Physicochemical Interactions Between Aroma Compounds and Milk Proteins: Effect of Water and Protein Modification

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ABSTRACT

The physicochemical interactions between aroma compounds and sodium caseinate were studied by complementary techniques involving the protein in aqueous solution at 25 or 75 g/L (exponential dilution and equilibrium dialysis) or in a solid state (sorption and infrared spectroscopy). No retention of acetone, ethyl acetate, and 2-propanol in aqueous solutions was found by exponential dilution and equilibrium dialysis. Diacetyl and benzaldehyde interacted with sodium caseinate through strong and weak bonds, as found by equilibrium dialysis. The results obtained by sorption differ from those obtained in aqueous solutions. The compounds that sorbed best to sodium caseinate were acetone and ethyl acetate, and their infrared spectra showed a negative shift of the carbonyl peak between the pure and sorbed state of the compounds, demonstrating their involvement in hydrogen bonds with the protein. In addition, 2-propanol and ethyl acetate were strongly bound to sodium caseinate in a dried state. For the other compounds, results from infrared spectroscopy indicated the presence of interactions between sodium caseinate and the ligand.
(**Key words**: aroma, casein, water, interactions)

INTRODUCTION

Interactions of aroma compounds with nonvolatile constituents can lead to decreased aroma perception and reduce consumer acceptability of food products (4, 8, 12, 15). In particular, the binding of flavor components by proteins can make it difficult to determine the appropriate amount of flavoring for food formulation. The factors contributing to protein binding need to be understood, and the nature of the interactions between aroma and protein needs to be established.

Bovine sodium caseinate, a well-characterized protein, was chosen as a useful model for investigating the interactions between aroma and protein (22). Products containing casein are widely used in the food industry because of their contribution to desirable flavor and because of their emulsifying, whipping, and hydrating properties, which impart desired viscosity characteristics (15). In low fat dairy desserts, sodium caseinate can serve as a fat replacement (1). Few studies (31) have reported on the behavior of aroma compounds in the presence of sodium caseinate.

Results of aroma retention, the quantification of the interactions between aroma and protein, and the determination of the nature of those interactions have many applications in the food industry. Voilley (35) used those data to study the role of physicochemical interactions between volatiles and different substrates in the aroma retention during drying and showed that these interactions could reduce the volatility of the aroma molecules contained in the encapsulating media.

The physicochemical behavior of the food constituents is controlled by their interactions with the solvent (i.e., mainly water); the latter may modify the reactivity of some protein sites (19). Le Thanh et al. (21) showed that, when the equilibrium relative humidity was raised from 11 to 90%, the quantity of sorbed volatile compounds increased from 5 to 93%. Modification of proteins by chemical treatment can also change the binding properties of the protein. Many previous works have dealt with the relationship between the conformational changes of proteins and their interactions with aroma components, including bovine serum albumin (5), soybean proteins (6), or

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β-LG (7, 29); those published studies demonstrate that this treatment in general affects both the binding affinity and the binding capacity of proteins for aroma compounds.

This study investigated the effect of protein hydration, pH, and ionic strength on the interactions between aroma and protein and the interactive properties of native and modified sodium caseinate. The study of the interactions between aroma and protein was carried out by complementary methods to demonstrate the retention of aroma components by sodium caseinate and to quantify and characterize the interactions between aroma and protein.

MATERIALS AND METHODS

Materials

Casein was purchased from Prospérité Fermière (Arras, France), and the reagents (reducing carbohydrates and sodium cyanoborohydride) were purchased from Merck (Darmstadt, Germany). The volatile compounds, acetone, diacetyl, benzaldehyde, ethyl acetate and 2-propanol were also obtained from Merck. Table 1 gives the physicochemical properties of these compounds.

Methods

Preparation of sodium caseinate. Casein was solubilized in distilled water at pH 7 with NaOH. The casein solution was filtered and precipitated at isoelectric pH 4.6 with HCl. The precipitated fraction was washed twice by distilled water. This procedure was executed twice, resulting in a total of four washings of the precipitate. The casein was filtered under vacuum (filter porosity of 114 mm), solubilized in distilled water at pH 7 with NaOH, and then freeze-dried during 48 h from a protein solution at 50 g/L. Visual examination of polyacrylamide gel electrophoretograms showed that no proteolysis occurred during freeze-drying. Sodium caseinate was stored at 5°C in a closed container until use.

Chemical modification of sodium caseinate solution. The glycosylated sodium caseinate was prepared according to methods of Lee et al. (18), using different reaction times to obtain a range of modification degrees. Freeze-dried sodium caseinate (30 g) and $NaCNBH_3$ (4 g) were dissolved in 300 ml of 0.2 *M* potassium phosphate buffer (pH 8.0). Monosaccharides (15 g) or disaccharides (30 g) were mixed, which corresponded to a molar ratio of carbohydrate to lysyl residues of 6. The degrees of glycosylation were highest after 120 h of coupling reaction at pH 8.0 and 37°C under agitation. The solutions were dialyzed once against 1.0 *M* NaCl and three times against distilled water and then freeze-dried. The determination of the modification degree consisted of a reaction between the unreacted ε-amino groups of the lysyl residues and 2,4,6-trinitrobenzenesulfonic acid. The derivatives absorb at 335 nm. The degree of glycosylation (DG) is given by

$$\text{DG (percentage)} = \frac{(A_C - A_a)}{A_a} \times 100 \quad [1]$$

where A_c = absorbance of unmodified sodium caseinate solution (control), and A_a = absorbance of modified casein solution (assay). This degree of glycosylation represents the percentage of glycosylated lysyl residues. For the bound carbohydrates—galactose, maltose, glucose, lactose, and fructose—the degree of glycosylation is 70, 54, 45, 43, and 13%, respectively.

Characterization of the sodium caseinate solution. The quantity of NaCl determined by electric conductivity was 3.5% (m/v). The pH of sodium

TABLE 1. Physicochemical properties of aroma compounds.

Aroma compound	p_i^s at 25°C[1]	Aqueous solubility	Hydrophobicity (log P)[2]
	(mm Hg)		
Acetone	231.2	Very soluble	–0.3
Diacetyl	57.6	250 g/L at 15°C	–2.0
Benzaldehyde	0.7	7.1 g/L at 25°C	1.5
Ethyl acetate	94.6	86 g/L at 20°C	0.6
2-Propanol	42.5	Very soluble	0.1

[1]Value of saturated vapor pressure calculated by the Antoine equation.

[2]Value of the logarithm of the partition coefficient between water and n-octanol calculated by the Rekker method (30).

caseinate solution was 7 for a protein concentration from 25 to 100 g/L (m/v); at this pH, 100% of the protein was soluble (11).

Measurement of the equilibrium between vapor and liquid. Exponential dilution was used to determine the equilibrium between vapor and liquid of the aroma compounds. This method consists of exhausting the liquid phase of volatile compounds in equilibrium with the vapor phase (20). An inert gas (helium) passed through the liquid phase (130 g of solution containing a molar fraction of less than 5.10^{-4} volatile compound) and carried the volatile compound into the headspace. The system was maintained at 25°C by a thermostat. A sample of the vapor phase was automatically injected into a gas chromatograph at regular intervals. The variation of the chromatographic peak area of the solute is an exponential function of time, provided the detector response is linear:

$$\log S = \log S_0 - \frac{p_i^s d}{RTN} \times \gamma_i^{\infty} t \qquad [2]$$

where S and S_0 = volatile peak areas, p_i^s = vapor pressure of pure compound i (Pascals), d = carrier gas flow rate (cubic meters per minute), R = gas constant (R = 8.314 J/K per mol), T = temperature (Kelvin degrees), N = number of moles of the liquid phase, γ_i^{∞} = activity coefficient of compound i, and t = time (minutes).

The activity coefficient was calculated from the values of the slope of the straight line obtained by plotting log S against time and represents the tendency for intermolecular interactions to develop between the component i and the major constituents of the liquid. The Henry constant, H_i, expresses volatility of aroma compounds and is calculated as

$$H_i = \gamma_i^{\infty} p_i^s. \qquad [3]$$

The particular device used allows the investigation of the equilibrium of viscous solutions (≤1000 mPa.s) and foaming systems such as aqueous solutions of proteins (33).

Measurement of binding. The interaction of the aroma compounds with native or glycosylated sodium caseinate was studied by an equilibrium dialysis method. The aqueous phase of protein and aroma compound were dialyzed at 25°C against distilled water in dialysis sacs. The volume of dialysis water was 50 times higher than that of the dialysis sacs. At equilibrium, the dialysis sacs were weighed to determine the final weight of the aqueous solution of sodium caseinate. Then, the concentrations of aroma compounds in the dialysis sacs and the dialysis external liquid were determined by gas chromatography. These data were analyzed by using the Scatchard equation (32),

$$\frac{\nu}{[L]} = K_a(n - \nu) \qquad [4]$$

where ν = number of moles of ligand bound per mole of protein, [L] = molar concentration of free ligand at equilibrium (moles per liter), K_a = intrinsic affinity constant, and n = number of binding sites per protein molecule. The Gibb's free energy of binding (ΔG), which is associated to the interaction between aroma and protein, was calculated from Equation [5].

$$\Delta G = -RT\ln K_a \qquad [5]$$

where T = temperature (Kelvin degrees), and R = gas constant (R = 1.987 cal/K per mole). The molecular mass of sodium caseinate is equal to, or greater than, 23,000 g/mol whether or not the protein is glycosylated. The measurements of binding were repeated twice. For diacetyl, the procedure of Wilkinson (36) was used to generate standard error terms for the number of binding sites, n, and the binding constant, K_a.

Chromatographic analysis of aroma compounds. A gas chromatograph (model 427; Packard, Downers Grove, IL) equipped with a flame ionization detector and an integrator (Hewlett-Packard 3380A; Avondale, PA) were used. The gaseous (exponential dilution) or liquid (equilibrium dialysis) samples were injected onto a 3-mm × 0.7-m Porapack Q column (Alltech, Deerfield, IL). Gas flow rates were as follows (output of the column at 160°C): helium, 65 ml/min; hydrogen, 56 ml/min; and air, 280 ml/min. The column temperature varied with the nature of the aroma compound. The injector and detector temperatures were 200 and 250°C, respectively, in exponential dilution and equilibrium dialysis experiments.

Preparation of complexes between aroma and protein for infrared spectroscopy. The preparation of the complexes was based on the procedure used by Maier (23). Sorption of the aroma compounds to sodium caseinate was monitored using static gravimetry in a dessicator containing silica gel to maintain a low and constant water activity. After equilibration, the quantity of the sorbed aroma compounds was determined by gas chromatography under the same

conditions as described previously. These measurements were made once before the use of the complexes for the infrared analysis. The sorption kinetics were controlled regularly at short intervals until the saturation of the protein in aroma compounds; this procedure permitted the equilibrium to be confirmed.

Infrared measurements. Infrared spectra of the aroma compounds and those of sodium caseinate, with or without aroma compounds, were obtained by using, respectively, single-beam and double-beam spectroscope (Perkin Elmer 580 B 5; Beaconsfield, England). The KBr pellets were prepared by mixing 2 mg of protein, with or without volatile compound, and 200 mg of KBr.

RESULTS AND DISCUSSION

Equilibrium Between Vapor and Liquid of Aroma Compounds

The equilibrium between vapor and liquid of the aroma compounds was first studied to determine the effect of sodium caseinate on their retention. The activity coefficient and Henry constant of the aroma compounds in water and in aqueous solutions of sodium caseinate at 25 and 75 g/L are given in Table 2. The activity coefficients in water decreased as follows: benzaldehyde, ethyl acetate, diacetyl, 2-propanol, and acetone. This variation was due to the physicochemical properties of the aroma compounds (Table 1) and, particularly, to their aqueous solubility; as the logarithm of the partition coefficient between water and n-octanol (log P) increased (i.e., the more apolar the molecule), its solubility decreased, but its activity coefficient increased. For acetone and 2-propanol, γ_i^∞ is 7.1; this result indicated that the presence of a carbonyl or alcohol group did not induce different interactions between the aroma molecule and water. However, the activity coefficient varied between acetone and diacetyl, which showed that interactions differed between these ketones and water.

The presence of sodium caseinate at 25 and 75 g/L did not affect the activity coefficients of acetone and ethyl acetate in relation to those of these compounds in water. For diacetyl, 2-propanol, and benzaldehyde, the activity coefficient varied as the concentration of sodium caseinate varied; for diacetyl and 2-propanol, the ratio γ_P/γ_W was higher than 1, which reflects the release of both molecules from sodium caseinate. However, benzaldehyde was retained by the protein, indicating the importance of the nature of the compounds and proteins on the aroma volatility. These results agree with those in the literature (16, 17, 24).

The effect of pH on the interactions between acetone and sodium caseinate in aqueous solution at 75 g/L was investigated. Acetone was considered a model compound to study binding as a function of the protein conformation; acetone has been used in the presence of substrates such as amino acids (25), casein (21), and saccharides and polyethylene glycol (34). At pH 3, the activity coefficient of acetone increased ($\gamma_P = 40.5$), which led to $\gamma_P/\gamma_W = 5.7$; at pH 7 and 10, γ_P did not vary significantly ($P \geq 0.05$). At pH 3, the release of acetone should be due to the overall positive charge of the protein and to the attractive forces among protein chains. From pH 7 to 10, the protein is increasingly unfolded and has an overall negative charge at pH 10; this changing struc-

TABLE 2. Henry constant (H_w)[1] and activity coefficients (γ_w and γ_p) of aroma compounds in water and sodium caseinate aqueous solutions.[2]

Aroma compound	Water		Sodium caseinate solution			
			At 25 g/L		At 75 g/L	
	H_w	γ_W	γ_P	γ_P/γ_W	γ_P	γ_P/γ_W
Acetone	2.2	7.1	6.8[a]	1.0	7.7[a]	1.0
Diacetyl	1.1	14.0	50.3*[,b]	3.6	93.9*[,a]	6.7
Benzaldehyde	1.4	1456.8	1864.6*[,a]	1.3	607.2*[,b]	0.4
Ethyl acetate	8.0	64.2	65.4[a]	1.0	66.8[a]	1.0
2-Propanol	0.4	7.1	10.4*[,b]	1.5	18.3*[,a]	2.6

[a,b]The values of activity coefficients for a given compound without a common superscript differ ($P < 0.05$).

[1]w = Water; p = sodium caseinate.

[2]At least three replicates were made.

*The activity coefficient in sodium caseinate solutions is significantly different from that in water ($P < 0.05$).

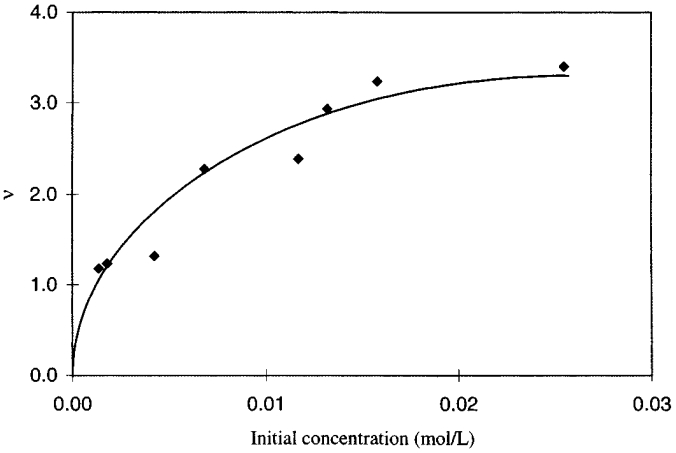

Figure 1. Binding curve of diacetyl in aqueous solution of sodium caseinate (75 g/L); ν = number of moles of ligand bound per mole of protein.

ture did not significantly affect the values of the activity coefficient for acetone. Hence, the unfolding of the protein did not create any binding sites for acetone; this binding would involve mostly interactions of physical nature, such as van der Waals interactions. The relationship between the aroma binding and the pH of the protein system has been investigated with other proteins, including bovine serum albumin (3), caseins (10), and whey proteins (27).

Binding Characterization

The determination of the binding capacity and affinity of sodium caseinate to aroma compounds by equilibrium dialysis was aimed at establishing the measurable binding properties of the protein. The binding curve of diacetyl obtained from equilibrium dialysis data at 25°C is given in Figure 1. The number of moles of ligand bound per mole of protein increased with the concentration of free ligand and maximum value tended to be 3.4 moles of diacetyl bound/mol of protein, corresponding to the saturation of the binding sites of the protein. The Scatchard plot exhibited two different slopes, suggesting the presence on sodium caseinate of two groups of binding sites for diacetyl (Figure 2). The values for the interaction between diacetyl and sodium caseinate indicated that the protein presented binding sites of high affinity (number of binding sites n_1 = 1.40 ± 0.04; intrinsic affinity constant K_{a1} = 4214.2 ± 975.6; ΔG = –4.92 kcal/mol) and weak affinity (number of binding sites n_2 = 4.6 ± 0.9; intrinsic affinity constant K_{a2} = 112.3 ± 31.8; ΔG = –2.57 kcal/mol) for diacetyl. Diacetyl was retained in the dialysis sac, even after exhaustive dialysis against water, suggesting a strong bonding between diacetyl and sodium caseinate. The addition of sodium azide at a concentration of 0.03 mol/L in the aqueous solution of sodium caseinate did not affect the binding between diacetyl and the protein.

At pH 7, chemical reactions between diacetyl and the primary amino groups of the protein (i.e., the terminal amino groups and mainly the ϵ-lysyl residues) were more likely to occur. At pH 7, the amino acids with a carboxyl group (aspartic and glutamic acids) are ionized and not reactive, and the hydroxyl functions are only involved in weak binding (i.e., hydrogen bonds) with the ligand.

The interaction between diacetyl and lysine was investigated by experiments using equilibrium dialysis with glycosylated sodium caseinate at 75 g/L. The chemical modification of protein resulted in the absence of diacetyl that was bound to galactosylated, maltosylated, glucosylated, and lactosylated sodium caseinate, regardless of the degree of modification (from 43 to 70%) or the chain length of bound carbohydrates. This result can be due to the inhibition of binding of diacetyl to the lysyl residues when the latter are glycosylated or to the steric hindrance caused by the bound carbohydrates. The absence of diacetyl bound to glycosylated sodium caseinate occurred with 10 g/L of protein. In addition, the low degree of modification that was obtained with fructosylated sodium caseinate (13% substitution of lysine) inhibited binding less than did the high degree of substitution of the other proteins: the number of moles of bound ligand in the presence of fructosylated

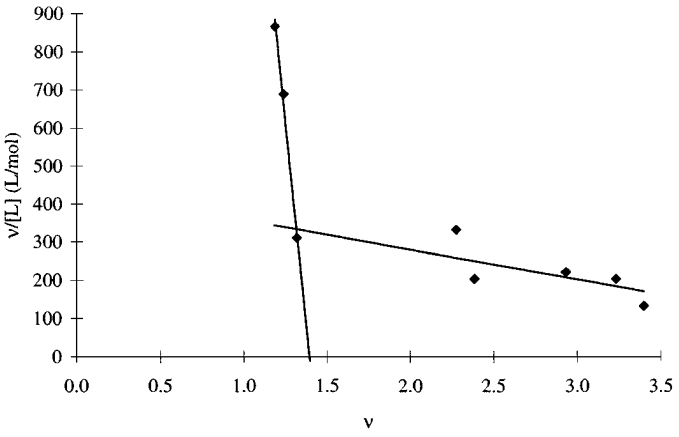

Figure 2. Scatchard plot of the binding of diacetyl to sodium caseinate in aqueous solution (75 g/L); ν = number of moles of ligand bound per mole of protein, and [L] = molar concentration of free ligand at equilibrium (moles per liter).

protein was 1.43. This behavior confirmed that diacetyl binding to the protein sites involved lysyl residues of the protein because these sites would have been glycosylated. An aqueous solution was prepared with 75 g/L of sodium caseinate and galactose, which was in a concentration equal to 70% of the lysine concentration. In this solution containing protein and free galactose and an initial concentration of diacetyl of 0.89 mol/L, the number of moles of ligand bound per mole of protein was reduced from 2.96 to 0.92, which is equal to 31% of the initial value (without galactose) compared with the 30% expected. This result is consistent with a competition between diacetyl and galactose for the same binding sites of sodium caseinate. The binding between a saccharide and an amino group increases strongly as temperature increases but occurs at any temperature (13).

The binding of benzaldehyde to sodium caseinate, as determined by equilibrium dialysis, revealed a constant increase in the quantity that was bound to the protein as a function of the initial concentration of benzaldehyde. Consequently, the binding behavior between benzaldehyde and sodium caseinate could not be investigated by the Scatchard model. The saturation of the binding sites was not reached; the initial concentration of benzaldehyde was limited by its aqueous solubility, in contrast to the behavior of diacetyl. Hence, the binding between benzaldehyde and sodium caseinate could not be characterized. These experiments were carried out in the presence of a protein precipitate induced by the interactions of benzaldehyde with sodium caseinate. This phenomenon was also observed for diacetyl and acetone at high concentrations; however, the amount of precipitate was less than that occurring with benzaldehyde. The carbonyl groups of aldehydes and ketones can react in a reversible manner with the amino groups of proteins to form a Schiff base; this procedure is applicable to a wide range of proteins (26). Precipitation was due to the reaction of the hydrophilic side chains of the amino groups with acetone, diacetyl, and benzaldehyde to yield hydrophobic side chains (i.e., alkyl and phenyl groups). The higher hydrophobicity of benzaldehyde could explain the extensive precipitation of sodium caseinate compared with that of the other aroma molecules. Another possible interaction is that between the aromatic ring of benzaldehyde and the hydrophobic protein sites. The removal of the free ligand from the dialysis sac, by an exhaustive dialysis, showed the presence of weak interactions between benzaldehyde and the protein; no volatile component was detected in the dialysis sac after this experiment. The measurement of protein solutions containing benzaldehyde by gas chromatography showed that benzaldehyde was fully recovered after dissolution of the precipitate by NaOH. Hence, the binding between benzaldehyde and casein seemed totally reversible, as in the case of diacetyl.

Because the native conformation of proteins can be affected by the presence of ions, the addition of sodium azide on the interactions between aroma and protein was investigated. In the presence of sodium azide in the dialysis bag, the protein precipitate disappeared. At 0.12 mol/L of benzaldehyde, the number of moles of ligand bound per mole of protein was 5.56 without sodium azide and was 0.05 when the concentration of sodium azide was 0.03 mol/L. Such variations indicated that sodium azide at low concentration slightly increased the hydration shell surrounding the protein. The solvation of the protein would lead to the inhibition of the binding of benzaldehyde to sodium caseinate because of hydrating water molecules at the sites of protein-ligand interactions. This decrease in binding of aroma molecules in the presence of low concentrations (<0.25 *M*) of salts was observed by Mohammadzadeh-K et al. (28) and Jouenne (14). Conversely, above this concentration range, the hydration shell decreased, and aroma retention was enhanced because ions compete with protein groups for water-binding sites. Damodaran and Kinsella (5) reported that the association of 2-nonanone to bovine serum albumin varied as the concentration, size, and charge of salts varied. Those authors (5) used concentrations in salts from 0.3 to 4 mol/L.

The effect of the chemical modifications of sodium caseinate on benzaldehyde binding was different from that on diacetyl binding. Benzaldehyde was not significantly associated with maltosylated, glucosylated, lactosylated, or fructosylated protein but was associated with galactosylated sodium caseinate. The addition of free galactose at an amount equivalent to a percentage of modification of 70% did not affect significantly the number of moles of ligand bound per mole of protein compared with effects of native sodium caseinate. Hence, the protein sites of the glycosylation reaction were not involved in benzaldehyde binding with the protein. It is inferred that chemical modification led to steric hindrance, preventing benzaldehyde from binding to sodium caseinate. Particular phenomena may be involved when the percentage of chemical modification is high (e.g., 70% modification in the case of the galactosylated sodium caseinate) because the fixation of benzaldehyde was improved. The effect of the degree of modification was studied by exponential dilution;

TABLE 3. Amounts of aroma compounds and water sorbed to sodium caseinate.

Aroma compound	Sorbed amount	
	Water	Aroma compound
	(g/100 g of protein)	
Acetone	...	7.4
Diacetyl	12.6	1.1
Benzaldehyde	8.2	0.6
Ethyl acetate	...	8.4
2-Propanol	8.3	5.3

the activity coefficient of benzaldehyde went from 9223 to 1390 for galactosylated sodium caseinate modified at 47 and 70%, respectively. This decrease in the activity coefficient of benzaldehyde indicated a higher retention of the compound by the galactosylated protein modified at 70%. This effect should not be due to the presence of galactose in the protein solution because the quantity of bound benzaldehyde did not vary significantly, regardless of the concentration of free galactose. A high degree of substitution might induce modifications in the binding of benzaldehyde.

Acetone, ethyl acetate, and 2-propanol were not significantly bound to sodium caseinate ($P \geq 0.05$). The results of our study confirm those obtained by exponential dilution; the ratio γ_P/γ_W of these molecules was 1 or greater than 1, demonstrating the absence of interactions or weak interactions with sodium caseinate. 2-Propanol and ethyl acetate are likely to interact with the protein through hydrogen bonding; acetone should form an imine with the protein amino groups (26) (i.e., the ϵ-amino groups of the lysine residues and the terminal amino groups). Such a reaction was not detectable by equilibrium dialysis. The retention of benzaldehyde by sodium caseinate was reported by both techniques. Exponential dilution showed the release of diacetyl from an aqueous solution of sodium caseinate at 75 g/L. However, the results of dialysis revealed the presence of bonding of diacetyl to sodium caseinate. This difference may be caused by the shear from air bubbles and the rotating screw used in exponential dilution method. This stirring would disrupt the interactions of low affinity but not affect the interactions of high affinity and would induce an increase in the activity coefficient in relation to that in water. Hence, the equilibrium dialysis can detect the physicochemical interactions of low and high affinities between aroma and proteins. Other thermodynamic models can be used to quantify the interactions between aroma and protein; Landy et al. (17) applied the Hill model to characterize the binding between esters and sodium caseinate and suggested a positive cooperation between the binding sites of the protein.

Influence of Protein Hydration

The equilibration time needed for sorption of the aroma components on dried sodium caseinate was 460 h. The affinity of the compounds for the protein decreased in the following order: ethyl acetate, acetone, 2-propanol, diacetyl, and benzaldehyde. Ethyl acetate and 2-propanol were strongly bound to sodium caseinate in a solid state: 3.2 and 1.5 g/100 g of protein, respectively. The amounts of the volatile compounds and water sorbed to sodium caseinate are given in Table 3. The amount of water sorbed to the protein varied with the nature of the compound because of a competition between the two volatile molecules.

Table 4 shows band frequencies, obtained from the infrared spectra, of the chemical functions of each compound and its corresponding shift. The carbonyl peak of acetone at 1714.6 cm^{-1} was shifted down to form a band at 1707.9 cm^{-1} (Figure 3A). Those data indicated that acetone interacted by hydrogen bonding to the electronegative atoms of sodium caseinate. The magnitude of shift was the same for diacetyl as for acetone, but the direction was positive. Hence, the interaction between diacetyl and sodium caseinate was different and must have involved both carbonyl groups of diacetyl because no band was observed at 1714 cm^{-1} (Figure 3B). Benzaldehyde did not bind to sodium caseinate in a comparable manner in relation to diacetyl; the magnitude of the shift was +18 cm^{-1} (Figure 3C). We suggest that benzaldehyde was bound to sodium caseinate through its aromatic group, which could lead to a change in C=O vibrational mode (i.e., its resonance frequency). The spectrum of pure and sorbed ethyl acetate is illustrated Figure 3D. The involvement of the carbonyl oxygen of ethyl acetate in hydrogen bonding was shown by the

TABLE 4. Band frequencies and shifts of aroma compounds in a pure and sorbed state.

Chemical group	Aroma compound	Band frequency		Shift
		Pure	Sorbed	
		(cm^{-1})		
Carbonyl	Acetone	1714.6	1707.9	–6.7
Carbonyl	Diacetyl	1714.6	1721.3	+6.7
Carbonyl	Benzaldehyde	1699.3	1717.9	+18.6
Ester	Ethyl acetate	1738.2	1731.6	–6.6
Alcohol	2-Propanol	3360.0	BB[1]	...

[1]Broad band maximum at 3280 cm^{-1}.

negative shift of –6.6 cm^{-1} between the pure and sorbed states of the compound. For the spectrum of 2-propanol, the OH group shift was difficult to quantify because of the broad band (maximum at 3280 cm^{-1}).

The quantity of sorbed aroma compounds was highest for compounds involved in hydrogen bonds with sodium caseinate (i.e., acetone and ethyl acetate). For 2-propanol, the infrared spectrum did not allow the quantification of OH shift, but the sorption results revealed the presence of strong interactions between 2-propanol and sodium caseinate in a solid state. For these three components, water seemed to have no inhibiting effect on aroma binding to the

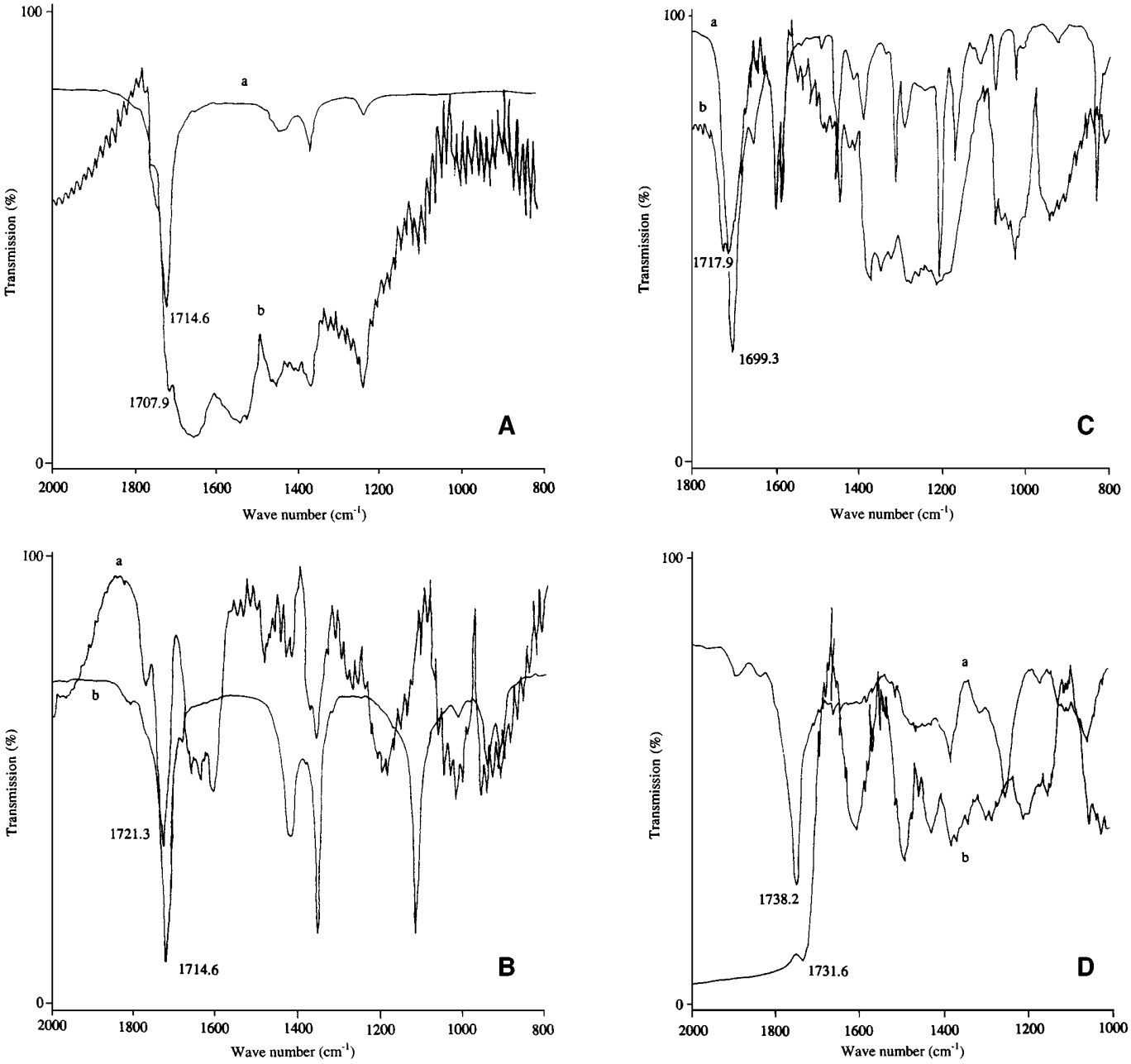

Figure 3. Infrared spectra for acetone (A), diacetyl (B), benzaldehyde (C), and ethyl acetate (D), both pure (a) and bound to freeze-dried sodium caseinate (b).

protein because their sorbed quantity is high in relation to that of diacetyl and benzaldehyde. Despite the low amount of bound diacetyl and benzaldehyde, the infrared spectra indicated interactions between aroma compounds and sodium caseinate. For diacetyl, lack of binding between the carbonyl groups of the volatile and ϵ-lysyl residues may reflect the importance of water molecules to catalyze the formation of the bond.

A few studies have shown that water content was important in relation to the aroma-binding properties of food constituents. Le Thanh et al. (21) reported, using sorption and headspace analysis, that acetone and ethyl acetate were released from glucose in solution but were retained by glucose in the solid state. The addition of water to a system containing oil, protein, and flavor compounds provided a decrease in volatility by increasing the binding of the aroma molecules by the proteins (9). Belton and Gil (2), using infrared and Raman spectroscopy, found that a disaccharide was excluded from the proximity of lysozyme by water, which caused the spectra of lysozyme to more closely resemble that of the hydrated protein than that of the dehydrated protein.

The physicochemical properties of the compounds and the quantity of water in the system are determinant parameters for the strength of interactions between macromolecules and ligands. The present study demonstrated quantitative and qualitative interactions between aroma and protein that showed the importance of hydrating water as a function of salts, the chemical modification of proteins, and the effect of pH on protein conformation. The importance of the physicochemical interactions between aroma compounds and sodium caseinate on aroma perception should be confirmed by sensory analysis, which would indicate interactions that affect acceptability of food products.

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