	0.438
$Mg(NO_3)_2$–6 H_2O	0.528
$SrCl_2$–6 H_2O	0.708
KCl	0.843
Pure water	1

2.2.1.3. Measurements at low water content. The quantity of aroma compounds adsorbed by the protein was obtained using a static method. The protein (0.2 g) and the aroma compounds were introduced in atmospheres of different water activities (0.11, 0.22, 0.33, 0.43). All the measurements were taken at constant temperature (25.0±0.5 °C). When the equilibrium was reached, the adsorbed aroma compound by the protein was quantified using the gas chromatographic technique (described below) after an solid–liquid extraction with hexane (density 0.658 at 20 °C; purity 98%) with an efficiency extraction upper than 99%.

2.2.1.4. Measurements at high water content. The sorption measurements of the aroma compounds in the protein solutions was conducted in dynamic conditions. The aqueous solutions of 3% β-lactoglobulin and a reference with distilled water (1 ml) were introduced in a thermostated cabinet (25.0±0.5 °C). A water and aroma compound saturated gas at a constant flow rate (30 ml min^{-1}) passed continuously through this cabinet (Fig. 1). The amount of adsorbed aroma compound was quantified by direct injection of a sample of the protein solution in the chromatograph. The quantity retained by he protein was the difference between the quantity adsorbed in the protein solution and the reference (distilled water).

2.2.2. Determination of the aroma flux

The kinetics of sorption of the aroma compounds allows us to determine the aroma flux at the interface of the vapor–aqueous solutions using the initial slope of these kinetics and considering the surface of the interface. All the experiments were conducted in open vessels (Fig. 1), the vapor phase was saturated in aroma compound and in water. Each aroma component was studied alone. Only the sorption experiments in pure water were realized with agitation of the solutions; it was not realizable in protein aqueous solutions because of the foaming.

2.2.3. Chromatographic analysis conditions

Quantitative analysis of the aroma compounds was done using a Chrompack CP 9000 chromatograph. Parameters for the gas chromatographic analysis were as follows:

1. Stainless-steel column (3 m length by 2.2 mm inner diameter) packed with Chromosorb W-AW, 100–120 mesh coated with 10% Carbowax 20 M.

2. Flow rates of carrier gas (N_2): 1.6×10^{-5} m^3/min; hydrogen: 2.5×10^{-5} m^3/min; and air: 25×10^{-5} m^3/min.
3. Flame ionization detector and injector temperatures were 200 and 190 °C, respectively.
4. Column temperature varied with the nature of the aroma compound (isotherms between 80 and 160 °C).

3. Results and discussion

The water sorption isotherm of β-lactoglobulin is given in Fig. 2. The behavior of this protein towards water was comparable with those of other proteins, notably of casein (Kinsella and Fox, 1986). At A_w values under 0.5, the water sorption did not exceed 10 g water per 100 g protein and at upper A_w values, the water sorption increased exponentially, even for weak changes in A_w values. On this curve, three parts could be distinguished: the first one located for A_w values under 0.25. In this region, Kinsella and Fox (1986) had quantified the water content of the hydration monolayer of β-lactoglobulin and have found a value of 6.7 g per 100 g protein. The second zone was situated between 0.25 and 0.7 A_w values. This region corresponded to a transition region between dry and humid states. Cayot and Lorient (1998) had found a total water quantity of about 18–25 g per 100 g of dry casein. Here, for β-lactoglobulin, at A_w value of 0.7, the water sorption did not exceed 15 g per 100 g dry protein (Fig. 2). The last region was located for A_w values higher than 0.75. The measured water content at A_w values of 0.84 and 0.99 were, respectively, equal to 20 and 40 g per 100 g of protein. This last result was in agreement with those of Kinsella and Fox (1986) who observed a water sorption equal to 40 g per 100 g of β-lactoglobulin using a calorimetric method. These authors had also shown that the surrounding conditions, particularly the pH value,

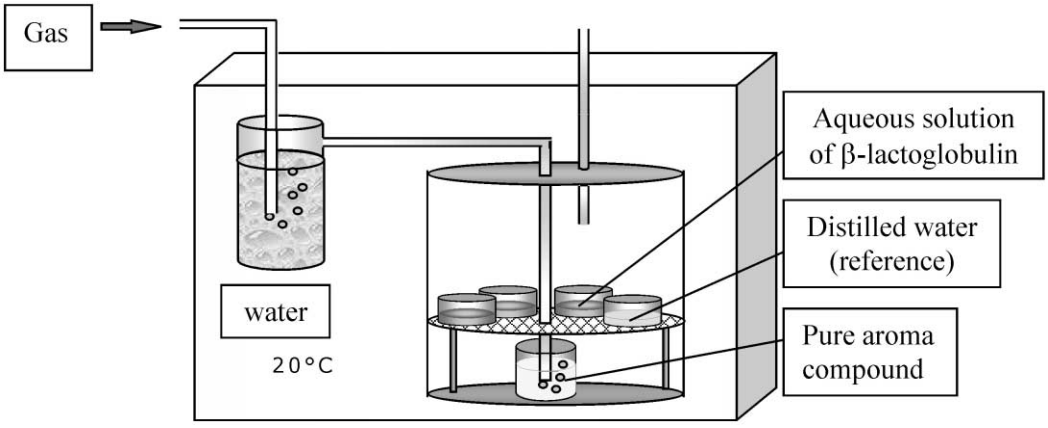
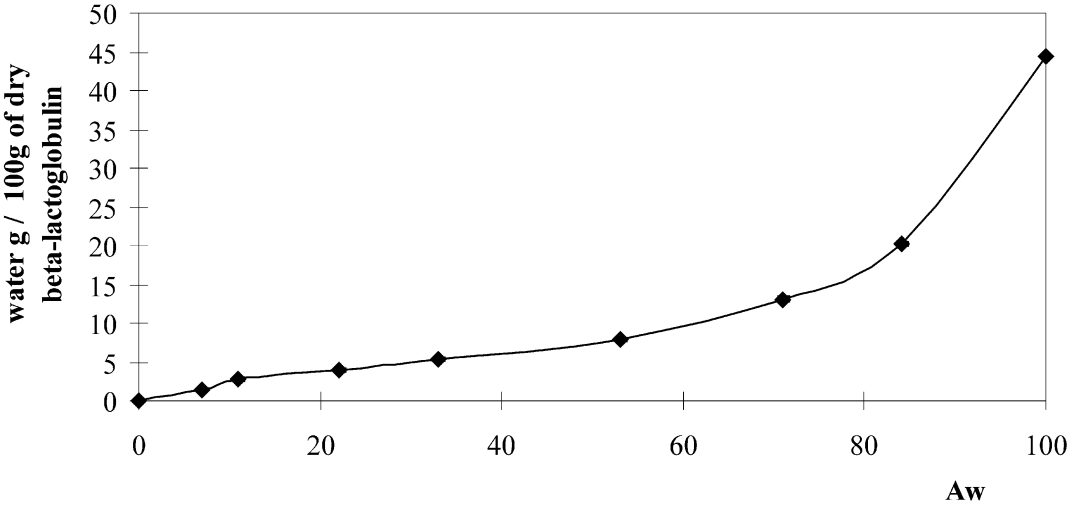


Fig. 1. System used to measure the aroma compounds sorption by the different aqueous protein solution.



standard deviation <0.2

Fig. 2. Water sorption isotherm of β-lactoglobulin at 25 °C.

may have an influence on the water sorption by the protein. They observed that β-lactoglobulin retained 40 and 60 g of water per 100 g of protein when it is respectively on dimeric and octameric conformations. Under our conditions (the aqueous protein solutions had a pH value = 3.4), the protein was present under a mixture of monomeric and dimeric forms (Casal, Köhler, & Mantsch, 1988). The adsorbed water quantity was close to that reported for the dimeric forms.

The determination of the water sorption isotherm of β-lactoglobulin indicates the conditions which permit the variation of the A_w while the water content of the protein is constant. For low A_w values (0.11, 0.22, 0.33, 0.43) the water content is quite stable (3–6 g/100 g of dry protein). The aim was to study the effect of A_w on the sorption of aroma compounds by the protein. The determination of the sorption of aroma compounds in aqueous solutions of protein allows us to compare the results at low and high water content. Two aroma compounds, 2-nonanone and *d*-linalool, were chosen for this study because of the differences in their physico-chemical characteristics (Table 2), particularly their vapor–liquid partition coefficients (K_{mol}) and activity coefficients (γ_i). The vapor–liquid partition coefficient represents the volatility of the aroma compounds in the medium and the activity coefficient shows the existence or the absence of interactions between the aroma and the medium. The partition coefficient is dependent on the water solubility and on the saturated vapor pressure of the aroma compounds.

After the production of the water sorption isotherm of β-lactoglobulin, the sorptions of the aroma compounds by the protein were studied. The adsorbed quantities of *d*-linalool and 2-nonanone, when the protein was not very hydrated, were very low [around 5–15 mg of aroma compounds per 100 g of dry protein (Fig. 3)]. In this region (between 0.11 and 0.43 A_w values), the quantities of adsorbed aroma compounds did not significantly vary. For constant water content, the sorption was not affected by water activity. Voilley (1975) had shown values in the same order of magnitude in the study of octanol sorption by sugars placed at low A_w values. Landy, Fares, and Voilley (1997) have found more important values in the study of aroma sorption by sodium caseinate (around 1 g/100 g of protein). These results show the importance of the nature of the substrate on the sorption of the aroma compounds at low A_w values.

The retention of 2-nonanone and *d*-linalool was also studied for A_w values close to 1 (the protein solution). The results are presented in Table 3 and are compared with the water sorption isotherm of β-lactoglobulin. When the water content was strongly increased, the sorption of the two aroma compounds by the protein increased significantly. Indeed, at low water content, the sorption of these two aroma compounds was in the order of 10 mg per 100 g of dry protein, whereas in solution the sorption reached 400 mg of aroma compound per 100 g of protein. The retention of aroma was multiplied 40 times, while the water sorption was 100 times more important than that of the aroma compounds.

These results illustrate the importance of the role of water on the interactions between aroma compounds and β-lactoglobulin. An explanation for the increase in the sorption of the aroma compounds in high water content may be found in the structural modifications undergone by the substrate during hydration. At weak water content, the protein was more rigid. However, when the water sorption was increased, a plasticization effect began to take place. This phenomenon led to a more open protein structure, and therefore, increased susceptibility to interactions with other molecules, such as aroma compounds. When the protein is in solution, we have to consider two phenomena: the dissolution of the aroma compounds in water, and following this, the interactions of the aroma compounds with the protein. These interactions depend on the intrinsic characteristics of the solutions, for example, pH. β-lactoglobulin is very sensitive to conformational changes (Pessen, Purcell, & Farrell, 1985; Timasheff, Mescanti, Basch, & Townend, 1966); the fixation of the ligands, aroma compounds in this study, could be affected by these changes (Jouenne, 1997). In order to describe the role of β-lactoglobulin on the aroma compound transfer at the interface air–aqueous solutions, we have determined the sorption kinetics in water of four aroma compounds (2-nonanone, *d*-linalool, benzaldehyde and iso-

Table 2
Physico-chemical characteristics of the aroma compounds at 25 °C

	Vapor–liquid partition coefficient (K_{mol})	Activity coefficient (γ_i^{∞})	Saturated vapor pressure [P_i^s (Pa)]	Hydrophobicity (log *P*)	Water solubility (g l^{-1})
d-Linalool	2.3	8631	27	3.5	2.6
2-Nonanone	33.6	58 036	59	2.9	0.4
Benzaldehyde	1.7	1436	120	1.5	7.1
Isoamyl acetate	36.3	5016	733	2.2	2.4

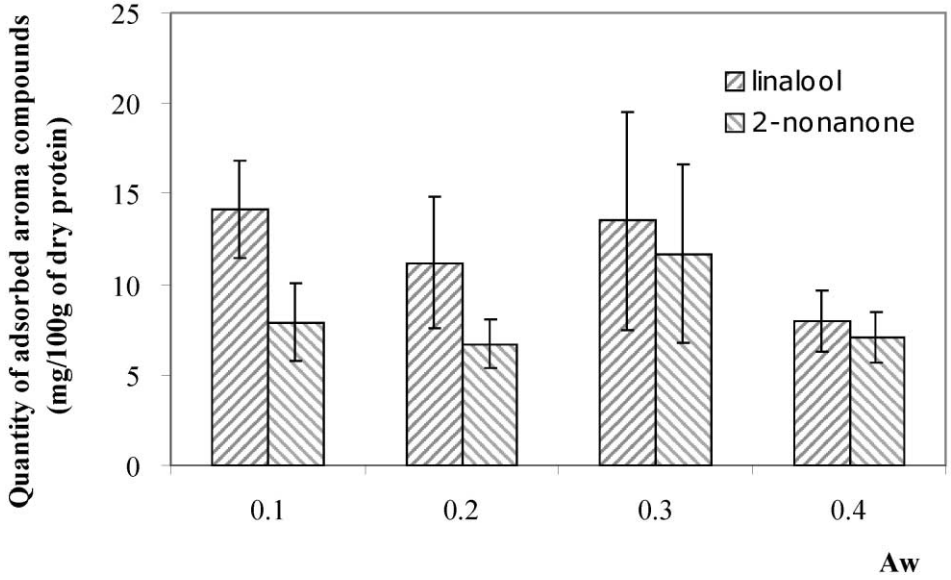


Fig. 3. 2-Nonanone and *d*-linalool adsorbed quantities by β-lactoglobulin at different relative humidities (25 °C).

amyl acetate). These aroma compounds were chosen due to their physico-chemical characteristics (Table 2), particularly their water solubilities. Isoamyl acetate and 2-nonanone have relatively high volatilities ($K_{mol} = 36.3$ and 33.6, respectively), while benzaldehyde and *d*–linalool have low volatilities in aqueous solution ($K_{mol} = 1.7$ and 2.3, respectively). The kinetics are discussed in terms of affinity and concentration differences. The transfer of the aroma compounds at the interface air–aqueous solution was studied in presence or in absence of β-lactoglobulin. The transfer coefficient depends on several parameters, and particularly on diffusion coefficient in the medium.

The kinetics of sorption of the aroma compounds (benzaldehyde, isoamyl acetate, *d*-linalool, 2-nonanone) in water are shown on the Fig. 4. The kinetics of sorption in water varied according to the aroma compound and the initial transfer rate was dependent on the nature of the volatile. The aroma flux at the interface vapor–water was calculated from the initial slope and is given in Table 4.

Table 3
Sorption of aroma compounds and water by β-lactoglobulin at 25 °C[a]

A_w	Aroma compounds adsorbed by β-lactoglobulin (mg/100 g)		Water adsorbed by β-lactoglobulin (g/100 g)
	d-Linalool	2-Nonanone	
0.11	14.1 (2.7)	7.9 (2.1)	2.8 (0.2)
0.22	11.2 (3.6)	6.7 (1.3)	3.9 (0.2)
0.33	13.5 (6.0)	11.7 (4.9)	5.3 (0.2)
0.43	8.0 (1.7)	7.1 (1.4)	nd
0.53	nd	nd	7.9 (0.2)
0.99	409.0 (48.0)	373.0 (4.0)	44.5 (3.9)

[a] Standard deviation indicated in parentheses. nd, not determined.

The flux of a solute, per surface unit, is proportional to the difference in concentration between the two phases. The initial flux would be favored by a large initial concentration. The initial concentration of the compound corresponds to that of the vapor phase which is saturated in water and in aroma compounds. Its behavior is similar to that of saturated vapor pressure P_i^s (Table 2).

The higher vapor phase concentrations and higher saturated vapor pressures observed in the case of isoamyl acetate and benzaldehyde explains their greater flux. Indeed the benzaldehyde vapor concentration was at least seven times higher than that of the other compounds studied. However, in the cases of 2-nonanone and *d*-linalool, the difference of concentrations did not sufficiently explain their aroma flux at the interface air–water: 2-nonanone, with a concentration 1.5 times higher than that of *d*-linalool, had a flux at interface vapor-water three times lower. Their close diffusion coefficients (6.6×10^{-10} and 5.8×10^{-10} m^2 s^{-1} for 2-nonanone and *d*-linalool, respectively) were not sufficient to explain this behavior. On the hand, the water solubility value, that represents the affinity of the volatile for the medium, was 6 times higher for *d*-linalool than that observed for 2-nonanone (Table 4). Since *d*-linalool has a higher affinity for water than 2-nonanone, it shows a higher retention in the aqueous medium and the flux at the interface air–aqueous solution is then lower.

After studying the transfer of these four aroma compounds at the air–water interface, we have introduced β-lactoglobulin into the solution and have observed the role of this protein. The transfer of the aroma compounds at the air–aqueous interface of the protein solution was also studied as a function of pH.

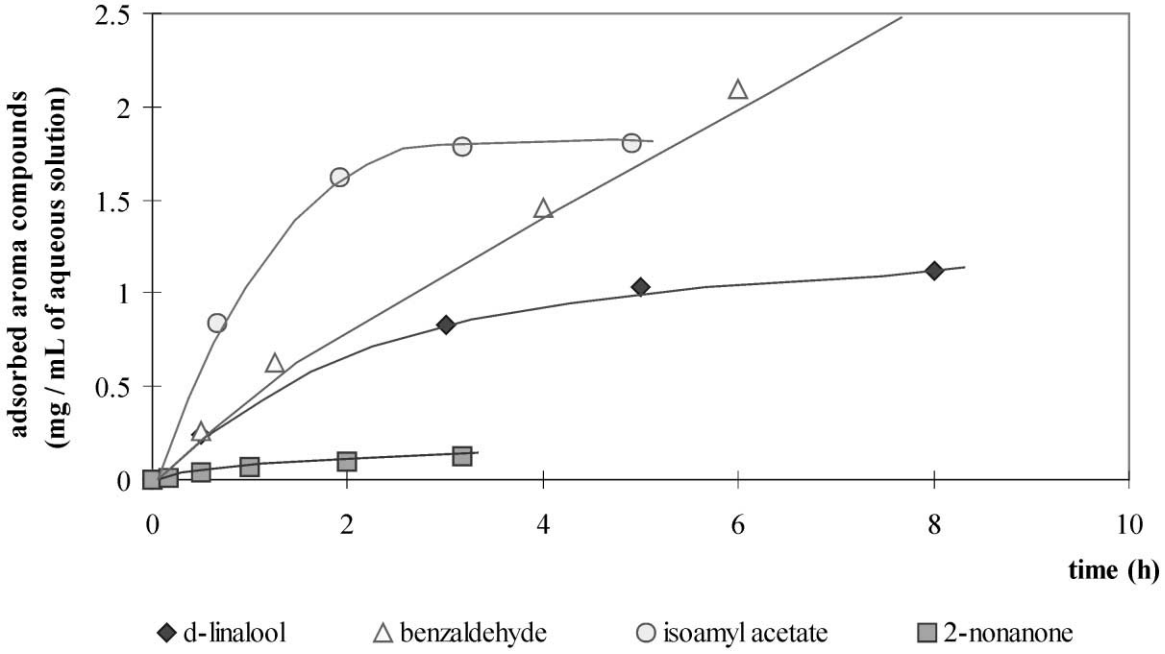


Fig. 4. Aroma compounds sorption kinetics in water at 20 °C (with agitation of the liquid phase).

The sorption kinetics of the aroma compounds in aqueous solutions with and without the protein, at various pH are presented in Figs. 5–8 for benzaldehyde, isoamyl acetate, *d*-linalool and 2-nonanone, respectively. Only the curves corresponding to the kinetics of sorption of the aroma compounds in water were drawn to better differentiate between those obtained in presence of the protein. For the four volatiles, the aroma compound quantities sorbed in water as a function of time were lower than those in the protein solutions. Then the presence of β-lactoglobulin in the aqueous phase modified the aroma compound sorption behavior. At the equilibrium we observed a retention rate of these four aroma compounds in decreasing order: 2-nonanone (39–49% upon the pH value), *d*-linalool (12–25%), benzaldehyde (6–20%) and isoamyl acetate (6–13%). The same order was found for the transfer kinetics. However, the influence of β-lactoglobulin on the sorption kinetics of the aroma compounds was greater in the case of 2-nonanone.

The initial aroma flux (calculated from the initial slope of the kinetics drawn in Figs. 5–8) at the air–water interface in presence or in absence of β-lactoglobulin at various pH values are given on the Table 4. The transfer rates at the air–aqueous interface solutions presented by the four aroma compounds were higher in the presence of protein compared with those in pure water. However, these four compounds did not display the same behavior with the addition of 3% β-lactoglobulin. For benzaldehyde and isoamyl acetate, the flux at the air–aqueous interface solution was fairly constant or slightly higher compared with those in pure water, whatever the pH value. *D*-linalool presented higher values (except at pH 9) in presence of the protein. The most affected aroma compound was 2-nonanone where the deviation, whatever the pH, reached high value (until 63% at pH 9).

Table 4
Aroma flux at the vapor–water interface and concentration in the vapor phase of the aroma compounds (20 °C)

Aroma compound	Aroma flux at the vapor water interface (mg h^{-1} cm^{-2})	Concentration in the vapor phase (μl ml^{-1})
d-Linalool	2.4	0.09
2-Nonanone	0.8	0.15
Benzaldehyde	3.8	1.68
Isoamyl acetate	9.5	12.21

Espinosa (1999) has observed that the increase of aroma flux at the interface in the presence of β-lactoglobulin was greater when the compound is better retained by the protein. This is particularly the case for *d*-linalool and 2-nonanone. This result shows the influence of the affinity of the solute for the aqueous phase on the flux. Furthermore, for a given compound, the initial concentration was always the same whatever the aqueous phase, as it is dependent on the saturation of the aroma in the vapor phase (Table 5).

Although protein conformations are expected to change with the pH (Casal et al., 1988; Jouenne, 1997; Kella, & Kinsella, 1988; Pessen et al., 1985; Shimizu, Saito & Yamauchi, 1985), no significant difference was recorded. This may be due to the relatively weak affinity changes between the aroma compounds and the protein at different pH values compared with the effect of the presence or absence

of β-lactoglobulin. The foaming properties of β-lactoglobulin may have a barrier effect on the transfer of the aroma compounds when the protein is absorbed at the air–water interface. Marin (1998) has observed changes in the foaming properties of β-lactoglobulin in the presence of aroma compounds. In the present study, the transfer behavior of these molecules could also be modified.

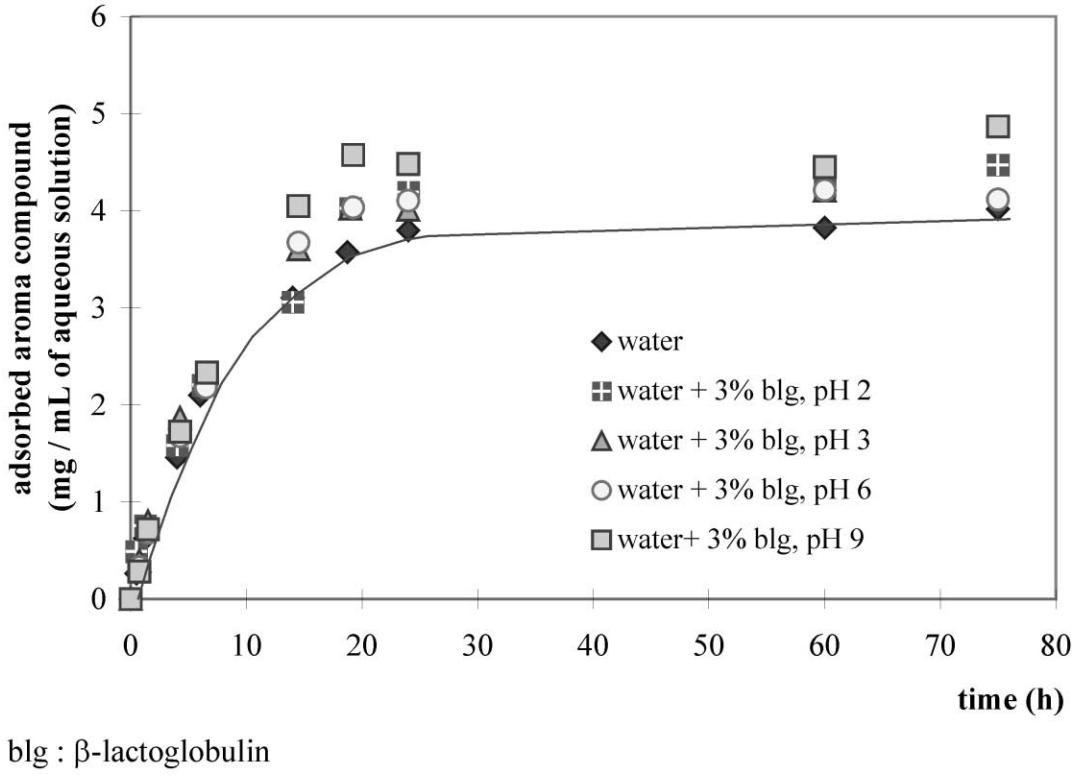


Fig. 5. Sorption kinetics of benzaldehyde in water with or without β-lactoglobulin (20 °C).

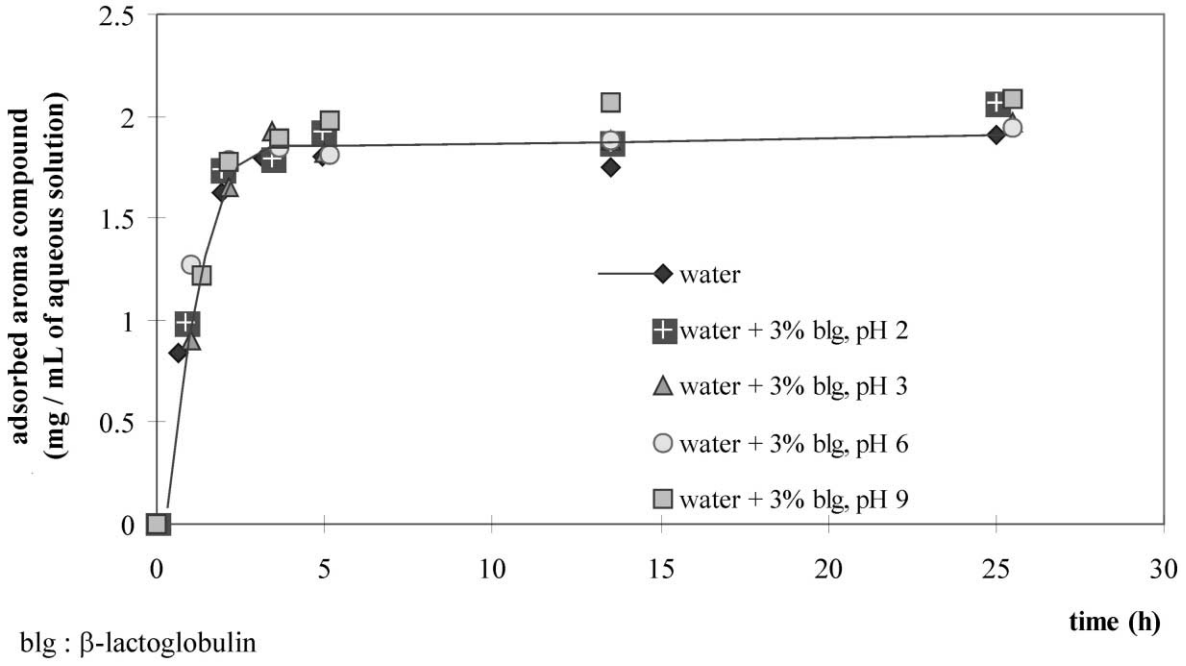


Fig. 6. Sorption kinetics of isoamyl acetate in water with or without β-lactoglobulin (20 °C).

4. Conclusion

The retention of the aroma compounds by the food matrix, especially by β-lactoglobulin, was studied in simple systems, taking into account the protein hydration and the pH value. These factors have an influence on the protein conformation. The hydration effect of β-lactoglobulin on the aroma retention was shown. At low water content, high A_w value variations did not alter the binding ability of aroma compounds on the protein. When the protein is highly hydrated (in solution), the aroma retention is 40 times higher than when the water content of the protein is less than 6%. The presence of β-lactoglobulin in the aqueous phase influenced the retention of the four aroma compounds, with a greater effect in the case of 2-nonanone. The

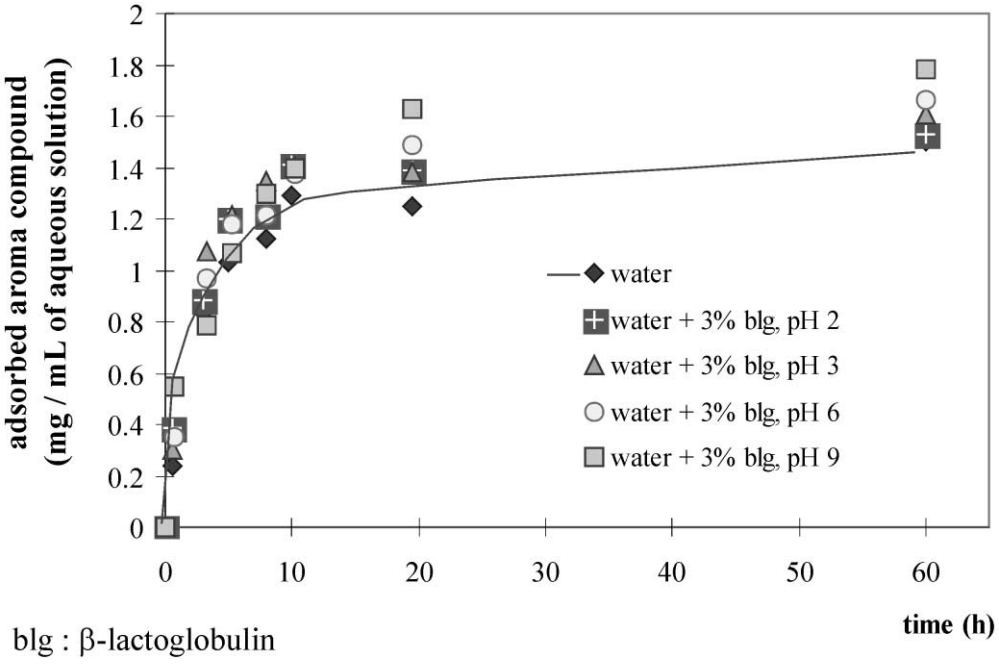


Fig. 7. Sorption kinetics of *d*-linalool in water with or without β-lactoglobulin (20 °C).

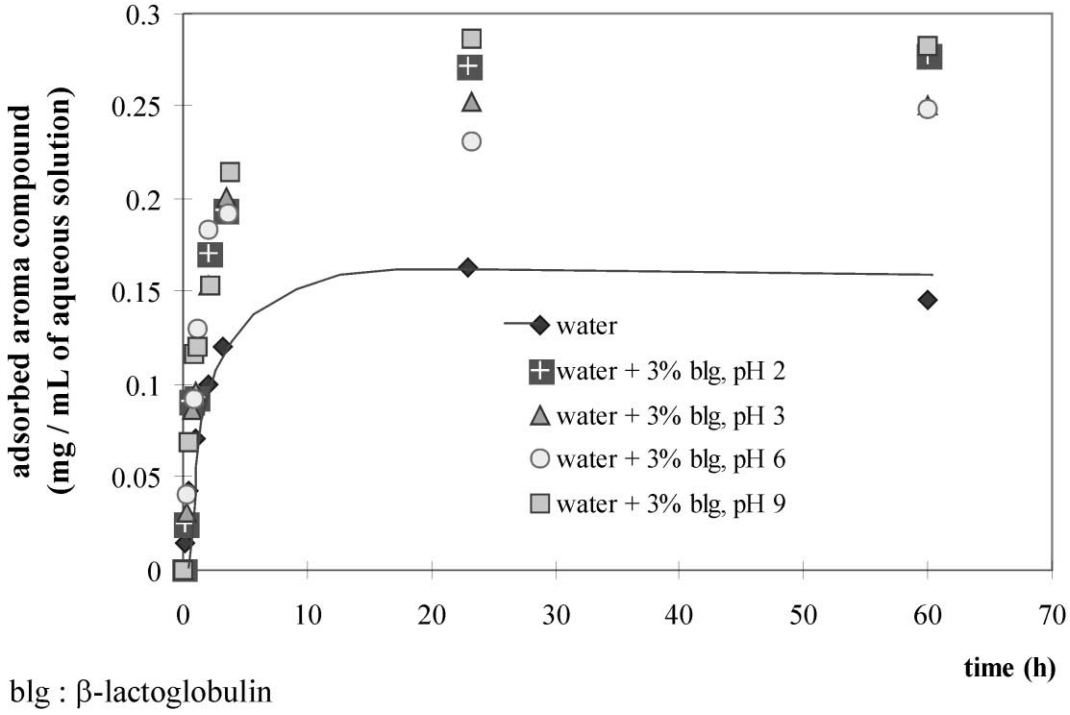


Fig. 8. Sorption kinetics of 2-nonanone in water with or without β-lactoglobulin (20 °C).

Table 5
Aroma flux at the vapor–aqueous solutions interface with or without β-lactoglobulin at various pH, 20 °C (mg h^{-1} cm^{-2})

Aroma compound	Water	3% β-Lactoglobulin aqueous solutions			
		pH 2	pH 3	pH 6	pH 9
Benzaldehyde	3.8	4.0 (+5)	4.1 (+8)	3.8 (0)	4.0 (+5)
Isoamylacetate	9.5	10.1 (+6)	9.4 (−1)	9.6 (+1)	9.3 (−2)
d-Linalool	2.4	2.7 (+13)	2.8 (+17)	2.6 (+8)	2.4 (0)
2-Nonanone	0.8	1.2 (+50)	1.0 (+25)	1.2 (+50)	1.3 (+63)

Deviation compared with the aroma flux at the vapor–pure water interface is indicated in parentheses. deviation = [(flux in presence of protein − flux in water)/flux in water] × 100.

study of the flux at the air–water interface has shown that the presence of protein induces an increase of the flux of *d*-linalool and 2-nonanone from water phase towards vapour phase compared to those obtained without protein. This flux appeared to be independent of the pH value in spite of the changes of the protein conformations.

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