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Influence of β-lactoglobulin, pH and presence of other aroma compounds on the air/liquid partition coefficients of 20 aroma compounds varying in functional group and chain length

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Abstract

The air/water partition coefficients of 20 aroma compounds varying in functional group and chain length were determined as a function of β-lactoglobulin concentration (0, 0.5, 0.7, 1.0, 2.0%), pH (3, 6, 9) and presence of other aroma compounds in the matrix. Air/liquid partition coefficients of dimethyl sulphide, 1-propanol, diacetyl, 2-butanone, ethyl acetate, 1-butanol, 2-pentanol, propyl acetate, 3-methyl-1-butanol, ethyl butyrate, hexanal, butyl acetate, 1-hexanol, 2-heptanone, heptanal, α-pinene, 2-octanone, octanal, 2-nonanol and 2-decanone were determined by static headspace gas chromatography. The majority of the compounds' partition coefficients decreased with β-lactoglobulin concentration, although not proportionally. Air/liquid partition coefficients of aldehydes and esters demonstrated a decline up to 90%, whereas alcohols were hardly affected by β-lactoglobulin. Larger chain length coincided with lower partition coefficients and thus more efficient retention. Increase in pH generally resulted in a more pronounced decrease in headspace concentrations, although α-pinene and alcohols showed diverting behaviour. The β-lactoglobulin concentration and pH did not demonstrate complementary effects but interacted with each other. Propyl acetate, ethyl butyrate, hexanal, heptanal and octanal increased each other's air/liquid partition coefficients.

Keywords: Aroma; β-lactoglobulin; Ligand binding; Static headspace gas chromatography; Volatile compound

1. Introduction

The amounts of aroma compounds delivered to the sensory system and, therefore, available for perception, are determined by the release of the compounds from the food matrix. The rate of release depends on the affinity of the aroma compounds for the food matrix. Certain major food components, and especially biopolymers, have a large impact on aroma release, as they interact with aroma compounds.

Milk proteins are often added to foods as protein-based fat substitutes to simulate the mouthfeel of fat. Some of them contain 100% whey proteins (Hansen & Booker, 1996). Among the whey proteins used in the food industry, β-lactoglobulin (βLG) is a common ingredient, as well as a well-defined and well-characterised protein. It is a globular protein of the lipocalin family and is composed of 10–15% of α-helix structures and of ca. 50% of anti-parallel β-sheet (Le Quéré, Lübke, Andriot, & Guichard, 2000). It is available in pure form, either from the bovine A or B variants or as a mixture of both. βLG is known for its interactions with a large variety of hydrophobic ligands. From its structural pattern, it has been suggested that small hydrophobic ligands may bind inside the central calyx. This hydrophobic pocket has been well established by X-ray studies (Papiz et al., 1986). The existence of more independent binding sites on the surface of the globular protein was proposed by Narayan and Berliner (1997). The existence of these two hydrophobic binding sites is a subject of continuous controversy regarding the main hydrophobic binding site of βLG towards hydrophobic ligands, particularly retinoids such as retinol. Recent crystallographic studies have proven that β-lactoglobulin binds long chain fatty acids inside the central calyx (Wu, Perex, Puyol, & Sawyer, 1999).

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The βLG binding behaviour of some aroma compounds, e.g. α-ionone, has been shown to be similar to retinol and fatty acids. However, the geometry of the molecules is important and determines the interactions within the cavity. It has also been reported that compounds as p-cresol, eugenol, 2-nonanone and γ-decalactone do not induce conformational changes to the protein and it is, therefore, likely that these compounds bind to the protein surface (Lübke, Guichard, & Le Quéré, 2000).

Most researchers have studied aroma compounds with a chain length of six carbon atoms and more. They found that these compounds were bound to some degree. Lowered concentrations of 'free' aroma in a food will result in lower concentrations in the headspace above that food and consequently reduce the amount of aroma available for perception. The extent of binding of various compounds in foods, not only the larger sized ones, but also the smaller ones, are of interest. Although the overall reduction in aroma headspace concentrations is a concern, as important are the proportions of the various compounds which make up the aroma. Changes in proportions are likely to lead to imbalanced aromas.

In the present study the influence of βLG, pH and presence of other aroma compounds was systematically evaluated for a group of 20 aroma compounds varying in functional group and chain length. The influence of βLG on the air/liquid partitioning of the compounds was examined at five concentrations and three pH levels. A multifactor approach taking into account various food matrix and aroma compound related factors was chosen to allow the study of the individual factors as well as their interactions.

2. Materials and methods

2.1. Sample materials

For the liquid food matrix distilled water and βLG was used. βLG was obtained from Fluka Chemie (Buchs, Switzerland). The 20 aroma compounds included: diacetyl, 2-butanone, ethyl acetate, 2-pentanol, hexanal, 1-hexanol, 2-heptanone, heptanal and α-pinene, which were supplied by Aldrich (Steinheim, Germany). Dimethyl sulfide, ethyl butyrate, 2-octanone and octanal were purchased from Merck (Hohenbrunn, Munich, Germany). 1-Propanol, propyl acetate, 1-butanol, butyl acetate and 3-methyl-1-butanol were supplied by Lancaster (Walkerburn, UK), 2-nonanol and 2-decanone were obtained from Fluka Chemie.

2.2. Sample preparation

The aroma compounds were added in triplicate to the water matrix for each βLG concentration level and pH, resulting in final concentrations of 0.001% v/v per aroma compound. The solutions were stored for 12 h at 4 °C in absence of light to allow equilibration. Preliminary experiments confirmed the solubility of the compounds at 0.001% concentration level.

2.3. Static headspace gas chromatography

For static headspace gas chromatography, 2 ml of sample was transferred into a 10-ml headspace vial. Three replicate vials for each matrix and concentration level were analysed. The samples were incubated at 37 °C and agitated at 750 rpm for 6 min in the automated headspace unit (Combipal-CTC Analytics system; JVA Analytical Ltd., Dublin, Ireland) of the gas chromatograph (GC; Varian CP-3800; JVA Analytical Ltd.). The GC was equipped with an injector at 225 °C, a BPX5 capillary column (60 m length, 0.32 mm i.d., and 1.0 μm film thickness; SGE, Kiln Farm Milton Keynes, UK; helium carrier gas 1.9 ml min^{-1}) and a flame ionisation detector (FID) at 300 °C. An initial oven temperature of −30 °C was used for 1 min, followed by a rate of 100 °C min^{-1} to 40 °C. The oven temperature was maintained at 40 °C for 4 min and was subsequently programmed to 90 °C at 2 °C min^{-1}, further to 130 °C at 4 °C min^{-1}, and finally at 8 °C min^{-1} to 270 °C.

Five concentrations of each of the compounds were analysed in triplicate for calibration, allowing quantification of the compounds in the air phase.

2.4. Air/liquid partition coefficient calculation

For determination of air/liquid partition coefficients of each of the compounds, air phase concentrations (w/v) were divided by the concentrations in the liquid phase (w/v).

2.5. Calculation of the reduction of the air/liquid partition coefficients

The reduction of the air/liquid partition coefficient of a compound in water consisting of x% βLG was calculated as follows:

$$\{(K_{0\%} - K_{x\%})/K_{0\%}\} \times 100$$

where $K_{0\%}$ is the air/liquid partition coefficient of the aroma compound in water without βLG, and $K_{x\%}$ is the compound's air/liquid partition coefficient in water with x% βLG.

2.6. Statistical analysis

Data of triplicate partition coefficient measurements were subjected to multivariate analysis of variance

(MANOVA) to determine significant differences between the samples. In addition, principal component analysis (PCA) was conducted on the air/liquid partition coefficients (O'Mahony, 1986). The significance level was $P < 0.01$ throughout the study.

3. Results and discussion

3.1. Influence of β-lactoglobulin concentration

The influence of βLG (0, 0.5, 0.7, 1.0, 2.0%) and pH (3, 6, 9) on the air/liquid partition coefficients of 20 aroma compounds was studied by measuring the static headspace concentrations of the compounds. The aroma compounds examined included six alcohols (1-propanol, 1-butanol, 3-methyl-1-butanol, 2-pentanol, 1-hexanol, 2-nonanol), five ketones (diacetyl, 2-butanone, 2-heptanone, 2-octanone, 2-decanone), four esters (ethyl acetate, propyl acetate, butyl acetate, ethyl butyrate), three aldehydes (hexanal, heptanal, octanal), a terpene (α-pinene), and a sulfur compound (dimethyl sulfide). The selection of the 20 compounds was based on their functional groups, chain length and odour properties (Arctander, 1994). The headspace concentrations were determined by static headspace gas chromatography, and the air/liquid partition coefficients subsequently calculated (Table 1). Air/liquid partition coefficients indicate the affinity of the aroma compounds for the specific matrix. Some of the air/water partition coefficients could be compared with independent literature values and showed similarity, e.g. those of ethyl acetate, propyl acetate and butyl acetate (Kieckbusch & King, 1979).

For an overview of the influence of βLG and pH on the retention of the aroma compounds, PCA was conducted. Sample scores in the PCA map (Fig. 1) showed that an increase in the βLG concentration resulted in higher negative scores on the first principal component. The scores correlated with a general decrease of the air/liquid partition coefficients, with a more pronounced decrease for the larger, hydrophobic compounds. The decrease of the air/liquid partition coefficients indicate increased retention with higher βLG concentrations. MANOVA of the data revealed that the βLG concentration had an overall effect on the partition coefficients [$F(4,600) = 705.586$, $P < 0.01$]. The compounds responded significantly different to the βLG at the three pH levels [$F(19,600) = 6991.567$, $P < 0.01$], which is clearly illustrated by the relative decrease of the air/liquid partition coefficients of the 20 compounds and the various conditions in Fig. 2. βLG changed the partition coefficients of 17 of the 20 aroma compounds significantly (Table 2). Nearly all compounds showed lower partition coefficients with increased βLG concentrations, except for a few smaller sized compounds. Ethyl acetate, propanol and butanol revealed slightly increased partition coefficients (<5%) at low βLG levels. This is likely to be due to a 'salting-out' effect. The effect of βLG was compound dependent, which is

Table 1
Air/liquid partition coefficients ($k \times 1000$) and coefficients of variance (CV) of 20 aroma compounds in water with various β-lactoglobulin concentrations at three pH levels ($n = 3$)

Compound		pH3					pH6					pH9				
		0%	0.5%	0.7%	1.0%	2.0%	0%	0.5%	0.7%	1.0%	2.0%	0%	0.5%	0.7%	1.0%	2.0%
1	Dimethyl sulfide	53.97	50.33	45.91	44.50	41.14	48.90	47.14	40.21	42.89	38.11	57.56	58.13	56.59	46.48	45.46
2	1-Propanol	1.23	1.22	1.18	1.15	1.16	1.15	1.18	1.15	1.16	1.15	1.95	1.90	1.89	1.88	1.86
3	Diacetyl	1.91	1.82	1.79	1.76	1.75	1.86	1.86	1.77	1.76	1.71	1.79	1.78	1.74	1.68	1.61
4	2-Butanone	3.76	3.60	3.54	3.44	3.45	3.74	3.77	3.63	3.62	3.55	3.79	3.52	3.53	3.40	3.39
5	Ethyl acetate	9.42	9.97	9.80	9.50	9.36	9.86	9.88	9.49	9.49	9.34	9.48	9.76	9.83	9.35	9.29
6	1-Butanol	0.89	0.91	0.90	0.90	0.90	0.86	0.84	0.84	0.84	0.83	0.89	0.88	0.87	0.86	0.86
7	2-Pentanol	1.48	1.47	1.44	1.42	1.40	1.49	1.46	1.40	1.38	1.36	1.47	1.42	1.41	1.37	1.32
8	Propyl acetate	12.93	12.88	12.48	12.09	11.69	13.07	12.60	11.86	11.90	11.51	12.55	11.96	11.85	11.03	10.74
9	3-Methyl-1-butanol	1.21	1.22	1.21	1.20	1.18	1.18	1.13	1.12	1.11	1.09	1.25	1.21	1.21	1.20	1.17
10	Ethyl butyrate	18.87	18.23	17.03	16.30	14.91	20.16	19.01	17.04	17.24	15.65	18.97	17.58	17.06	15.17	14.21
11	Hexanal	17.67	17.29	15.58	14.94	13.23	16.81	15.12	12.07	12.46	10.34	18.03	15.37	13.79	11.51	9.34
12	Butyl acetate	16.51	15.64	14.66	14.01	12.92	16.30	14.40	13.01	13.07	11.97	15.78	14.81	14.30	12.83	12.01
13	1-Hexanol	0.53	0.45	0.45	0.45	0.45	0.48	0.46	0.45	0.45	0.45	0.48	0.45	0.45	0.45	0.45
14	2-Heptanone	9.38	9.00	8.36	7.96	7.17	9.08	8.10	7.32	7.21	6.42	9.33	7.93	7.58	6.89	6.26
15	Heptanal	20.94	18.41	15.67	14.50	11.59	19.82	16.01	11.95	12.11	9.08	19.86	15.55	13.30	10.45	7.80
16	α-Pinene	5.23	2.33	1.32	0.39	0.17	1.49	0.44	0.30	0.18	0.12	0.25	0.94	0.73	0.25	0.16
17	2-Octanone	12.36	10.59	9.43	8.61	7.06	12.51	10.45	8.87	8.56	6.72	12.47	10.45	9.61	8.05	6.63
18	Octanal	20.34	14.20	11.14	9.55	6.51	19.36	8.16	5.52	5.40	3.41	19.10	4.87	3.98	2.82	1.91
19	2-Nonanol	0.79	0.82	0.74	0.72	0.66	0.61	0.60	0.59	0.59	0.59	0.64	0.60	0.59	0.64	0.64
20	2-Decanone	25.74	14.49	10.36	8.15	4.45	26.13	10.22	6.55	5.95	3.23	29.10	10.17	8.08	5.19	3.32
	CV [%]	12.51	1.69	0.90	0.82	2.98	5.35	13.02	8.54	2.59	2.46	10.01	1.50	1.92	9.71	10.18

indicated by the MANOVA results which showed significant interaction between βLG concentration and the compounds [$F(76,600) = 78.227$, $P < 0.01$]. The response of the compounds to βLG varied considerably (>factor 100) and resulted in a considerable imbalance of the total aroma.

The effect of βLG is in agreement with other studies, e.g. Roozen and Legger (1998) reported a difference of ca. 75% in the air/liquid partition coefficient of 2-nonanone in water with and without 2% βLG. Up to 95% binding at 2% βLG was observed (for α-pinene), which is very likely to exert a considerable effect on perception of the specific compounds. Guichard and Langourieux (2000) reported significant odour changes of 2-heptanone and 2-octanone in water with addition of 1% βLG. Andriot, Harrison, Fournier, and Guichard (2000) conducted mathematical modeling to estimate relative partition coefficients for 2-heptanone, 2-octanone and 2-nonanone in water with βLG at pH3. The present partition coefficients are slightly lower than those in their paper, which can be explained by the fact that the values were based on water with 50 mM NaCl. Andriot, Marin, Feron, Relkin, and Guichard (1999) showed that the presence of salt decreased binding of aroma compounds. The latter authors assumed that the decreased binding was a consequence of a salting out effect or due to a change in polarity at the protein surface by the presence of salt.

Reduction in headspace concentration of the aroma compounds in the present study is more pronounced with higher βLG concentrations, but not proportionally. The aroma concentration appeared to be more limited than the number of binding sites (protein concentration). At the concentrations used in the present study (0.001% = 10 ppm) it is not likely that saturation of βLG takes place according to studies of Charles, Bernal, and Guichard (1996). In contrary, Andriot et al. (2000) showed that the dynamic release of 2-octanone from water was changed proportionally with the βLG concentration. This effect on aroma release may have originated from mass transfer factors rather than partitioning factors.

The effect of βLG on the partition coefficients was selective, it was more pronounced for the larger sized compounds (Table 1; Fig. 2). Especially α-pinene (95%), 2-octanone (47%), octanal (90%) and 2-decanone (89%) headspace concentrations were reduced efficiently by βLG at 2% level and pH9, but also at lower concentration levels. These results are in agreement with data of Andriot et al. (2000), who reported that larger methyl ketones were more retained than smaller ones.

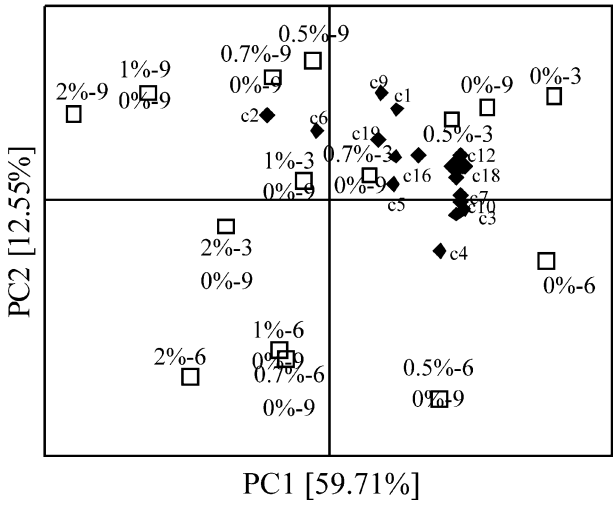

Fig. 1. Results of principal component analysis: scores of samples (β-lactoglobulin concentration-pH; □) and loadings of 20 aroma compounds (▲) on the first (PC1) and second principal component axes (PC2). Reference numbers refer to compounds in Table 1.

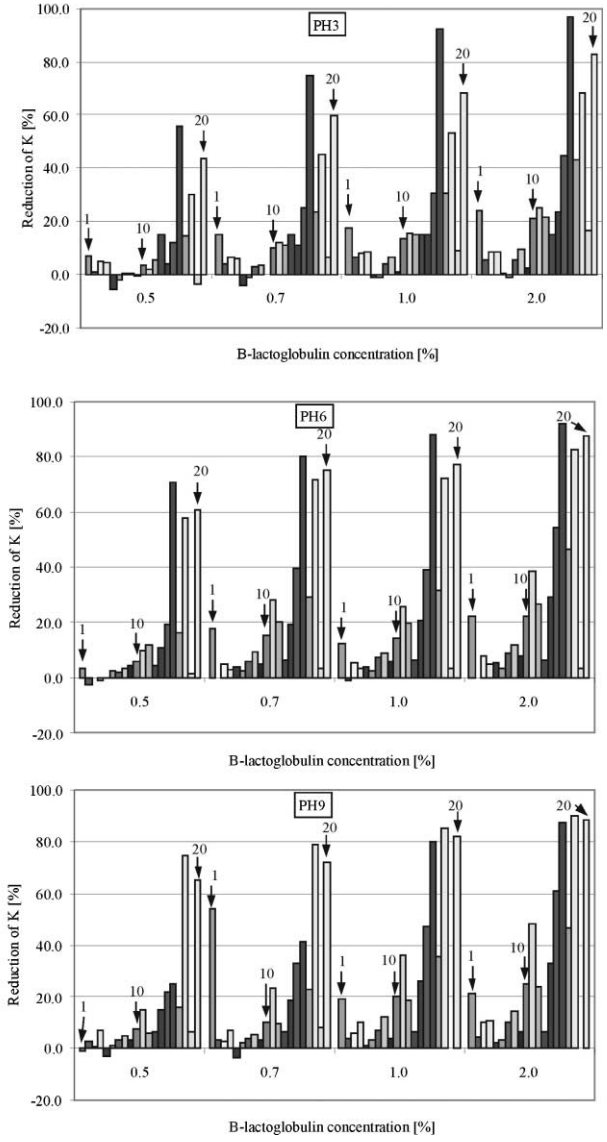

Fig. 2. Reduction of air/liquid partition coefficients (K) of 20 aroma compounds in water with various β-lactoglobulin concentrations (0.5, 0.7, 1.0, 2.0%) and at three pH levels ($n = 3$). Reference numbers refer to compounds in Table 1.

Apart from the general difference caused by chain length, a difference was observed among the functional group series: alcohols, ketones, aldehydes and esters. The linear relationship between the chain length of the homologous series of the compounds and their air/liquid partition coefficients for the various βLG concentrations and pH levels were evaluated: correlation coefficients, and slope and intercept values are shown in Table 3. The intercept values at 0% βLG indicate the affinity of the compounds for the water matrix, high values coincide with high air/liquid partition coefficients and thus relatively low affinity for water of the smaller compounds of the various groups. Smaller sized esters showed highest affinity for water, followed by alcohols, ketones and aldehydes, respectively.

Reasonable correlations between the number of carbon atoms and air/liquid partition coefficients were found for the ketones, aldehydes and esters. Alcohols were not bound as efficiently as the other compounds (Fig. 2). Chain length of the alcohols was not correlated with extent of binding, which is indicated by its low correlation coefficient and slope value (Table 3). The differences in slope value for the group of compounds with higher βLG concentrations show the affinity of the larger compounds for βLG. Slope values changed more pronounced with βLG concentration for aldehydes and esters. The ketones showed a smaller change and alcohol slope values did not change at all. The results of the ketones and aldehydes are likely to be related to increased binding to βLG, which was demonstrated by Sostmann and Guichard (1998). They reported a linear relationship between binding constants and the number of carbon atoms for ketones and unsaturated aldehydes. In contrary to present results, they showed same for unsaturated alcohols.

Table 2
Analysis of variance results: probability levels [%] associated with *F*-values of the two factors β-lactoglobulin concentration (BLG) and pH for the air/liquid partition coefficients of 20 aroma compounds in water with various β-lactoglobulin concentrations at three pH levels[a]

	BLG	PH
Dimethyl sulfide	**0.0**	**0.0**
1-Propanol	5.7	**0.0**
Diacetyl	**0.0**	**0.0**
2-Butanone	**0.0**	**0.0**
Ethyl acetate	**0.0**	6.6
1-Butanol	53.8	1.4
2-Pentanol	**0.0**	**0.0**
Propyl acetate	**0.0**	**0.0**
3-Methyl-1-butanol	**0.0**	**0.0**
Ethyl butyrate	**0.0**	**0.0**
Hexanal	**0.0**	**0.0**
Butyl acetate	**0.0**	**0.0**
1-Hexanol	**0.1**	42.7
2-Heptanone	**0.0**	**0.0**
Heptanal	**0.0**	**0.0**
α-Pinene	**0.0**	**0.0**
2-Octanone	**0.0**	27.0
Octanal	**0.0**	**0.0**
2-Nonanol	11.4	**0.0**
2-Decanone	**0.0**	**0.5**

[a] In bold: significant probabilities at a 1% level.

3.2. Influence of pH

The three pH levels were separated consistently on the PCA map (Fig. 1). They were mainly separated on the second principal component, with highest scores for pH9, followed by pH3. Samples of pH6 showed lowest scores on the second principal component. Out of the three pH levels, the pH3 samples correlated best with the loadings of the volatile compounds. The correlation indicated that highest air/liquid partition coefficients were found for pH3, and more diverting ones for the two other pH levels. However, the diversion of pH6 and pH9 was not in the same direction. Especially alcohols (c2, c6, c9, c19) correlated reasonably well with pH9 samples. It implies that alcohols were more strongly retained at pH6 than at pH9. MANOVA revealed a significant effect of pH for 16 out of the 20 compounds [$F(2,600) = 80.703$, $P < 0.01$, Table 2]. The effect was compound related, which was demonstrated by the significant interaction between pH and compounds [$F(38,600) = 36.302$, $P < 0.01$]. Fig. 2 demonstrates the relative effect of pH on the partition coefficients of the compounds. For most compounds, except for α-pinene (c16) reduction of the partition coefficient was lowest at pH 3, higher at pH6 and highest at pH9. α-Pinene showed a different pattern with lowest reduction at pH9 and similar values at pH3 and pH6.

Similarities in response to pH have been reported in literature for other compounds. Jouenne and Crouzet (1998) reported increased affinity with increase of pH for limonene, β-ionone, α-ionone and terpenyl acetate up to pH9. The same authors (2000) demonstrated consistently lower activity coefficients of methyl ketones and ethyl esters with higher pH levels in the pH 3–9 range. Additionally, Roozen and Legger (1998) showed that the air/water partition coefficient of 2-nonanone decreased when the pH was increased from 3.5 to 7.4.

The effect of the pH on binding of most compounds by βLG can be explained by the structure release at higher pH levels. It has been reported that βLG undergoes three pH dependent conformational transitions (Wong, Camirand, & Pavlath, 1996). The first reversible transition occurs at pH 4–6. Only a contraction of the protein and ionisation of two groups takes place. The protein generally exists as a dimer resulting from the association of the monomer at the respective α-helical segments at the isoelectric pH of 5.2. Between pH 3.5 and 5.2 βLG tends to form octamers. Below pH 3.5, βLG dissociates into monomers due to electrostatic

repulsion between the subunits and is acid stable, resisting denaturation at pH2. At pH2 and low ionic strength, βLG has a monomeric form, exhibits a highly structured β-sheet core and less ordered regions, which results in a partially folded structure (Molinari et al., 1996). Therefore, βLG has a more rigid conformation at low pH (Shimuzu, Saito, & Yamauchi, 1985).

The second reversible conformational change occurs between pH 6.5–7.8 and is called the Tanford transition (Hambling, McAlpine, & Sawyer, 1992). There is an expansion of the volume of the protein and a variation in the shape, an increased dissociation of dimer to monomer. One buried carboxyl per subunit becomes exposed and ionised. The free sulfydryl also becomes more accessible when the pH is raised from 6 to 8. No large change in the folding of the polypeptide occurs, rather a subtle rearrangement of side chains has been observed. The increase in flexibility could improve the accessibility to the binding sites (Kella & Kinsella, 1988). Above pH 8, alkali denaturation occurs. As the pH rises from 8 to 9, the sedimentation coefficient decreases to a value consistent with a monomeric structure and it remains at this value up to pH 10. Modifications of the ternary structure of the protein becomes irreversible after pH 9.3–9.5 and only a residual structure of the β-barrel could persist (Casal, Köhler, & Mantsch, 1988).

According to present results, especially larger aldehydes and esters benefit from the conformational change (larger slope difference Table 3). The alcohols and α-pinene, however, show a more diverting behaviour. The change in retention of most of the aroma compounds with pH, indicates the importance of the conformation of the protein for the exposure of binding sites. The conformation of the protein certainly will affect the accessibility of the hydrophobic pocket, but it could also influence the accessibility of the secondary external binding sites to some extent as has been extensively discussed by Jouenne and Crouzet (2000). The opposite behaviour of α-pinene with more extensive binding at pH3 might imply a binding site at the surface rather than in the interior of the protein.

3.3. Interaction between β-lactoglobulin concentration and pH

Statistical analysis of the data presented in Sections 3.1 and 3.2 demonstrated significant interactions between βLG concentration and pH [$F(8,600) = 7.629$, $P < 0.01$)]. These results imply that the effect of the pH depends on the concentrations βLG and vice versa. The effects of the two factors are obviously not complementary, but should be considered an integrated phenomenon. In addition, significant interactions between βLG concentration, pH and compounds were determined [$F(152,600) = 2.630$, $P < 0.01$)], which complicates the situation even further.

3.4. Presence of other aroma compounds

The influence of the presence of other aroma compounds on the air/liquid partition coefficients of compounds in water with βLG was evaluated (Fig. 3). The compounds were added together and individually to

Table 3
Relationships determined by linear regression between the chain length of homologous series of alcohols, ketones, aldehydes and esters, and air/liquid partition coefficients ($k \times 1000$) of aroma compounds in water with various β-lactoglobulin concentrations and at three pH levels: Pearson's correlation coefficients, slopes and intercepts

	pH3					pH6					pH9				
	0%	0.5%	0.7%	1.0%	2.0%	0%	0.5%	0.7%	1.0%	2.0%	0%	0.5%	0.7%	1.0%	2.0%
Correlation coefficient															
Alcohols	−0.47	0.44	−0.49	−0.50	−0.56	−0.55	−0.47	−0.58	−0.59	−0.59	−0.65	−0.71	−0.71	−0.69	−0.69
Ketones	0.94	1.00	0.97	0.87	0.32	0.93	0.93	0.67	0.59	0.06	0.92	0.93	0.83	0.50	0.11
Aldehydes	0.77	−0.71	−0.86	−0.90	−0.96	0.79	−0.81	−0.87	−0.89	−0.94	0.58	−0.86	−0.89	−0.92	−0.95
Esters	0.97	0.95	0.95	0.95	0.94	0.93	0.87	0.85	0.85	0.82	0.94	0.93	0.92	0.92	0.90
Slope															
Alcohols	−0.08	−0.07	−0.08	−0.08	−0.09	−0.10	−0.09	−0.10	−0.10	−0.10	−0.13	−0.19	−0.19	−0.17	−0.17
Ketones	0.25	0.55	0.80	0.90	0.43	0.24	0.75	0.76	0.70	0.08	0.21	0.73	0.80	0.61	0.16
Aldehydes	1.34	−1.55	−2.22	−2.70	−3.36	1.28	−3.48	−3.28	−3.53	−3.47	0.54	−5.25	−4.91	−4.35	−3.72
Esters	4.19	3.54	3.05	2.85	2.27	4.27	3.48	2.80	2.87	2.24	4.03	3.31	3.01	2.38	1.95
Intercept															
Alcohols	1.40	1.37	1.38	1.37	1.41	1.45	1.35	1.42	1.42	1.40	1.68	2.05	2.04	1.98	1.95
Ketones	4.04	2.04	0.95	0.94	4.87	4.11	1.12	2.23	2.84	6.87	4.38	1.42	1.52	3.66	6.48
Aldehydes	10.31	27.45	29.67	31.86	33.96	9.74	37.46	32.77	34.70	31.87	15.25	48.68	44.69	38.68	32.36
Esters	−7.58	−4.38	−2.54	−1.98	0.29	−7.59	−4.27	−1.87	−2.15	0.35	−6.95	−3.85	−2.53	−0.42	1.32

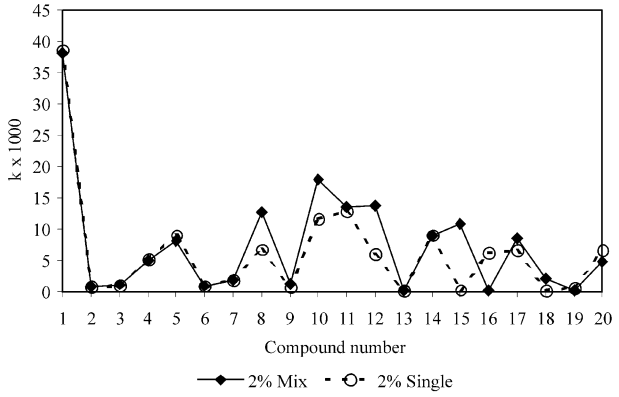

Fig. 3. Air/liquid partition coefficients of 20 aroma compounds added individually and in a mixture to water with 2% β-lactoglobulin ($n = 3$). Compound numbers refer to compounds in Table 1.

water with 2% βLG at pH6. Some compounds showed increased partition coefficients when present with others, i.e. propyl acetate, ethyl butyrate, butyl acetate, hexanal, heptanal and octanal. Remarkable is that they are all either esters or aldehydes. These effects are solely related to the presence of βLG as preliminary experiments demonstrated no effect of the compounds on each other in a 100% water matrix. The esters and aldehydes showed also highest affinity for the βLG and the results indicate some form of competition for the binding sites at βLG. The results differ from data of Jouenne, Chalier, and Crouzet (2000) who, although they reported competition, reported competition for compounds with different functional groups (ketones and esters). They showed that the competition was concentration and pH dependent, which is probably related to the accessibility to the binding sites. In the present work, again α-pinene demonstrated diverting behaviour with the compound showing lower partition coefficients when present in a mixture. Increased concentrations of 'free' aroma in the solution seem to increase the affinity of α-pinene for the matrix. The same was shown for this compound with increased binding at the lowest pH, which coincided with higher concentrations of 'free' aroma in the matrix

4. Conclusions

Static headspace experiments showed considerable effects of βLG concentration, pH, functional groups of compounds, chain length of compounds, as well as presence of other aroma compounds on the air/liquid partitioning of 20 aroma compounds. MANOVA indicated significant interactions between βLG concentration, pH and type of compounds. Therefore, the combined effect of all factors should be considered for correction of food aromas imbalanced by βLG.

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