



Contents lists available at ScienceDirect

International Dairy Journal

journal homepage: www.elsevier.com/locate/idairyj

Inherent and process-induced hydrophobicity influences aroma retention in fat-free dairy matrices

Andrej Heilig^{a,*}, Sümeyye Çetin^a, Kerstin Erpenbach^b, Judith Höhn^c, Jörg Hinrichs^a

^a Department of Dairy Science and Technology, Institute of Food Science and Biotechnology, Universität Hohenheim, Garbenstr. 21, 70599 Stuttgart, Germany

^b CSM Deutschland GmbH, Theodor-Heuss-Allee 8, 28215 Bremen, Germany

^c Chemisches und Veterinäruntersuchungsamt Stuttgart, Schaflandstr. 3/2, 70736 Stuttgart, Germany

ARTICLE INFO

Article history:

Received 10 July 2010

Received in revised form

24 December 2010

Accepted 4 January 2011

ABSTRACT

It was shown that both inherent and process-induced hydrophobicity can influence the aroma retention in fat-free dairy matrices. The extent and direction to which these parameters take effect depends on the composition of the dairy matrix as well as on the hydrophobicity (log P) of the aroma compounds. The phase ratio variation (PRV) method was used to determine the matrix–air partition coefficient K_{MG} . For the hydrophobic aroma compound limonene, K_{MG} increased when the ratio of casein protein to whey protein was increased in model milk, while the less hydrophobic ethyl hexanoate and the hydrophilic diacetyl remained unaffected. This was attributed to the hydrophobic character of the casein micelle. Upon acidification with glucono- δ -lactone, K_{MG} of diacetyl decreased significantly while K_{MG} of ethyl hexanoate and limonene slightly increased. This was attributed to the predominance of hydrophobic interactions in acidified dairy matrices, which were analysed via bonds analysis using destabilising buffer solutions.

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1. Introduction

Along with texture and taste, the aroma properties of a food product are crucial for consumer acceptance. The interplay of ever changing consumer demands, reformulations in food composition and new production techniques requires a high understanding of the aroma compound–dairy matrix interaction to maintain constant product quality.

The extent to which the dairy matrix composition influences retention and release of a given aroma compound strongly depends on the physico-chemical properties of the compound (Voilley & Souchon, 2006). In most cases the term “aroma” describes a multitude of single aroma compounds that are present in appropriate amounts to generate the desired aroma impression in a dairy matrix of defined composition. When this composition is altered, the retention and release of each aroma compound will be differently affected and the respective effect has to be met accordingly. Otherwise, such an adaptation is likely to result in an unbalanced and unpleasant aroma profile (de Roos, 2006).

As the economic relevance of low caloric dairy products will further increase, it is important to understand the aroma–matrix

interaction in fat-free dairy based matrices. In such matrices, higher contents of whey protein (Van Ruth & Villeneuve, 2002) or casein protein (Fischer & Widder, 1997; Landy, Druaux, & Voilley, 1995) have been shown to lower the concentration of many aroma compounds in the gas phase, i.e., to increase aroma retention in the matrix under equilibrium conditions. Most studies that investigated the aroma–dairy matrix interaction either focused on very simplified matrices or on highly complex varieties. With regard to the former, matrices consisting of pure β -lactoglobulin (Andriot, Harrison, Fournier, & Guichard, 2000; Andriot, Marin, Feron, Relkin, & Guichard, 1999; Lubke, Guichard, Tromelin, & Le Queré, 2002; Reiners, Nicklaus, & Guichard, 2000; Tavel, Andriot, Moreau, & Guichard, 2008; Van Ruth & Villeneuve, 2002), α -lactalbumin (Fabre, Aubry, & Guichard, 2002) or sodium caseinate (Fares, Landy, Guillard, & Voilley, 1998; Kuehn, Zhu, Considine, & Singh, 2007; Landy et al., 1995) were investigated to establish a fundamental understanding of the dairy protein–aroma interaction on a molecular level, but largely neglected the complex physico-chemical characteristics of the dairy protein matrix. With regard to complex matrices, real food-like systems such as (model-) yoghurt (Brauss, Linforth, Cayeux, Harvey, & Taylor, 1999; Decourcelle, Lubbers, Vallet, Rondeau, & Guichard, 2004; Deleris, Lauverjat, Trelea, & Souchon, 2007; Deleris, Zouid, Souchon, & Trelea, 2009; Kopjar, Andriot, Saint-Eve, Souchon, & Guichard, 2010; Kora, Souchon, Latrielle, Martin, & Marin, 2004; Lubbers, Decourcelle, Martinez,

* Corresponding author. Tel.: +49 711 459 24438; fax: +49 711 459 23617.

E-mail address: ha-lth@uni-hohenheim.de (A. Heilig).

Table 1
Concentration and supplier specification of the strawberry aroma-based composition of volatile compounds used in this study.

Compound	% (w/w) in aroma	ppm (w/w) in final product	log P octanol/water ^a	CAS-No.
Limonene ^b	1.20	120	+4.5	138-86-3
Ethyl hexanoate	0.60	60	+2.8	123-66-0
(Z)-3-hexenyl acetate	0.60	60	+2.4	3681-71-8
γ -Decalactone	0.30	30	+2.4	706-14-9
Methyl cinnamate	0.30	30	+2.2	1754-62-7
Ethyl-2-methylbutanoate	1.00	100	+2.1	7452-79-1
Hexanoic acid	1.80	180	+1.8	142-62-1
Ethyl butanoate	0.80	80	+1.8	105-54-4
(Z)-3-hexenol	1.00	100	+1.6	928-96-1
2-Methyl butyric acid	1.80	180	+1.1	116-53-0
Furaneol	0.27	27	+0.3	3658-77-3
Diacetyl ^b	1.20	120	-1.3	431-03-8
Propylene glycol	89.13	–	–	57-55-6

^a log P (hydrophobicity) calculated by (ACD/Labs) Software V8.14 (Advanced Chemistry Development Inc., Toronto, Canada).

^b Compounds were added to commercial strawberry aroma.

Guichard, & Tromelin, 2007; Lubbers, Decourcelle, Vallet, & Guichard, 2004; Merabtine, Lubbers, Andriot, Tromelin, & Guichard, 2010; Nongonierma et al., 2007; Nongonierma, Springett, Le Queré, Cayot, & Voilley, 2006; Perreault et al., 2010; Saint-Eve, Juteau, Atlan, Martin, & Souchon, 2006), (model-) custard (Gonzalez-Tomas, Bayarri, Taylor, & Costell, 2007, 2008; Guth & Rusu, 2008; Lethuaut et al., 2005; Lethuaut, Weel, Boelrijk, & Brossard, 2004; Martuscelli, Savary, Pittia, & Cayot, 2008) and (model-) milk (Fabre et al., 2002; Meynier, Garillon, Lethuaut, & Genot, 2003; Relkin, Fabre, & Guichard, 2004; Seuvre, Espinosa-Diaz, Cayot, & Voilley, 2004) gave results that could be of direct benefit to the manufacturer. As many of the systems investigated in the above named studies either contained fat, hydrocolloids and sugar alone or in combination, or were subjected to different mechanical treatments, it is difficult to link the observed effects to the fundamental principles that lie beneath the aroma compound–dairy matrix interaction.

How aroma compounds are distributed under equilibrium promotes understanding of the physico-chemical principles of the aroma–matrix interaction. The distribution of different aroma compounds between the matrix phase and the gas phase under equilibrium conditions is expressed by the matrix–gas partition coefficient K_{MG} , which describes the ratio of the concentration of the aroma compound between the matrix phase C_M and the gas phase C_G . Recent research successfully applied the phase ratio variation (PRV) method to determine the partition coefficient in various food matrices (Deleris et al., 2007, 2009; Jouquand, Ducruet, & Giampaoli, 2004; Kopjar et al., 2010; Martuscelli et al., 2008; Merabtine et al., 2010; Saint-Eve et al., 2006; Savary,

Guichard, Doublier, & Cayot, 2006; Tehrani, Mouawad, & Desobry, 2007).

The present study aimed to clarify aroma compound–dairy matrix interactions depending on protein phase properties by specifically investigating the effect that two of the major compositional and processing parameters, namely the casein to whey protein ratio and the acidification process, have on the aroma compound distribution under equilibrium conditions. In contrast to previous studies, the adopted combination of whey protein isolate and micellar casein instead of skim milk powder, single casein fractions or caseinates allowed for the specific adjustment of the casein protein to whey protein ratio while at the same time maintaining the structural integrity of the casein micelle. Non-acidified aromatised model milks consisting of micellar casein, native whey protein and ultrafiltration permeate were studied with regard to the K_{MG} -values of differently hydrophobic aroma compounds, namely diacetyl, ethyl hexanoate and limonene. Subsequently, the effect of model milk acidification with glucono- δ -lactone on the K_{MG} -value was determined and linked to the physico-chemical properties of the acidified dairy matrix.

2. Materials and methods

2.1. Aroma composition

The aroma composition used for this study was based on a commercial strawberry aroma from Symrise AG, Holzminden, Germany. To extend the log P range of the aroma compounds under investigation, limonene and diacetyl, obtained from Symrise AG, were added. The final composition of the propylene glycol based aroma used in this study is reported in Table 1.

2.2. Model dairy matrix constituents

Table 2 lists the raw materials used to prepare the differently composed model dairy matrices.

2.3. Preparation of flavoured model dairy matrices

Ultrafiltration permeate was produced by reconstituting 5.2 g 100 g⁻¹ ultrafiltration permeate powder in distilled water. Model milk solutions were prepared by dissolving micellar casein powder and whey protein isolate powder in the ultrafiltration permeate at room temperature. By choosing appropriate amounts of micellar casein and whey protein isolate powder, the casein protein to whey protein ratio (CWR) in the model milk solutions was adjusted by taking into account the actual casein protein and whey protein content of the respective powders.

Table 2
Specification of the model dairy matrix constituents.

Constituent	Commercial name, company, location	Dry matter (% w/w)	Protein (% w/w)	Fat (% w/w)	Lactose (% w/w)	Ash (% w/w)
Sweet whey ultrafiltration permeate powder	Bayolan PT, BMI, Landshut, Germany	96.1 ^c	3.5 ^c	0.1 ^c	83.0–87.0 ^b	8.2 ^c
Whey protein isolate powder	DSE 5627, Fonterra, Hamburg, Germany	94.5 ^c	93.6 ^{b,c,d}	0.3 ^b	0.5 ^b	1.6 ^c
Micellar casein powder ^a		96.5 ^c	69.8 ^{c,e}	0.5 ^c	17.8 ^c	7.6 ^c

^a Micellar casein concentrate was produced in-house by microfiltration–diafiltration following the method described by Kersten (2001). Micellar casein concentrate was spray dried at a temperature of 190 °C to obtain micellar casein powder.

^b Supplier specification.

^c In-house analysis according to VDLUFA VI, C 35.6, 1985 (dry matter); IDF 185:2002 (protein); IDF 9C:1987 (fat); IDF 198:2007 (lactose), VDLUFA IV, C 10.2, 2000 (ash).

^d β -Lactoglobulin 66.9%, α -lactalbumin 17.4%, glycomacropeptide 4.4%, bovine serum albumin 2.2%, IgG 1.4%, supplier specification.

^e Whey protein 0.2%, in-house analysis according to IDF 178:2005.

All solutions were batch pasteurised at 65 °C with a holding time of 30 min at this temperature. Once the heating time was completed, the solutions were immediately cooled down to around 10 °C in ice water and then stored overnight at 4 °C. The dynamic light scattering measurement of a pasteurised model milk solution with a CWR of 100:0 showed a mean particle diameter of 187 nm, which is in the range of casein micelle size reported in the literature (Horne & Dalgleish, 1985; Kruif, 1998; O'Connell & Fox, 2000). The pH of the model milk solutions was 6.75 ± 0.06. After storage, model milk solutions were flavoured with 1.0% (w/w) of the aroma composition. Flavoured model milks at pH 6.75 were liquid independent of their protein content or CWR.

Model yoghurt matrices with a protein content of 4, 8 and 12% were produced from flavoured model milk solutions (CWR = 80:20) by slowly adding 1.64%, 2.72% and 3.66% (w/w) of glucono-δ-lactone GDL (Art.-No. 49210, CAS 604-69-3, Sigma-Aldrich, Munich, Germany) within 3 min. By further agitation for 7 min with a magnetic stirrer, complete dissolution of the GDL was ensured. The solution was filled into headspace vials and then incubated in a 35 °C water bath for 300 min to reach a final pH of 4.31 ± 0.05. Once the incubation time was completed, the model yoghurts were cooled to 10 °C in a 10 °C water bath and stored overnight at 10 °C until headspace analysis. After storage, the pH of the model yoghurt matrices was 4.23 ± 0.01. After acidification, all matrices were in a gelled state, comparable to set-type yoghurt, independent of their protein content.

2.4. Determination of the matrix/gas partition coefficient K_{MG}

2.4.1. Gas chromatography headspace analysis

Vials containing flavoured model milk or model yoghurt were equilibrated for 15 and 30 min, respectively, at 40 °C in an automatic headspace sampler QHSS40 (QUMA Elektronik & Analytik GmbH, Wuppertal, Germany). During equilibration, vials containing liquid samples were gently agitated using the QHSS40 integrated shaker. Using the procedure described by Savary et al. (2006), it was checked that the aroma compound concentration in the headspace had reached its maximum after the respective equilibration times. Following equilibration, 1 mL of headspace was automatically withdrawn. Only one headspace injection was made per vial, and at least three vials per sample volume were analysed. The valve temperature was set to 90 °C and the tube temperature to 150 °C.

Headspace analysis was performed on a CP-3800 gas chromatograph (Varian Deutschland GmbH, Darmstadt, Germany), equipped with a split/splitless injector CP-1177 and a flame ionisation detector (FID). For samples containing less than 12% protein, a split-ratio of 1:50 was selected, while for samples with 12% protein, a split-ratio of 1:20 was chosen. The injector temperature was set at 240 °C. The FID was operated at 250 °C with H₂ and synthetic air at flow rates of 28 mL min⁻¹ and 300 mL min⁻¹, respectively. N₂ was used as make up gas with a flow rate of 30 mL min⁻¹. The volatile compounds were injected into a deactivated silica-coated pre-column with an inner diameter of 0.53 μm and a length of 5 m, which was followed by an HP-FFAP capillary column with an inner diameter of 0.32 mm, a film thickness of 0.25 μm and a length of 30 m (Agilent Technologies, Waldbronn, Germany). The carrier gas was H₂ at a flow rate of 2 mL min⁻¹. The oven program started at 40 °C for 5 min, followed by heating up with 5 °C min⁻¹ to 100 °C and 40 °C min⁻¹ to 240 °C with a holding time of 5 min.

2.4.2. Analysis of headspace gas chromatography data

The determination of the matrix/gas partition coefficient K_{MG} was performed by means of the phase ratio variation (PRV) method

as described by Ettre, Welter, and Kolb (1993). Different volumes (50, 75, 100, 150, 200, 500, 1000, 2000 μL) of the flavoured dairy matrix were transferred into headspace vials of 22 mL (QUMA Elektronik & Analytik GmbH, Wuppertal, Germany). After filling, the vials were immediately sealed with PTFE septa in metallic caps (QUMA Elektronik & Analytik GmbH). For model yoghurt matrices, the sealed vials were incubated as described above.

As an indirect analytical method, the PRV method employs the coherence of volatile compound concentration in the headspace, given by the chromatographic peak area A , and the phase ratio β among headspace volume V_G and matrix volume V_M , to determine the partition coefficient K .

$$1/A = \left(\frac{1}{f_i \cdot C_M} \cdot K \right) + \left(\frac{1}{f_i \cdot C_M} \right) \cdot \beta \quad (1)$$

with f_i as a proportional factor and C_M as the initial aroma compound concentration in the matrix. This linear equation can be simplified to

$$1/A = a + b \cdot \beta \quad (2)$$

After plotting $1/A$ against β , the matrix/gas partition coefficient K_{MG} is calculated from the slope b and the intercept a .

$$K_{MG} = a/b \quad (3)$$

Most studies that employ the PRV method focus on the release instead of the retention of aroma compounds and report the gas/matrix partition coefficient K_{GM} instead of the K_{MG} -value (Deleris et al., 2007, 2009; Jouquand et al., 2004; Kopjar et al., 2010; Martuscelli et al., 2008; Merabtine et al., 2010; Saint-Eve et al., 2006; Savary et al., 2006).

$$K_{GM} = \frac{1}{K_{MG}} \quad (4)$$

2.5. Analysis of bonding conditions

The relative amounts of protein stabilised by either disulfide bonds (SS), hydrophobic interactions (HY), calcium bridges (CA) or electrostatic interactions (EI) were determined according to a slightly modified method described by Hinrichs and Keim (2007), Keim and Hinrichs (2004) and Keim, Kulozik, and Hinrichs (2006) for pressure, rennet and acid-induced casein gels. This method uses various destabilising buffer solutions to cleave the above named protein–protein interactions. Experimental conditions, i.e., buffer composition, buffer to sample ratio, reaction time, centrifugation settings etc. were in accordance with Keim (2005). After centrifugation, the supernatant was removed and the relative amount of protein dissolved in the supernatant $C_{P,dis}$ was calculated as

$$C_{P,dis} = \frac{m_{sup} \cdot C_{P,sup} - m_{buf} \cdot C_{P,buf}}{m_{mdm} \cdot C_{P,mdm}} \cdot 100 \quad (5)$$

where $C_{P,dis}$ is the amount of dissolved protein in %, m_{sup} is the mass of supernatant in g, $C_{P,sup}$ is the protein content of the supernatant in %, m_{buf} is the mass of destabilising buffer in g, $C_{P,buf}$ is the protein content of the buffer in %, m_{mdm} is the mass of model dairy matrix in g and $C_{P,mdm}$ is the protein content of the model dairy matrix in %.

In our study, the supernatant obtained after centrifugation was filtered through a Chromofil RC-45/25 syringe filter with 0.45 μm pore width (Macherey-Nagel GmbH, Düren, Germany) to ensure the removal of protein lumps. It was checked beforehand that filtration did not remove dissolved protein from the supernatant.

2.6. Statistics

All K_{MG} -values reported in this study were calculated as the mean of at least three independent PRV analysis from at least two fully independent experiments. The matrix/gas partition coefficient K_{MG} of each analysis was derived from separate linear regression as described by Atlan, Trelea, Saint-Eve, Souchon, and Latrille (2006). Bonding condition results represent the mean of at least two fully independent experiments with two independent analysis each. Student's t-test ($p < 0.05$) and analysis of variance (ANOVA) was used to determine significant differences among the K_{MG} -values and bonding types. Error bars displayed in Figs. 1–3 represent the 95% confidence interval (Statgraphics Plus Version 5.1, Statpoint Technologies Inc., Warrenton, USA).

3. Results and discussion

3.1. Performance of the headspace gas chromatography and phase ratio variation method

Using an aroma composition yields a headspace in which all of the aroma compounds are present simultaneously. Under the above named experimental conditions, only seven (limonene, ethyl hexanoate, (Z)-3-hexenyl acetate, ethyl-2-methylbutanoate, ethyl butanoate, (Z)-3-hexenol and diacetyl) of the 12 added aroma compounds were detected and hence analysed regarding the matrix/gas partition coefficient. Under comparable experimental conditions, other authors (Martuscelli et al., 2008; Saint-Eve et al., 2006; Savary et al., 2006) reported similar restrictions with regard to the non-detectable aroma compounds γ -decalactone, methyl cinnamate, hexanoic acid, 2-methyl butyric acid and furaeol, which are attributed to a combination of low volatility and low concentration of the named compounds.

A single headspace analysis will result in peak area values for all of the detectable aroma compounds. Albeit following the principles of the PRV method consequently yields K_{MG} -values for all of these compounds, only the results obtained for diacetyl, ethyl hexanoate and limonene will be presented and discussed here in detail. These three compounds were selected by their hydrophilic (diacetyl,

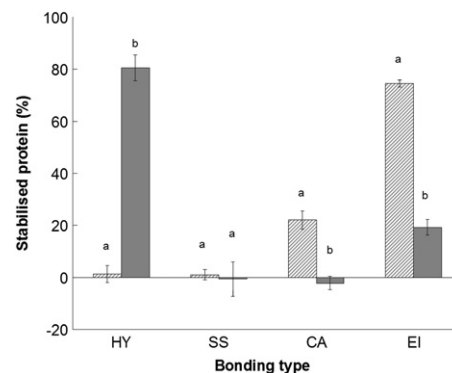


Fig. 2. Amount of protein in % of total protein that is stabilised by hydrophobic interactions (HY), disulfide bonds (SS), calcium bridges (CA) and electrostatic interactions (EI) in a fat-free model dairy matrix with a total protein content of 4% (CWR = 80:20) at different pH (▨ = 6.75, ■ = pH 4.23). Bars with different indices a - b at a given bonding type differ significantly ($p < 0.05$).

log $P = -1.3$), hydrophobic (ethyl hexanoate, log $P = +2.8$) and highly hydrophobic (limonene, log $P = +4.5$) character to represent the entity of the detectable compounds.

3.2. Casein protein to whey protein ratio

The share of casein protein in a model milk solution (pH 6.75) with 4% of total protein was varied between 0%, 20%, 40%, 60%, 80% and 100%. For a casein share of 80%, we obtained K_{MG} -values of 696 ± 114 , 27.0 ± 3.3 and 22.7 ± 2.3 for diacetyl, ethyl hexanoate and limonene, respectively. As Fig. 1 shows, it is only for limonene that a directional influence of the casein protein to whey protein ratio (CWR) can be observed. The higher the share of casein protein, the higher is the retention of limonene in the model milk solution.

As limonene is by far the most hydrophobic of the investigated aroma compounds, this observation may be best explained by the build-up of the casein micelle. Three major models have been proposed to explain the kinetics and the properties of the casein micelle: the submicelle model (Walstra, 1990), the Holt-model

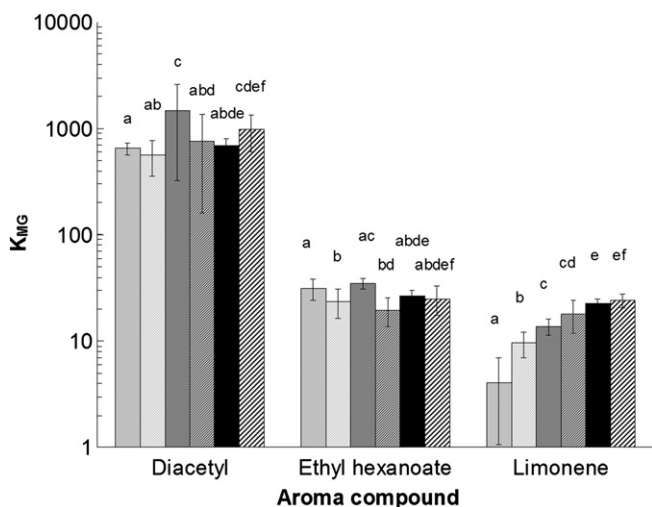


Fig. 1. Matrix/gas partition coefficient K_{MG} of diacetyl, ethyl hexanoate and limonene depending on the share of casein protein in % (■, 0%; ▨, 20%; ▩, 40%; ▪, 60%; ▫, 80%; ▬, 100%) in a fat-free model milk solution (pH 6.75) with a total protein content of 4%. Bars with different indices a - f at a given aroma compound differ significantly ($p < 0.05$).

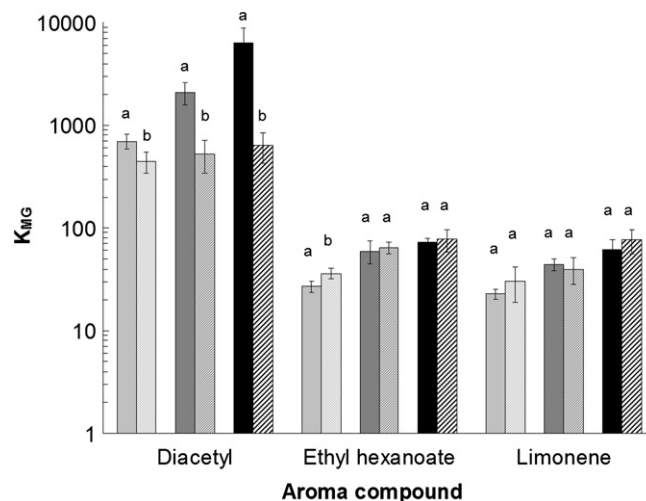


Fig. 3. Matrix/gas partition coefficient K_{MG} of diacetyl, ethyl hexanoate and limonene depending on the total protein content (CWR = 80:20) and pH of fat-free model dairy matrices (▨ = 4% protein and pH 6.75, ▨ = 4% protein and pH 4.23, ▩ = 8% protein and pH 6.75, ▩ = 8% protein and pH 4.23, ▪ = 12% protein and pH 6.75, ▪ = 12% protein and pH 4.23). Bars with different indices a - b at a given aroma compound differ significantly ($p < 0.05$).

(Holt, 1992) and the dual-binding model (Horne, 1998). Amidst their differences, the above named models give considerable attention to the existence of highly hydrophobic compartments within the casein micelle (Fox & Brodtkorb, 2008). These originate from the adsorption of $\alpha_{s1/52}$ casein molecules, which molecular structure contains two hydrophobic regions. Accordingly, conditions that favour the break-up of hydrophobic interactions, such as the addition of reagents like SDS (Lefebvre-Cases et al., 1998) or low temperature (Fox & Hoynes, 1976) will lead to the disintegration of the casein micelle. It was therefore suggested that the casein micelle acts as a natural carrier for hydrophobic substances like vitamin D₂ (Semo, Kesselman, Danino, & Livney, 2007). Vitamin D₂ is highly hydrophobic, with a log P of 7.3 (Wishart et al., 2008). That the retentive effect of a higher casein share is not seen for aroma compounds other than limonene may be due to the limited hydrophobic potential of the casein micelle.

An analysis of the micellar casein powder showed that there was some residual fat present in the powder (0.5 g 100 g⁻¹). Nevertheless, the observed effect of increased retention at higher casein content cannot be attributed to an, along with the casein content, increased fat content of the solution. As our own research on the aroma retention effect of milk fat has shown, ethyl hexanoate responds to an increased fat content in the same way that limonene does. As for ethyl hexanoate no effect is seen, the increased retention of limonene at higher CWR is therefore attributed to the characteristics of the casein micelle. We will present our results regarding the effect of milk fat content on the aroma–matrix interaction in a forthcoming paper.

3.3. Acidification

Previous research has pointed towards hydrophobic interaction between proteins as the driving force in acid gel formation (Hinrichs & Keim, 2007; Lefebvre-Cases et al., 1998; McMahon, Du, McManus, & Larsen, 2009). It was therefore investigated to which degree the hydrophobic stabilisation of a dairy matrix can lead to an increased retention of aroma compounds.

Fig. 2 displays the amount of protein in a model dairy matrix, in % of the total protein, that is stabilised by either hydrophobic interactions (HY), disulfide linkages (SS), calcium bridges (CA) or electrostatic interactions (EI) at a pH of 6.75 (model milk, liquid) or 4.23 (model yoghurt, gelled). As can be seen, the shift in pH is accompanied by a change in bonding conditions. At pH 6.75, most of the protein is associated by CA (22.1 ± 3.5%) and EI (74.6 ± 1.4%), while HY (1.3 ± 3.3%) and SS (1.0 ± 2.0%) can be neglected. Once the model milk has been acidified to pH 4.23 with GDL, most of the protein in the resulting gel network is stabilised by HY (80.7 ± 5.0%), along with a small share of EI (19.3 ± 3.1%). We have furthermore found that the proportion of protein that is stabilised by either HY, SS, CA or EI at a given pH remains constant regardless of the total protein content of an equally constituted and processed dairy matrix (results not shown).

Fig. 3 displays the K_{MC} -values of diacetyl, ethyl hexanoate and limonene in acidified and non-acidified dairy matrices with a protein content of 4%, 8% and 12%. The data show that the K_{MC} -value, i.e., the retention of diacetyl, ethyl hexanoate and limonene within the liquid model milk (pH 6.75) increases with increasing protein content. While for ethyl hexanoate and limonene, this increase in retention is also observed in the gelled state, hydrophilic diacetyl shows a very distinct behaviour at pH 4.23.

As can be seen from Fig. 3, higher protein contents do not increase diacetyl retention if the dairy matrix is acidified. This observation is best explained by the hydrophobic character of the acidified matrix. It was already mentioned that the relative amount of the total protein which is stabilised by HY remains constant regardless of the total

protein content. Nevertheless, the more protein is present in the acidified matrix, the more protein is stabilised by means of hydrophobic interactions. Consequently, more hydrophobic sites are exposed and interact with each other, which leads to increased hydrophobicity within the matrix. In such an environment, the hydrophilic diacetyl is expelled from the matrix. This effect has previously been proposed by Nongonierma et al. (2006), but not been quantified so far. The described effect is comparable with that observed for fat containing matrices, in which the retention of diacetyl decreases with increasing fat content, i.e., increased matrix hydrophobicity, and vice versa (Guyot et al., 1996; Haahr, Bredie, Stahnke, Jensen, & Refsgaard, 2000; Saint-Eve et al., 2006).

Our findings are, furthermore, in agreement with those of Perreault et al. (2010) and Merabtine et al. (2010). Perreault et al. (2010) found that in GDL-induced skim milk gels, the retention of the hydrophobic aroma compounds ethyl butanoate and ethyl hexanoate increased, while the retention of the less hydrophobic ethyl acetate (log P = 0.7) and pentan-2-one (log P = 0.9) was not affected by acidification to pH 4.5. It has to be mentioned though that the latter compounds are still far more hydrophobic than diacetyl (log P = -1.3). Merabtine et al. (2010) observed that in GDL-induced skim milk gels the retention of only the most hydrophobic aroma compounds under investigation, i.e., 1-nonanol, linalool, ethylbutanal, octanal and ethyl butanoate, showed a significant positive correlation with their log P value (log P = 1.6–3.4). They attributed this behaviour to hydrophobic zones formed by the proteins in the dairy gels, and assumed hydrophobic van der Waals interactions of these highly hydrophobic aroma compounds with the dairy proteins in acid gels.

4. Conclusions

An increased CWR increased the retention of the highly hydrophobic limonene in liquid model milk solutions, while the less hydrophobic ethyl hexanoate and the hydrophilic diacetyl were not affected. This observation is explained by the presence of hydrophobic binding sites which result from the build-up of the casein micelle. Albeit the magnitude of the here observed effect of CWR on the retention of highly hydrophobic compounds is rather small, if compared, for example, with the effect of fat content or overall protein content described in the literature, it is significant and may become of greater importance at protein contents higher than the here investigated 4%. In this context, the casein micelle's potential as a carrier for hydrophobic substances deserves more investigation in the near future. Acidification of model milks to pH 4.23 led to the expulsion of hydrophilic diacetyl, which is caused by an increased hydrophobicity of the acidified dairy matrix resulting from hydrophobic protein–protein interactions. The distribution of the hydrophobic aroma compounds ethyl hexanoate and limonene remained unaffected by acidification.

The obtained results show that the extent to which the aroma–matrix interaction takes effect highly depends on the physico-chemical properties of the involved aroma compounds and dairy protein matrices.

Further research of the fundamental principles underlying the dairy matrix–aroma interaction at equilibrium and dynamic conditions is needed. The expected results will facilitate the systematic adaptation of aroma compositions on changes in the composition and processing of dairy matrices.

Acknowledgments

We wish to thank Bernd Köhlnhofer, Katja Buhr and Peter Schieberle from DFA (Deutsche Forschungsanstalt für Lebensmittelchemie) for the close cooperation within this research

project on the aroma–dairy matrix interaction. This research project was supported by the FEI (Forschungskreis der Ernährungsindustrie e.V., Bonn), the AiF and the Ministry of Economics and Technology. AiF-Project No.: 15158 N.

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