Interactions between Methyl Ketones and β-Lactoglobulin: Sensory Analysis, Headspace Analysis, and Mathematical Modeling

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Interaction of flavor compounds with proteins is known to have an influence on the release of flavor from food. Hydrophobic interactions were found between β-lactoglobulin and methyl ketones; the affinity constant increases by increasing the hydrophobic chain. Addition of β-lactoglobulin (0.5 and 1%) to aroma solutions (12.5, 50, and 100 μL L^{-1}) of three methyl ketones induces a significant decrease in odor intensity. The chosen methyl ketones were 2-heptanone (K_b = 330), 2-octanone (K_b = 950), and 2-nonanone (K_b = 2440). The release of these flavor compounds (50 μL L^{-1}) was studied by static headspace in water solution (50 mM NaCl, pH 3) with different concentrations of β-lactoglobulin (0, 0.5, 1, 2, 3, and 4%). Increasing the concentration of protein increases the retention of volatiles, and this effect is greatest for 2-nonanone, the compound with the highest affinity constant, and lowest for 2-heptanone. A mathematical model previously developed to describe flavor release from aqueous solutions containing flavor-binding polymers (Harrison, M.; Hills, B. P. *J. Agric. Food Chem.* **1997**, *45*, 1883−1890) was used to interpret the data. The model assumes that the polymer−flavor interaction is reversible and the rate-limiting step for release is the transfer of volatiles across the macroscopic gas−liquid interface. This model was used to predict the equilibrium partitioning properties and the rate of release of the three methyl ketones. Increasing the affinity constant leads to decreased release rates and a lower final headspace aroma concentration.

Keywords: *Binding; aroma; protein; odor intensity; mathematical modeling; interfacial mass transfer; partitioning*

INTRODUCTION

The quality of a particular food is primarily determined by the quantity of flavor released from the product matrix during consumption. However, a large percentage of flavor present in a food is bound to the other ingredients that constitute the food matrix. This reduces the quantity of free flavor available for release and, hence, perception. It is well-known that proteins interact with volatiles both reversibly (O'Neill and Kinsella, 1987; Sostmann and Guichard, 1998) and irreversibly (Hansen and Heinis, 1991, 1992).

The magnitude of these protein−volatile interactions depends greatly on the physical and chemical nature of the molecules involved. For example, short acids and methylpyrazines (Pelletier et al., 1998) were found not to interact with β-lactoglobulin, whereas methoxypyrazines do interact with β-lactoglobulin (Reiners et al., 2000). Hydrophobic interactions occurred between esters and β-lactoglobulin, because the global affinity increased when the length of one of the two hydrophobic chains increased (Pelletier et al., 1998). The cocrystallization of β-lactoglobulin with palmitate demonstrated binding to the central cavity (Wu et al., 1999). Dufour and Haertlé (1990) have shown that retinol and its derivatives interact specifically with β-lactoglobulin at a 1:1 molar ratio at pH 7.1. Charles et al. (1996) have found one binding site of 2-nonanone per β-lactoglobulin dimer at pH3. Moreover, Reiners et al. (2000) showed that the odor intensity of eugenol in water solution decreased by addition of β-lactoglobulin, whereas no significant difference was found for vanillin, which has a lower affinity for this particular protein.

A more extensive interpretation of the data can be accomplished through the use of mathematical models. Using such an approach, Harrison and Hills (1997) developed a model to describe flavor release from aqueous solutions containing aroma-binding macromolecules. The model was based on two main assumptions: first, the transport of volatiles across the gas−liquid interface can be described by the penetration theory of interfacial mass transfer; and, second, the rate of exchange of volatiles between the bound and unbound states is extremely fast and hence not rate limiting. This work was later extended to investigate the effect of saliva flow on release from such systems (Harrison, 1998).

In general, the theory predicts that the main effects of adding binding polymers to an aqueous solution, is to reduce both the extent and rate of flavor release. Furthermore, the initial rate of release is primarily determined by the macroscopic interfacial mass transfer coefficient, which will depend greatly on the viscosity of the liquid mixture and, hence, the type of polymer introduced. This model predicted that the ingredient−aroma interaction was a major physical factor influencing both the rate and the extent of release from liquid

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foods. Although the model highlighted which physicochemical parameters were important for understanding flavor release from these systems, the relative importance of these factors can only be determined experimentally.

In an attempt to verify the theoretical model, Bakker et al. (1998) studied the effect of viscosity on flavor release from aqueous solutions by varying the gelatin concentration present in a liquid solution. These authors observed that the mass transfer coefficient decreased linearly with increasing gelatin concentration. More importantly, though, they found that the mass transfer coefficient was proportional to the inverse square root of the stirring rate. This result, therefore, confirms the validity of applying the penetration theory of interfacial mass transfer to describe flavor release from liquid systems. However, this study concentrated more on the viscosity effects of the liquid gelatin, rather than on the physicochemical binding of the ingredient−aroma interaction.

The aim of this paper is, therefore, to combine the methods of mathematical modeling and headspace and sensory analysis to develop a more comprehensive understanding of the influence of volatile−protein interactions on flavor release and perception. Furthermore, combining mathematical modeling with headspace analysis will allow the theory proposed by Harrison and Hills (1997) to be tested. By considering a simple aqueous phase containing only one flavor and one protein, complications of multiple interactions, between ingredients, can be avoided. For this particular study, we have chosen to focus on the interaction of β-lactoglobulin with three methyl ketones: heptanone, octanone, and nonanone, which are known to be flavor impact compounds in yogurt (Ott et al., 1997). The next section briefly reviews the theoretical model, proposed by Harrison and Hills (1997), used to interpret the results, highlighting in particular the main predictions of this model.

FLAVOR RELEASE FROM AQUEOUS SOLUTIONS

Binding. In an aqueous solution containing only a single volatile and protein, a proportion of total amount of volatile present will bind to the protein. Therefore, only the free flavor will be freely available for release. The total flavor in the solution is given by

$$c_{tf} = c_{bf} + c_{ff} \tag{1}$$

where c_{tf}, c_{bf}, and c_{ff} are the total, bound, and free flavor concentrations, respectively, in the aqueous phase. Assuming first-order kinetics we can write

$$c^e_{bf} = K_b c^e_b\, c^e_{ff} \tag{2}$$

where c^e_{bf} and c^e_{ff} are the equilibrium concentrations of the bound and free flavors, respectively, c^e_b is the equilibrium concentration of binder present in the solution, and K_b is the global equilibrium binding constant. The effect of the volatile−protein interaction is to reduce the free equilibrium flavor concentration, available for release, by a factor of $(1 + K_b c^e_b)$. This is reflected in the gas−liquid partition coefficient, which is also reduced by the same factor to produce an effective partition coefficient, K^{eff}_{ga}:

$$K^{eff}_{ga} = \frac{c^e_g}{c^e_{ff}} = \frac{K_{ga}}{1 + K_b c_b} \tag{3}$$

In eq 3 c^e_b can in effect be regarded as the total concentration of binder in the mixture, c_b, and therefore becomes time-independent.

Table 1. Description of Symbols with Corresponding Default Values Used in the Calculations

symbol	description	value
A_{ga}	gas−liquid surface area (m^2)	5×10^{-4}
$c_a(t)$	volatile concn in aq phase (mg/cm^3)	
$c_g(t)$	volatile concn in gaseous phase (mg/cm^3)	
$c_{tf}(0)$	initial volatile concn in aq phase (mg/cm^3)	0.041
h_D	gas−liquid mass transfer coefficient (m/s)	
K_{ga}	gas−liquid partition coefficient	
K^{eff}_{ga}	effective gas−liquid partition coefficient	
K_b	volatile−protein global binding coefficient (M^{-1})	
c_b	concn of β-lactoglobulin in aq phase (M)	
v_g	vol of gas phase (m^3)	3×10^{-5}
v_a	vol of aq phase (m^3)	1×10^{-5}
t	time (s)	

Theory of Flavor Release from Solutions. Transport of free volatiles from a liquid phase, to the gaseous phase, can be described by the penetration theory of interfacial mass transfer. If the volatiles are released into a closed headspace, then an analytical solution for the concentration of flavor in the gas phase, $c_g(t)$, can be derived from three basic equations describing chemical binding, mass conservation, and interfacial mass transfer:

$$c_g(t) = \frac{c_{tf}(0)}{[(1 + K_b c_b)/K_{ga} + v_g/v_a]}\left[1 - \exp\left\{-\frac{A_{ga} h_D}{v_g}\left(\frac{1}{K_{ga}} + \frac{v_g}{v_a}\frac{1}{1 + K_b c_b}\right)t\right\}\right] \tag{4}$$

A description of the parameters used in eq 4, with default values, can be found in Table 1.

The release curve (eq 4) has two important features that provide valuable information on the mass transfer coefficient, h_D, and the binding constant, K_b. Analysis of eq 4 shows that the important aspects of release are the initial rate of release, given by

$$c_g(t) = \frac{h_D A_{ga}}{v_g}\frac{c_{tf}(t)}{1 + K_b c_b} t \tag{5}$$

and the equilibrium headspace concentration

$$c_g(\infty) = \frac{c_{tf}(0)}{\left[\frac{1 + K_b c_b}{K_{ga}} + \frac{v_g}{v_a}\right]} \tag{6}$$

The value of eq 6 is approximately equal to the effective partition coefficient, K^{eff}_{ga}, given by eq 3.

MATERIALS AND METHODS

Materials. Commercial β-lactoglobulin was obtained from Besnier Bridel Aliments (Chateaulin, France) (purity > 90%). The powder was dispersed in NaCl (50 mM) solution. The pH was adjusted at pH 3 with HCl (1 N). Methyl ketones were

kindly supplied by International Flavors and Flagrances (I.F.F., Longvic, France). Their purity was evaluated by GC-MS (>95%). Aroma solutions were prepared daily in NaCl (50 mM) solution adjusted at pH 3 with HCl (1 N).

Headspace Analysis. Analyses were done in triplicate in amber flasks (40 mL) closed with mininert valves (Supelco, Bellefonte, PA). The samples were composed of 5 mL of aqueous aroma solution and 5 mL of either NaCl solution or protein solution. For each methyl ketone, the final concentration was 50 μL L^{-1}. For protein, four final concentrations were studied (0.5, 1, 2, 3, and 4%). Analyzed solutions, with or without protein, were stirred and equilibrated at 30 °C for different times (15−2700 s). Only one sample per flask was made. Vapor phase samples (1 mL) were taken with a gastight syringe (1 mL, SGE) and injected onto a Carlo Erba 8000 gas chromatograph equipped with a DB-Wax column (J&W Science, i.d. 0.32 mm, 30 m, film thickness = 0.5 μm). Temperatures of the injector and detector were, respectively, 250 and 260 °C. The H_2 carrier gas velocity was 1.9 mL min^{-1}. The FID signal was sampled every 50 ms using a PC-driven four-channel plug-in acquisition board developed in the laboratory (Almanza and Mielle, 1990). After analysis, the data were processed using software developed in the laboratory (Almanza et al., 1989).

Sensory Analysis. Sensory evaluations were performed in a testing room equipped with computerized booths. Data acquisition and data treatment were conducted with the Fizz software (Biosystemes, Dijon, France). Sixteen panelists (8 males and 8 females) were trained to estimate the odor intensity of methyl ketones in aqueous solutions with or without protein. Samples (20 mL) were presented in brown flasks (60 mL) closed with screw caps. Their odor intensities were evaluated after 1 h of equilibration time at 21 °C.

In three training sessions, one methyl ketone per session, panelists were trained to range the eight references in increasing order of concentrations of methyl ketone (from 0.78 to 100 μL L^{-1}, with a concentration step of 2). After this classification step, they were familiarized with estimating the odor intensity of methyl ketones in the presence of protein, applying the matching test according to Rousseau et al. (1996).

In three tasting sessions, the odor intensities of methyl ketones were measured on a linear scale according to eight reference samples. Panelists were asked to sniff the reference game classified by increasing order of concentration and coded from 1 to 8 and to memorize the intensity of odor perceived. Following this training period, subjects had to estimate the odor intensities of nine samples: three aroma concentrations (12.5, 50, and 100 μL L^{-1}) were tested in β-lactoglobulin solutions (0, 0.5, and 1%). The nine samples coded with three-digit random number were placed following a presentation order based on a Latin square. Panelists had to evaluate the odor intensity of the methyl ketone, with abstraction of the protein odor, by comparison with the reference sample; they reported their position on a continuous linear scale overlapping the eight reference points, reaching from <1 to >8. In each tasting session, two methyl ketones were tested in duplicate. For example, in the first session, half of panelists were first given 2-heptanone and the other half 2-octanone.

Sensory data were subjected to a three-way analysis of variance testing the factors as follows: protein concentration, flavor concentration, judges considering this factor as random, and all interactions. Sample means were calculated and compared by the Newman−Keuls test.

RESULTS AND DISCUSSION

Headspace Analysis. The release study was conducted over a period of 2700 s: the release curves (Figure 1) show that the equilibrium is reached after ~900 s for the three methyl ketones. The release of three methyl ketones depends on the quantity of protein present in the aqueous solution. The rates and extent of volatile released into the headspace was found to decrease with increasing protein concentration. For example, the release of 2-octanone seems to be proportional to the protein concentration: increasing the protein concentration increases the retention from 10 to 60%. In the case of 2-nonanone the retention is greater (40−75%), reaching a maximum at a protein concentration of 3%. These results are in agreement with those previously observed by Jouenne and Crouzet (1996).

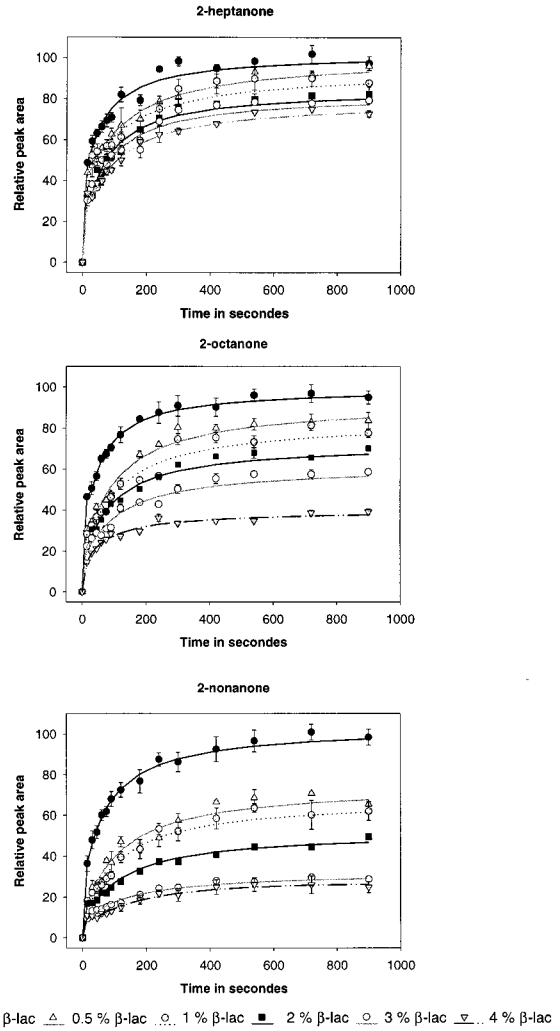

Figure 1. Release of three methyl ketones (50 μL L^{-1}) in water solution (50 mM NaCl, pH 3) with different amounts of β-lactoglobulin (0−4% w/w).

Analysis of the equilibrium concentrations, for the pure water systems, shows that the gas−water partition coefficient increases for compounds possessing a longer carbon chain (Figure 2), that is, increasing hydrophobicity. Furthermore, Figure 1 shows the equilibrium concentrations decrease as β-lactoglobulin is gradually added to the aqueous phase. Quantitative analysis of the volatile−protein interaction is achieved by applying eq 3 to plot the normalized effective partition coefficient as a function of the β-lactoglobulin concentrations (Figure 3). In Figure 3 the global binding coefficients have been used as the fitting parameters. The values for the global binding coefficient, K_b, obtained from these experiments are 330, 950, and 2440 for heptanone, octanone, and nonanone, respectively (Figure 4), and fall between the values obtained by Sostmann and Guichard (1998) and Kinsella (1989) (Figure 3). A greater value of K_b corresponds to a stronger interaction between the protein and flavor volatile.

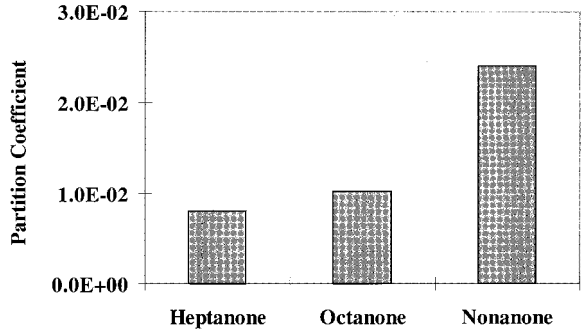

Figure 2. Experimentally determined partition coefficients of three methyl ketones.

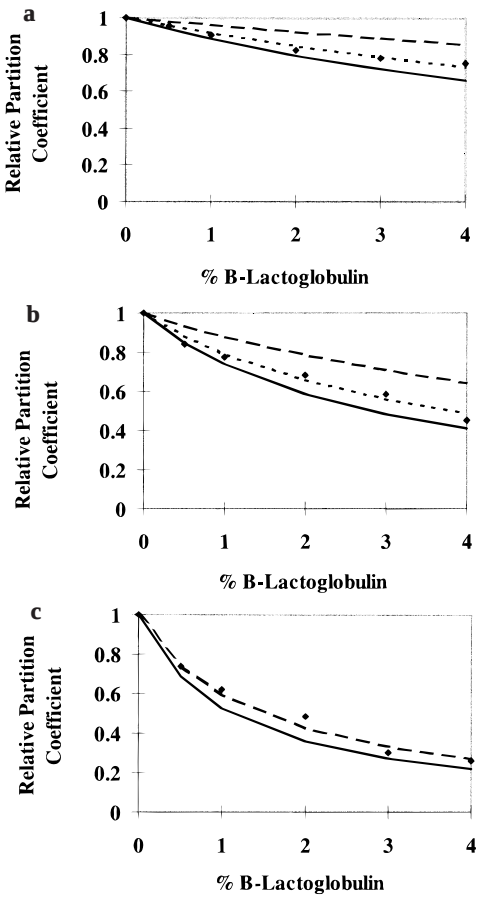

Figure 3. Ratio K_{ga}^{eff}/K_{ga} for heptanone, octanone, and nonanone as a function of percentage of β-lactoglobulin concentration: (dotted line) best fit; (solid line) Sostmann and Guichard (1998); (dashed line) Kinsella (1989).

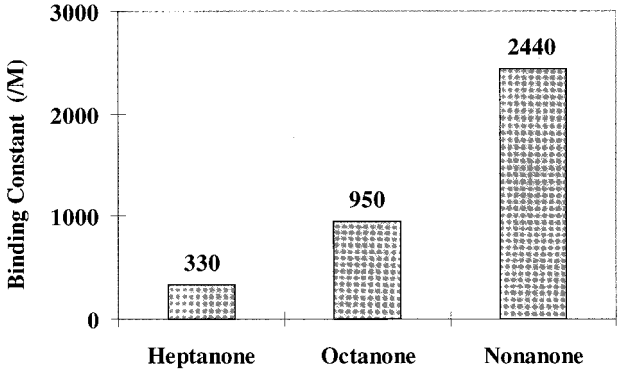

Figure 4. Experimentally determined global binding affinity values for the interaction between β-lactoglobulin and three methyl ketones.

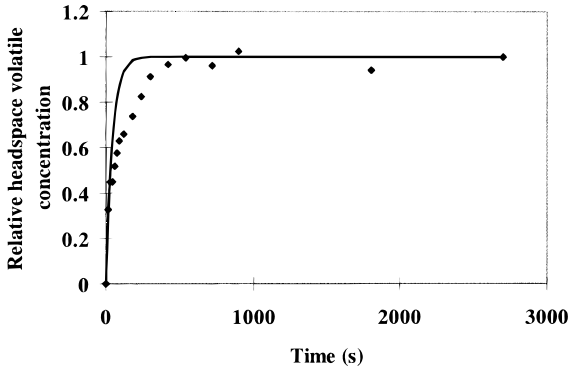

Figure 5. Modeled time-dependent octanone release from an aqueous solution containing 2% β-lactoglobulin solution.

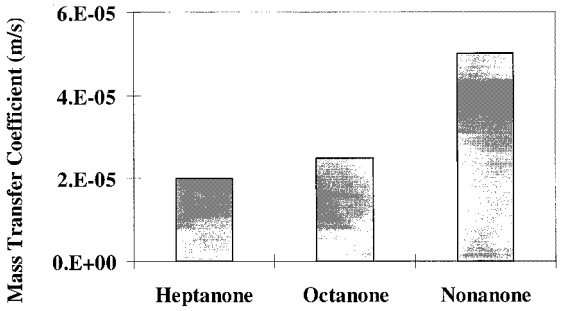

Figure 6. Mass transfer coefficient of release of three methyl ketones from aqueous solutions of β-lactoglobulin.

Further quantitative information can be extracted from Figure 1 by modeling the complete release curves (Figure 5), using the mass transfer coefficient as the fitting parameter (Figure 6). On first inspection of Figure 5 the correlation between theory and experiment is not ideal, and it could be argued that a more suitable fit could be obtained. Deviations from the desired theoretical curves are a consequence of inefficient stirring in the aqueous phase. Consequently, once the liquid surface layers are depleted of free volatiles, diffusion gradients develop in the surface layers of the liquid,

Table 2. Results of Analysis of Variance Obtained in Sensory Analysis

	2-heptanone		2-octanone		2-nonanone	
factor	*F* value	*p* value[a]	*F* value	*p* value[a]	*F* value	*p* value[a]
aroma concn (A)	102.92	<0.0001***	107.36	<0.0001***	113.17	<0.0001***
β-lactoglobulin concn (BLG)	19.38	<0.0001***	11.48	<0.0001***	4.96	0.0138*
judge (J)	3.79	<0.0001***	4.98	0.0002***	4.92	<0.0001***
A × BLG	0.16	0.9576	1.03	0.4002	0.71	0.5852
A × J	2.08	0.0023**	1.33	0.1348	1.31	0.1500
BLG × J	0.97	0.5196	1.21	0.2273	1.85	0.0091**
A × BLG × J	1.33	0.0887	0.54	0.9964	1.13	0.2822

[a] *, significant at $p \leq 5\%$; **, significant at $p \leq 1\%$; ***, significant at $p \leq 0.1\%$.

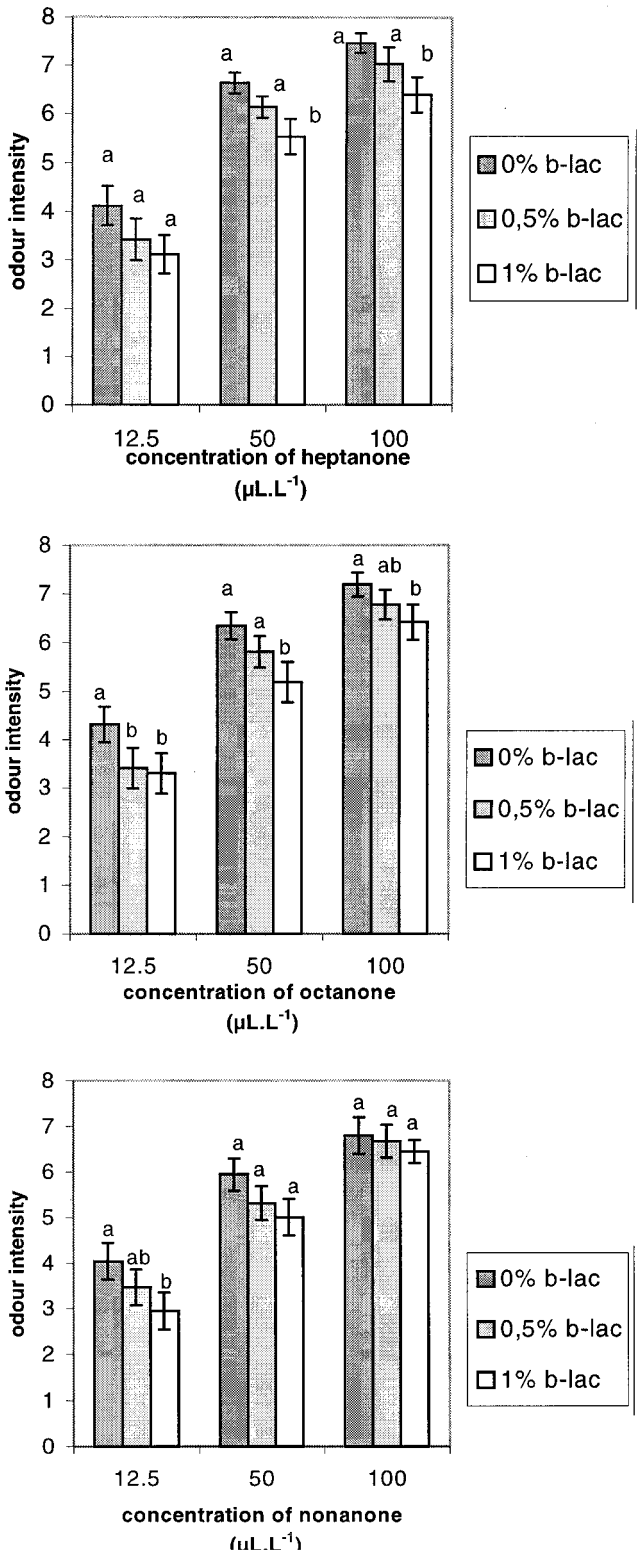

Figure 7. Odor intensities of methyl ketones in the presence of different amounts of protein. Different letters mean that the results are significant at the 5% level (Newman−Keuls test).

thus reducing the flavor release rates. However, it is the initial rate of release (eq 5) and the equilibrium concentration (eq 6) that are the important features of these curves.

Sensory Analysis. Addition of β-lactoglobulin to aroma solutions produces a significant decrease in perceived odor intensities (Figure 7), performing the test of Newman−Keuls at $p = 0.05$. The addition of protein (1%) reduces significantly ($p < 5\%$) the odor intensity of 2-heptanone (50 and 100 μL L^{-1}). Whatever the concentration of 2-octanone, the differences are significant ($p < 5\%$) between the solution with (1%) and without protein. In the case of nonanone, the difference between 0 and 1% of protein is significant only for the lowest aroma concentration (12.5 μL L^{-1}).

A three-way analysis of variance was performed to establish the impact of the following factors: aroma concentration, concentration of β-lactoglobulin, judge, and their interactions on the perceived odor intensities. Results of the variance analysis for the three methyl ketones are reported in Table 2. For each methyl ketone, three-way analysis of variance shows significant effects of the aroma concentration, of the protein concentration, and of the judge factor. The judge effect was significant for all of the compounds, meaning that subjects used the scale in a different way. This difference could be due to the protein odor and the difficulties of the panelists to evaluate the odor intensity of methyl ketones without taking onto account the odor of protein. Moreover, the significance of the BLG−judge interaction observed for 2-nonanone could be due to the odor of protein. The aroma and protein effects were significant at $p < 0.1\%$ except for 2-nonanone, for which the effect of protein concentration was significant at $p < 5\%$. These observations mean that subjects are able to distinguish the difference between the aroma note due to the ketones and that due to the protein. Moreover, the F value corresponding to the aroma concentration was higher than the F value of the judge factor, showing greater importance of volatile compounds. For 2-heptanone, the aroma−judge interaction, significant at $p < 1\%$, shows that the judges evaluate the samples in a different way. The interaction between aroma and protein concentrations was not significant: the addition of protein produces the same effect for the three concentrations of methyl ketones. The interactions between the three factors tested were not significant: the tasters ranked the samples in the same order. Addition of protein produces a significant decrease of the odor perception of methyl ketones. Moreover, this effect is not well correlated with the retention of the aromas for the protein. In fact, the panelists had some difficulties perceiving differences between the odor of 2-nonanone and that of the protein, a result that contrasts those obtained for the other ketones.

Conclusions. This paper focuses on the effects of protein−flavor interactions on the rates of volatile release from liquid solutions. A multidisciplinary approach, using headspace analysis, mathematical modeling, and sensory studies, was used to investigate the specific interaction between β-lactoglobulin and three methyl ketones. The time-dependent headspace concentrations were obtained under well-defined experimental conditions, where all volumes, concentrations, and the interfacial surface area were known. It was, therefore, possible to apply a previously developed mathematical model to fit the experimental data. In general, there was good agreement between the experiments and theory, thus providing further evidence supporting the model proposed by Harrison and Hills (1997). Using this model, partition coefficients, dissociation constants, and mass transfer coefficients were quantified for the interaction between β-lactoglobulin and three methyl ketones.

In general, these simple release experiments show that the ingredient−aroma interaction is a major physical factor influencing both the rate and extent of release from liquid foods. Volatiles with longer chain lengths bind more strongly to the protein, illustrated by the increased binding affinity. Conversely, such compounds are released from the aqueous solution at a faster rate, due to the increased hydrophobicity with increasing chain length. The relative importance of these opposing factors is easily probed by combining controlled release experiments with mathematical modeling and, thus, enhances the understanding of interactions between flavor volatiles and food ingredients. This work further emphasizes the need for careful release measurements to obtain the relevant partition coefficients, mass transfer coefficients, and binding constants. Once obtained, these values can be used in conjunction with mathematical models to develop predictive simulations of flavor release during eating (Harrison, 2000). Understanding protein−flavor interactions will help to explain differences in odor perception of flavor compounds incorporated into different matrices.

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