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Flavour retention by lipids measured in a fresh cheese matrix

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Interactions between lipids and several aroma compounds (aldehydes, methylketones, esters, and dimethyldisulfide) were studied in a real food system composed of fresh cheese, triolein, and water. The concentration of 'free' ligands was measured with a dynamic headspace-gas chromatographic system. From our results, we conclude that retention of all flavouring molecules increases with the amount of triolein. This phenomenon is highly influenced, however, by the structure of the aroma compound, as demonstrated by RP-HPLC lipophilicity determinations. Within the same chemical family there exists a linear relationship between the lipophilicity index k_w and retention, indicating that this physicochemical property should be taken into account in choosing the best internal analytical standard. © 1998 Published by Elsevier Science Ltd. All right reserved

INTRODUCTION

The acceptability of food depends greatly on its sensory properties (colour, surface structure, texture, taste, flavour, etc.). Among these, aroma seems the most important. Essential are not only the nature and concentration of the aroma compounds but also their interactions with non-volatile macromolecules such as sugars, proteins, and lipids. This phenomenon has recently been reviewed by Bakker (1995).

Protein–aroma interactions have received much attention in the last decade. Several studies have demonstrated reversible, nonspecific, hydrophobic binding of most volatile compounds tested (ketones, alcohols, vanillin, aldehydes, terpenes) to proteins such as bovine serum albumin, β-lactoglobulin, fava bean protein (Arai *et al.*, 1970; Franzen and Kinsella, 1974; King and Solms, 1979; Damodaran and Kinsella, 1981*a*,*b*; Dumont and Land, 1986; O'Neill and Kinsella, 1987*a*,*b*; Ng *et al.*, 1989; Dufour and Haertlé, 1990; O'Keefe *et al.*, 1991*a*,*b*). Some specific interactions have also been demonstrated, such as interaction between β-ionone and β-lactoglobulin (Dufour and Haertlé, 1990) or irreversible pH-dependent binding of aldehydes and diacetyl to proteins (Overbosch *et al.*, 1991).

As regards the interactions of aroma compounds with sugars, added mono- and disaccharides usually cause the vapour pressure to increase (Nawar, 1971; Buttery *et al.*, 1971; Kieckbusch and King, 1979; Bakker, 1995). Polysaccharides, on the other hand, usually decrease aroma compound volatility (Maier, 1975; Voilley *et al.*, 1977; Le Thanh *et al.*, 1992). When appropriate functional groups are present, flavouring compounds can form hydrogen bonds with the macromolecules (Kinsella, 1990). In the case of starch, unbranched helical structures can also entrap some volatile compounds by forming low-energy bonds such as Van der Waals and hydrophobic interactions (Godshall and Solms, 1992). The ability to form such inclusion complexes with small molecules has been extensively studied in the case of all cyclodextrin structures (Szente and Szejtli, 1988; Chang and Reineccius, 1990; Divakar, 1990).

Of all food ingredients, lipids probably have the strongest organoleptic impact. Ebeler *et al.* (1988), studying menthone and isoamyl acetate in soybean oil, found lipids to significantly reduce headspace aroma concentration and hence perceived flavour intensity. Earlier, Buttery *et al.* (1973) found that the air–oil partition coefficients determined for a series of aliphatic aldehydes and ketones decreased as the molecular weight of the flavouring compound increased. Kieckbusch and King (1979) obtained similar results for butyl, pentyl, and hexyl acetate. A comparable decrease was observed by Schirle-Keller *et al.* (1994) for limonene, ethyl heptanoate and diacetyl in an emulsified oil/water system. Sensorial and analytical experiments indicate that parameters such as fat composition and concentration, emulsion characteristics and temperature,

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significantly modify interactions between lipids and small molecules (Land, 1978; Kieckbusch and King, 1979; Lee, 1986; Linssen *et al.*, 1993; Salvador *et al.*, 1994; Schirle-Keller *et al.*, 1994).

Most of the above-mentioned experiments involved simple model systems. In this paper, we focus on a real fat-containing food system composed of fresh cheese, triolein, and water. We have used dynamic headspace analysis to show how triolein affects the release of methylketones, esters, aldehydes, and dimethyldisulfide. We relate our results to the aroma lipophilicity index determined by RP–HPLC.

MATERIALS AND METHODS

Preparation of the food matrix

Lipids. Triolein [1,2,3-tris(cis-9-octadecenoyl)glycerol] (98% pure) was purchased from Aldrich Chemicals (Belgium). Deodorised triolein was obtained by heating to 80°C at 60 mm Hg in the presence of helium. It was then stored at 4°C until used in the headspace experiments.

Fresh cheese samples with a low lipid content (1%) were purchased from a local market and stored at 4°C.

Flavours. Benzaldehyde, 2-methyl butanal, 3-methyl butanal, hexanal, 2-butanone, 2-hexanone, 2-heptanone, ethyl acetate, isoamyl butyrate and dimethyldisulfide were purchased from Aldrich Chemicals (Belgium). Butanal and ethyl propionate came from Fluka Chemika. An aqueous solution of these aroma compounds (± 10 ppm) was prepared in volatile-free deionized water (Milli-Q water purification system, Millipore, Bedford, MA).

Sample preparation. Blends of fresh cheese (20% w/w), triolein, and ultrapure water were prepared with increasing amounts of lipid and accordingly decreasing quantities of water. We thus prepared a 'light' system without triolein, called '0% triolein' and a 'high-fat' system without water, called '80% triolein'. Four intermediate compositions were also tested: 10, 20, 33, and 60% triolein. For each triolein content, volatile compounds were added at increasing concentrations (between 40 and 400 ppb according to the flavouring compound). An internal standard (2-methyl pentanal) was also added to each sample just to monitor the reproducibility of our injections, for a similar lipid content. Prior to dynamic headspace analysis, 60 μl of a 10 ppm aqueous solution of 2-methyl pentanal and 30, 60, or 90μl of a diluted aroma-compound solution

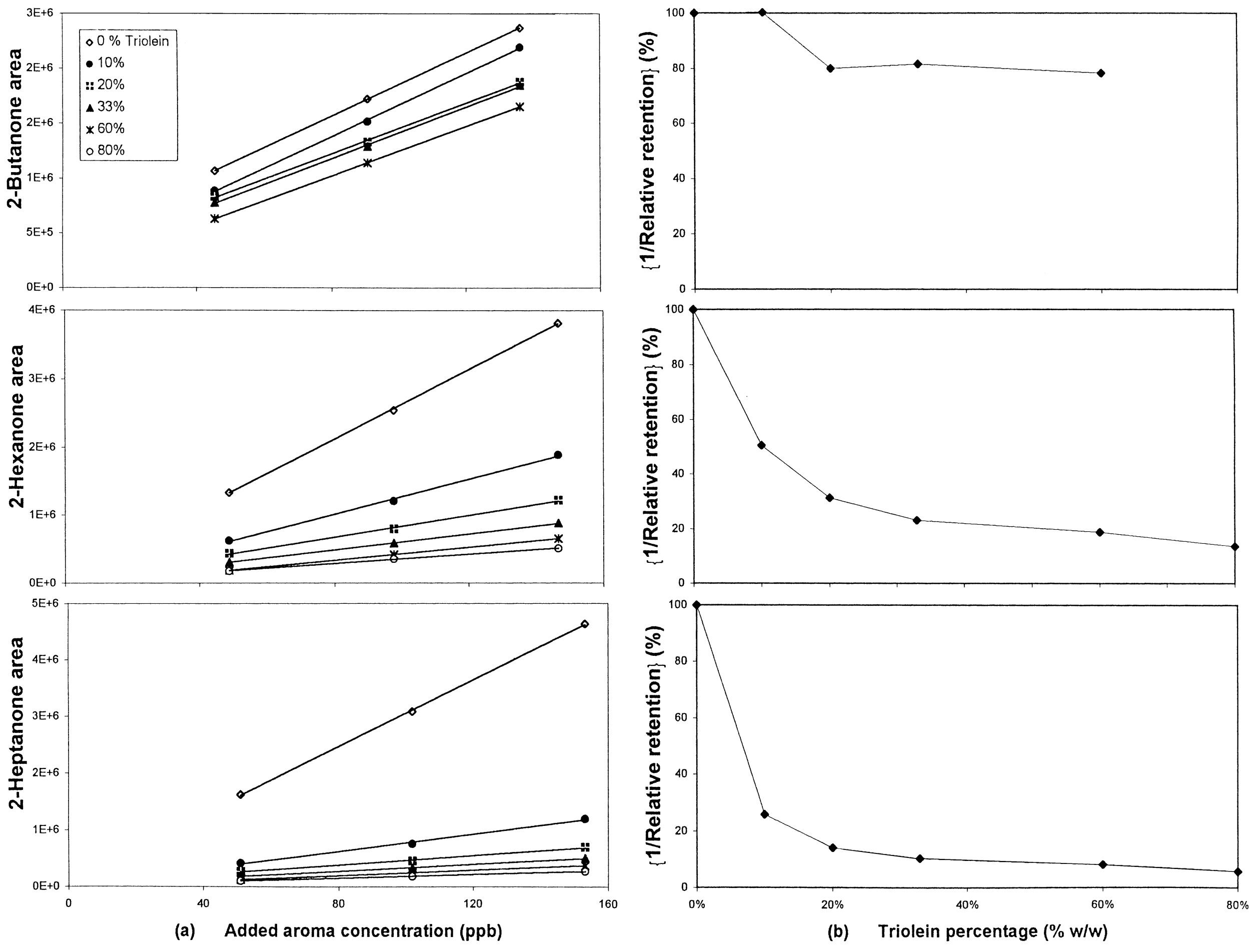

Fig. 1. (a) Linear relationships between chromatographic areas and aroma methylketone concentrations at different percentages of triolein. (b) Relationship between the inverse of the relative retention (%) and the percentage of triolein (%).

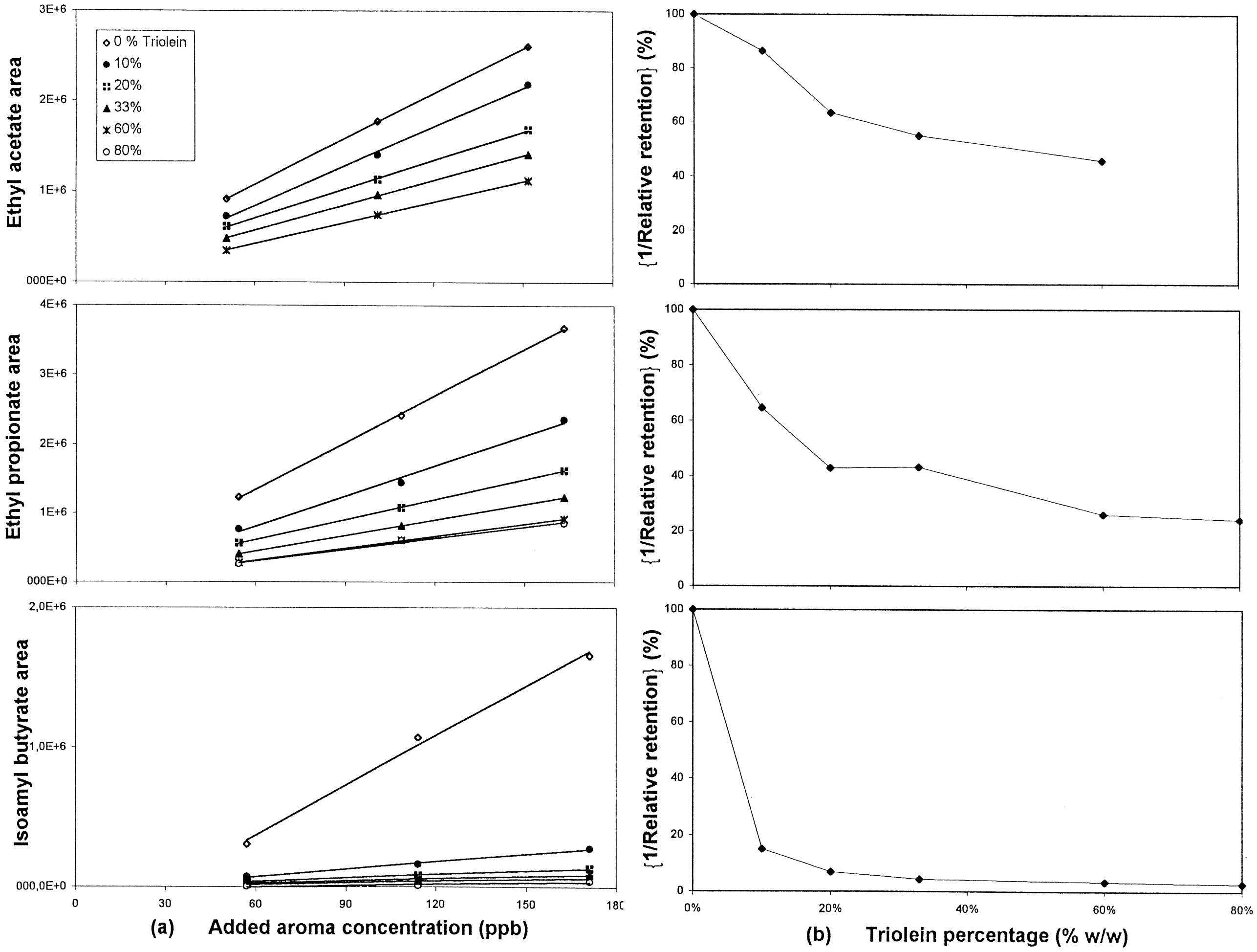

Fig. 2. (a) Linear relationships between chromatographic areas and aroma ester concentrations at different percentages of triolein. (b) Relationship between the inverse of the relative retention (%) and the percentage of triolein (%).

(final concentration between 40 and 400 ppb) were added to 7 g of the blend of fresh cheese, triolein, and water, then mixed with an Ultra-Turrax (Janke and Kunkel Gmbh, Ika-Labortechnik, Ultra-Turrax® T25; 9500 rpm) for 30 s at 4°C. Portions of this mixture (5 g) were finally poured into the 25 ml purge vessel (surface area = 4.2 cm^2) for dynamic headspace extraction.

Dynamic heasdspace analysis

Dynamic headspace operating conditions. A Chrompack purge-and-trap injector was used. Samples were injected into the chromatographic column in three steps as follows: (1) precooling of the cold trap (CP-SIL 8 CB capillary column, 0.53 mm i.d.; film thickness, 5 μm): the trap was cooled for 1 min in a stream of liquid nitrogen; (2) purging of the sample: the temperature of the purge vessel was set at 60°C. The sample was purged with helium gas (12 ml min^{-1}) for 15 min. The gas stream was passed through a condenser kept at −15°C by means of a cryostat (Colora WK 15) to remove water vapour and then through an oven at 200°C. The volatiles were finally concentrated in the cold trap maintained at −95°C (liquid nitrogen); (3) desorption of the volatiles: cooling was stopped, and the surrounding metal capillary was rapidly heated to 220°C for 5 min. The carrier gas swept the trapped compounds into the analytical column.

Gas chromatographic analytical conditions. A Hewlett-Packard Model 5890 gas chromatograph equipped with a flame ionization detector and an integrator (Shimadzu C-R1A) was used. Analysis of volatile compounds was carried out on a 50 m×0.32 mm, wall-coated, open tubular (WCOT) apolar CP-SIL5 CB capillary column (film thickness, 1.2 μm). Oven temperature, initially kept at 33°C for 16.5 min, was programmed to rise from 33 to 160°C at 2°C min^{-1} and thereafter from 160 to 200°C at 20°C min^{-1}, remaining at the maximum temperature for 9 min. Helium carrier gas was used at a flow rate of 1.0 ml min^{-1}. Injection and detection temperatures were 200 and 220°C, respectively. The minimum peak area for data acquisition was set at 5000 μV.s. Each sample was analysed in duplicate. Assessment of the technique's reproducibility has been previously described (coefficients of variation under 10% for five analyses of the same standard mixture; Collin *et al.*, 1993).

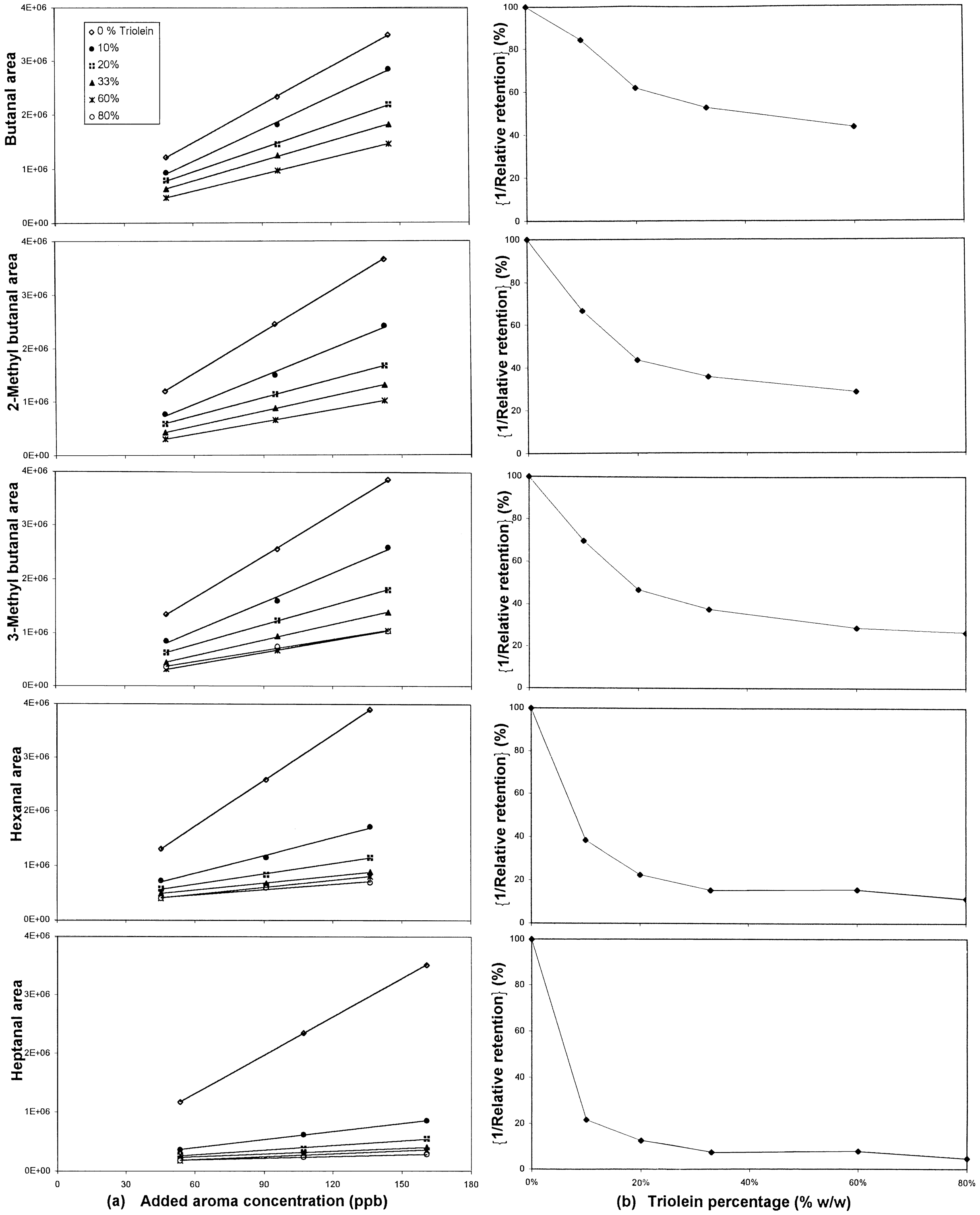

Fig. 3. (a) Linear relationships between chromatographic areas and aroma aliphatic aldehyde concentrations at different percentages of triolein. (b) Relationship between the inverse of the relative retention (%) and the percentage of triolein (%).

Lipophilicity measurements

Lipophilicity was measured by RP-HPLC with a chromatograph equipped with a Waters model 510 isocratic pump and a Waters WISP 710 B autosampler. The detector was a Perkin-Elmer LC 75 operating at 270 and 212 nm for methylketones and esters and dimethyldisulfide, respectively. The Guard-Pak insert was packed with μBondapak® C18, particle size 10 μm (Waters). The column (25 cm×4 mm ID) was prepacked with LiChrosorbRP-18, particle size 10 μm (Merck). A Digital 380 PC equipped with the Waters 840 acquisition program (version 6.0) was used as an integrator for peak recording and to calculate retention times. The mobile phase

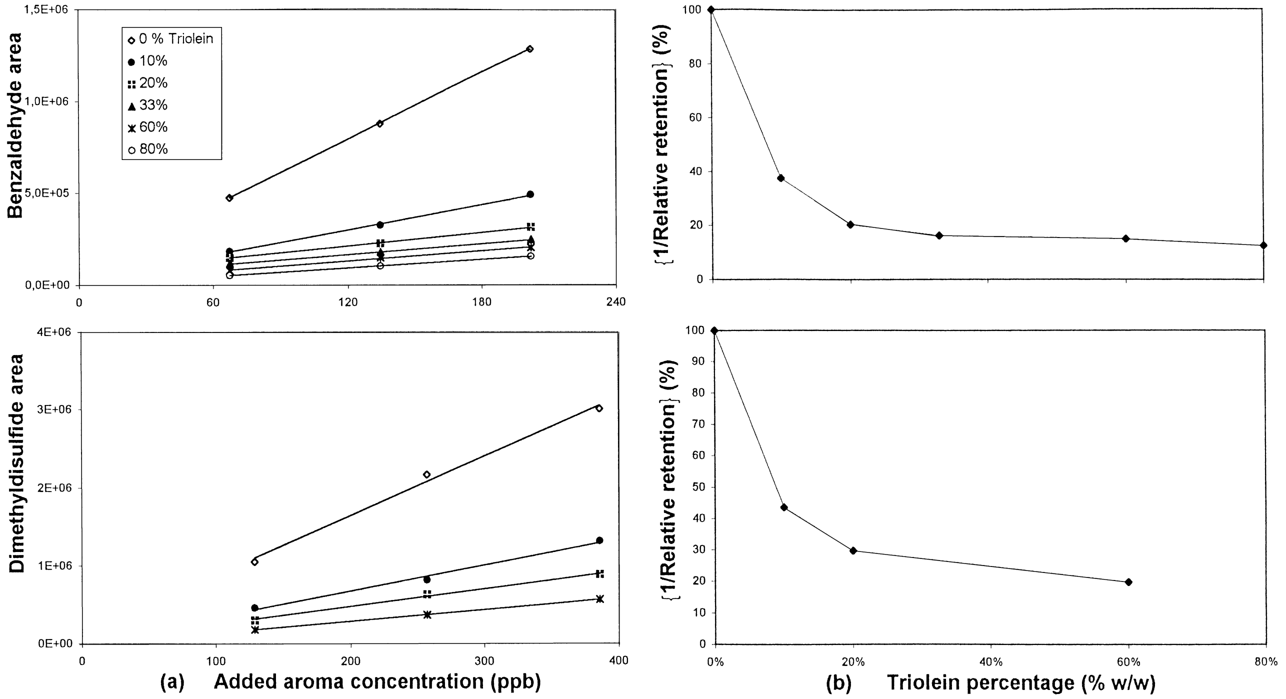

Fig. 4. (a) Linear relationships between chromatographic areas and aroma benzaldehyde and dimethyldisulfide concentrations at different percentages of triolein. (b) Relationship between the inverse of the relative retention (%) and the percentage of triolein (%).

was made up volumetrically from various combinations (30–70%) of methanol (analytical grade, LabScan), and a solution containing MOPS (3-morpholinopropane sulphonic acid, Sigma, Belgium) buffer (0.01 M) and *n*-decylamine (Sigma) (0.2% v/v). MOPS buffer was chosen to avoid ion-pair formation; *n*-decylamine was used as a masking agent to eliminate silanophilic interactions (El Tayar *et al.*, 1985*b*). The pH of the aqueous solution was adjusted beforehand to 7.4 by addition of HCl. The methanol/aqueous solution mixture was filtered with a Millipore HAWP filter (0.45μM). Retention times (t_r) were measured at room temperature with a 1.5 ml min^{-1} flow rate. The column dead time (t_0) was determined with uracil. The capacity factor was defined as $k=(t_r-t_0)/t_0$. Log k for 100% water (log k_w) was linearly extrapolated from results obtained for different mobile phase compositions (El Tayar *et al.*, 1985*a*,*b*).

RESULTS AND DISCUSSION

The dynamic headspace method was used to quantify triolein–aroma compound interactions in a fresh cheese system (20% w/w). Thirteen dairy-product aroma compounds (Collin *et al.*, 1993; Schieberle *et al.*, 1993) were selected: three methylketones (2-butanone, 2-hexanone, and 2-heptanone), three esters (ethyl acetate, ethyl propionate, and isoamyl butyrate), three straight chain aldehydes (butanal, hexanal, and heptanal), two branched aldehydes (3-methyl butanal and 2-methyl butanal), an aromatic aldehyde (benzaldehyde), and a sulfur compound (dimethyldisulfide). For each triolein content (0, 10, 20, 33, 60, and 80% w/w), increasing amounts of these flavouring substances were added prior to chromatographic analysis.

The linear relationship between the measured chromatographic area of each substance and the aroma concentration was plotted for each triolein ratio (Figs 1(a)–4(a)).

In the figures, the slope of each line includes both the detector response coefficient and the aroma recovery factor. From these results, the inverse of the relative retention was calculated by dividing the slope of the straight line obtained for the blend containing added triolein by the slope of the '0% triolein' samples. These data were plotted versus the triolein content, as shown in Figs 1(b)– 4(b) (Piraprez and Collin, 1995).

For the three investigated methylketones (Fig. 1(b)), the higher the fat content the lesser the slope of the calibration curve. Even for aqueous molecules such as 2-butanone, triolein seems to be able to modify the interaction with the matrix. Similar results were obtained for the esters (Fig. 2(b)), the six aldehydes (Figs 3 and 4(b)), and dimethyldisulfide (Fig. 4(b)). As suggested by Fig. 5, the higher the molecular weight of the flavouring substance, the stronger the aroma–lipid interaction. Even for very similar structures such as 2- or 3-methyl butanal and hexanal, significant variations were still measured, indicating how difficult it might be to choose an optimal internal standard for quantitative analyses.

To further probe the nature of these flavour–food matrix interactions, we used Reversed-Phase High Pressure Liquid Chromatography (RP-HPLC) to determine the lipophilicity of all the above aroma compounds

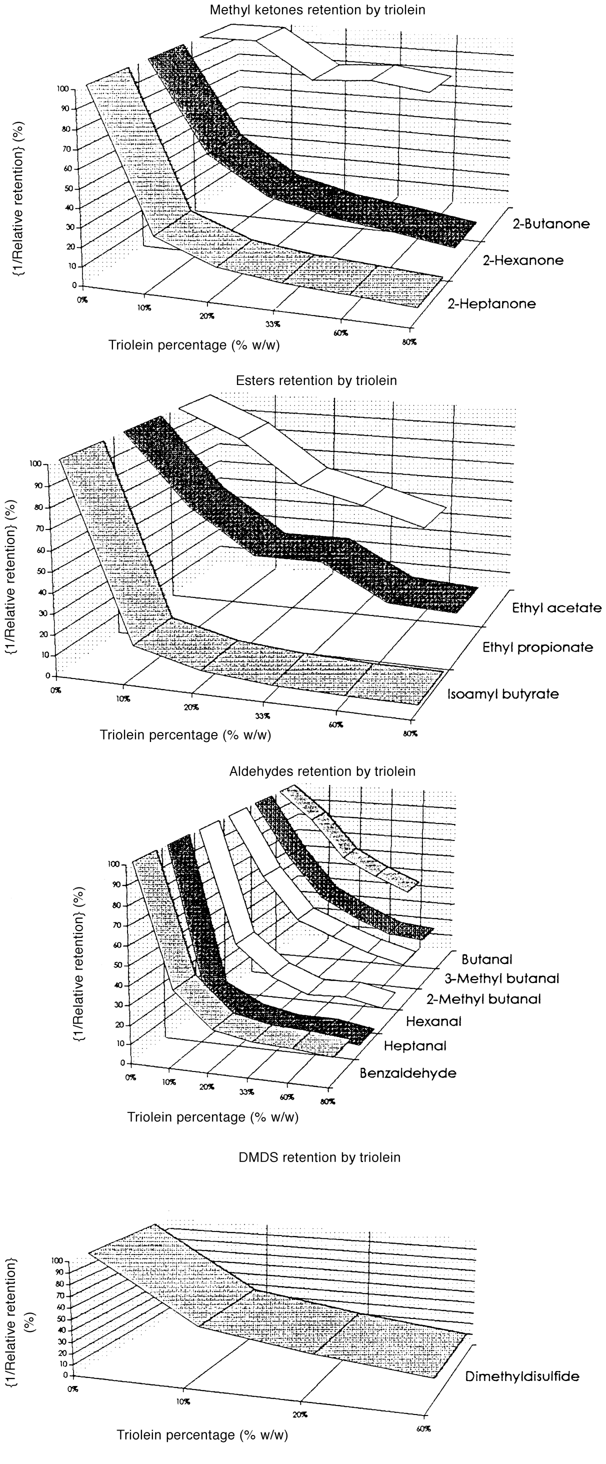

Fig. 5. Aroma retention by triolein in fresh cheese.

except the aldehydes. For the latter, we used Rekker's theoretical calculations (Rekker and de Kort, 1979). The RP-HPLC method yields a capacity factor, k_w, whose logarithm correlates with the logarithm (log P) of the usual partition coefficient (El Tayar *et al.*, 1985*b*). El Tayar and coworkers (El Tayar *et al.*, 1985*a*) have shown that this parameter includes both hydrophobic and enthalpic contributions when an RP-18 column is used.

As shown in Table 1, the retention at a given percentage of triolein was directly proportional to the aroma capacity factor. Comparing lipophilicities could be a means of selecting an optimal internal standard for accurate quantification without the tedious elaboration of calibration curves for samples of different lipid content. If the chosen internal standard's hydrophobicity is very different from that of the compound of interest, as often occurs, we recommend correcting the quantitative data by a capacity factor ratio $k_w/k_{w\mathrm{IST}}$.

Table 1. Linear relationships between the aroma capacity factor (k_w) and aroma retention according to the percentage of triolein in the fresh cheese samples

All the aroma compounds except aliphatic aldehydes (lipophilicity estimated): $n=8$	r^2
Retention (10% triolein) = $0.27\,k_w$	0.907
Retention (20% triolein) = $0.63\,k_w$	0.928
Retention (33% triolein) = $1.17\,k_w$	0.967
Retention (60% triolein) = $1.58\,k_w$	0.983
Retention (80% triolein) = $1.46\,k_w$	0.941
All the aroma compounds : $n=13$	r^2
Retention (10% triolein) = $0.26\,k_w$	0.888
Retention (20% triolein) = $0.58\,k_w$	0.886
Retention (33% triolein) = $1.06\,k_w$	0.909
Retention (60% triolein) = $1.32\,k_w$	0.847
Retention (80% triolein) = $1.43\,k_w$	0.885

CONCLUSION

The retention of methylketones, esters, aldehydes, and sulfur compounds in fresh cheese increases with the triolein content. Yet this phenomenon is highly influenced by aroma compound structure, as demonstrated by the RP–HPLC lipophilicity data. In quantitative analysis, the k_w value could be a good indicator, either for selecting the optimal internal standard, or for calculating appropriate corrections.

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