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Flavour release of aldehydes and diacetyl in oil/water systems

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Abstract

The concentration- and time-dependent release of three C_6 -aldehydes, six C_9 -aldehydes and diacetyl was studied in model systems. The systems were water, rapeseed oil and oil-in-water emulsions. Dynamic headspace sampling was used to collect the volatile compounds. In the concentration-dependent release experiment, the $C₆$ -aldehydes were released in equal proportions from the aqueous and the emulsion systems, but in lower amounts from the pure oil. The amounts of $C₉$ -aldehydes released decreased with increasing oil content. All aldehydes were released more rapidly from the aqueous system than from the pure oil. The release over time for diacetyl and (E,E) -2,4-hexadienal showed a linear relationship in all systems. The other compounds followed an exponential relationship between the time and the fraction released in the aqueous systems. It was demonstrated that the release of the volatile compounds was dependent on the chain length, the degree of unsaturation as well as the characteristics of the model system. \odot 2000 Elsevier Science Ltd. All rights reserved.

1. Introduction

Release of flavour compounds during consumption is a key quality parameter of food and beverages. Several studies have shown that the perceived flavour of a food differs from the odour before ingestion (Taylor, 1996; Taylor & Linforth, 1996, 1998). Factors such as the physicochemical properties of the flavour compounds and the food matrix as well as the mastication of the food influence the release. Lipids are part of the matrix of many food products and modify the physical properties of foods, thereby affecting mouthfeel, appearance, and structure. Lipids may also act as flavour precursors, solvent for flavour compounds, and flavour release modulators (Drewnowski, 1992). Food lipids have been reported to influence the perception of flavour in food products in terms of both flavour release and textural changes (Tuorila, Sommardahl, Hyvoenen, Leporanta & Merimaa, 1995). Reduction of the food fat level is continually being advised by health authorities in an attempt to reduce the incidence of diseases related to high fat consumption (Morrissey, 1997). A reduction in fat content will, however, affect the perceived flavour and might result in higher loss of flavour during processing

and storage due to the increase in volatility of flavour compounds (Roos, 1997).

In bulk oil, the volatile compounds are present in, and released from, only one phase. In emulsions they may be distributed and released from several phases: the aqueous phase, the oil phase and the interface (Druaux & Voilley, 1997). Many volatile flavour compounds possess a lower vapour pressure in oil and, therefore, a higher odour threshold than they do in aqueous systems (Buttery, Guadagni & Ling, 1973). The partition coefficient between the product and vapour phases describes the potential extent of the release of flavour compounds, and lipids affect the partitioning of flavour compounds between product and vapour phase (Druaux & Voilley, 1997). Apart from the partition coefficient, the resistance to mass transfer is a major factor in determining the rate at which equilibrium is achieved and the extent of flavour release (Buttery et al., 1973). Release of hydrophobic flavour compounds from the oil phase of a food proceeds at a lower rate than from the aqueous phase. This may be attributed to the higher resistance to mass transfer in oil than in water. Furthermore, in oil-in-water emulsions flavour compounds have to diffuse from the oil to the aqueous phase before they can be released from the aqueous phase of the emulsion to the vapour phase (Roos, 1997).

Theoretical analyses of the release of flavour compounds from emulsions have been made by several groups

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(Harrison, Hills, Bakker & Clothier, 1997; McNulty & Karel, 1973a, 1973b), but little work has been reported on the volatility of flavour compounds from emulsions (Landy, Courthaudon, Dubois & Voilley, 1996). In the present study, aldehydes and diacetyl were chosen because they are important flavour compounds in several lipid-containing foods such as salmon (Milo & Grosch, 1996), butter (Schieberle, Gassenmeier, Guth, Sen & Grosch, 1993) and mayonnaise (Jacobsen et al., 1999). Flavour release of diacetyl has been intensively studied in water (Bakker, Boudaud & Harrison, 1998; Land & Reynolds, 1981; Yven, Guichard, Giboreau & Roberts, 1998) and also in emulsions (Harrison & Hills, 1997; Harrison et al., 1997; Salvador, Bakker, Langley, Potjewijd, Martin & Elmore, 1994). However, a systematic study concerning the effect of lipids on the dynamic release of aldehydes in relation to their degree of unsaturation and chain length has not been reported. This study aimed to evaluate the release of different aldehydes and diacetyl from water- and lipid-containing systems using a dynamic headspace sampling technique to simulate dynamic processes during mastication.

2. Materials and methods

2.1. Materials

Rapeseed oil was purchased from Jacob Full House Catering (Copenhagen, Denmark). The volatile compounds used were diacetyl [95%, Food Chemicals Codex (FCC)], hexanal (97%, FCC), (E)-2-hexenal (95%, FCC), (E,E)-2,4-hexadienal (95%), nonanal (95%, FCC), (Z)-6 nonenal (92%), (E,E)-2,6-nonadienal (93%), (E)-2-nonenal (93%), (E,Z) -2,6-nonadienal (92%, FCC), and (E,E) -2,4-nonadienal (85%), all obtained from Aldrich-Chemie (Steinheim, Germany). The purity of the compounds was checked by gas chromatography-mass spectrometry $(GC-MS)$ and no volatile contaminants were detected. A stock solution of the volatile compounds was prepared in 96% ethanol. Dilutions in ethanol were made from the stock solution to obtain the different concentration levels. The solubility of the flavour compounds in water was checked by visual inspection at solute levels at least 50 times above the concentrations used in the experiments, i.e. at about 12.5 mg/g (maximal concentration in experiments: $170 \mu g/l$). Complete solubilisation was confirmed if only one phase could be seen, i.e. no droplets of lipophilic material on the surface and no turbidity of the solution.

2.2. Preparation of model systems

Oil-in-water (o/w) emulsions were prepared in volumes of 100 ml using an Ultra-Turrax T25 (Janke & Kunkel IKA-Labortechnik, Staufen, Germany). Water was stirred with the Ultra-Turrax for 20 s after which rapeseed oil with emulsifier was added under vigorous mixing (13,500 rpm). Tween-20 (Sigma, St. Louis, USA) was used as emulsifier at a level of 0.3% (w/w) in all oilcontaining systems. A homogenisation time of 3 min was employed. During preparation, the emulsions were covered with nitrogen. The same batch of each emulsion was used throughout an experiment. Each experiment was carried out in duplo using two different batches of each emulsion.

The following model systems were prepared: (A) water, (B) 1% rapeseed o/w emulsion + 0.3% (w/w) Tween-20, (C) 10% rapeseed o/w emulsion + 0.3% (w/w) Tween-20, (D) rapeseed oil and (E) rapeseed oil + 0.3% (w/w) Tween-20.

2.3. Characterisation of emulsions

The emulsions were visually inspected through a light microscope at 40 times magnification. The average droplet size was determined and the presence of aggregates of oil droplets was examined. In both 1% o/w emulsions and 10% o/w emulsions, 70% of the droplets were between 10 and 50 μ m, 20% were between 2.5 and 9 μ m and the remaining were between 50 and 60 μ m. No aggregation was observed and the droplet size distribution of the emulsions was constant for at least 5 days.

2.4. Headspace sampling

The volatile compounds were sampled by a dynamic headspace technique (Refsgaard, Haahr & Jensen, 1999). For headspace sampling, approximately 30 mg of the diluted mixture of volatile compounds dissolved in ethanol were placed in a 100 ml pear-shaped flask and, immediately prior to the headspace sampling, 5 ml of the model system were added. The model system and flavour solutions were swirled for 5 s before sampling. The following parameters were used: purge flow through solution: 340 ml nitrogen/min, purge time: 10 min, sampling temperature: 37° C. The volatile compounds were collected on traps (Perkin-Elmer, CN, USA) packed with 225 mg Tenax GR (Chrompack, Bergen op Zoom, The Netherlands).

2.5. Concentration-dependent release

The release of the volatile compounds was measured as a function of the initial concentration, after a collection time of 10 min from the different model systems. The concentration-dependent release curves for each compound were made in triplicate at five different concentrations. These included systems where no volatile compounds were added. In the bulk oil and the oil with Tween-20, concentrations of the solution of volatiles were varied between 115 and 860 μ g/l. In the other systems,

the concentrations of the added solution of volatiles varied between 15 and 170 µg/l. The loss of volatiles during collection was examined by analysing four traps in series. Only diacetyl showed a breakthrough (30%) to the second trap after 10 min collection time and therefore data for diacetyl are not included in this part of the study.

2.6. Release of compounds over time

The purge parameters as described above were used. The Tenax traps were exchanged after 30, 60, 90, 120, 240, 360, and 600 s. Experiments were performed in triplicate. For the bulk oil and the oil with Tween-20 the concentration of each volatile compound was between 450 and 550 mg/l and for diacetyl around 1 mg/l. In the three other systems, the concentrations of volatile compounds were between 60 and 80 mg/l and for diacetyl around $160 \mu g/l$. No breakthrough was observed for diacetyl at the short collection times used in this experiment. Time-dependent release curves showed the accumulation of the fractions released at each time interval, the fraction being calculated as the released amount of a compound divided by the initial amount present in the system.

2.7. Thermal desorption and $GC-MS$

An ATD-400 automatic thermal desorber with a Tenax GR-packed cold-trap (Perkin-Elmer, CN, USA) was used for thermally desorbing the collected volatiles. Helium was used as carrier gas with a flow of 1.3 ml/ min. The transfer line of the ATD was connected to a 5890 IIA gas chromatograph (Hewlett-Packard, CA, USA) equipped with a DB 1701 column $(30 \text{ m} \times 0.25)$ $\text{mm} \times 1.0 \text{ µm}$, J&W Scientific, CA, USA) coupled to a HP 5972A mass selective detector. The temperature programme used was: 65° C for 1 min, 65 to 90 $^{\circ}$ C at 4° C/min and 90 to 240 $^{\circ}$ C at 20 $^{\circ}$ C/min and finally hold at 240° C for 3.75 min. The GC-MS transfer line temperature was kept at 280° C. Ionisation energy of the mass spectrometer was set at 70 eV in the EI mode and the detector operated with a mass range between 30 and 250 with a scan rate at 3.35 scans/s.

2.8. Quantification of volatile compounds

Quantification of the compounds released from the model systems was done by selected ion monitoring. The target ion represents a specific MS fragmentation ion of each compound. The target ions were verified on the basis of two or three qualifier ions and the chromatographic retention time. Quantification of the amounts collected on the trap was done using calibration curves for known amounts of each volatile compound. The calibration curves were determined by applying 1.0 µl of the flavour solution at five concentration levels directly to Tenax tubes. Determinations were made in triplicate with relative standard derivations ranging from 0.1 to 4% and with correlation coefficients for all compounds between 0.993 and 0.998.

2.9. Statistical analyses

To compare several regression lines for the concentration-dependent release curves for each compound in each of the five model systems, a covariance analysis was performed as described by Montgomery (1991). The covariance analysis tests for differences in the intercept, the effect of the system, the regression coefficient and for the error. A significance level at 0.05 was used.

3. Results and discussion

3.1. Effect of concentration on release

The release of aldehydes and diacetyl from oil/water model systems was measured as function of the concentration in five different systems, namely: (A) water; (B) 1% rapeseed oil-in-water emulsion + 0.3% (w/w) Tween-20 (C) 10% rapeseed oil-in-water emulsion +0.3% (w/w) Tween-20; (D) rapeseed oil +0.3% (w/w) Tween-20; (E) rapeseed oil.

Fig. 1 shows typical concentration-dependent release curves after 10 min headspace sampling obtained in the systems B-D for hexanal, nonanal and $(E.E)$ -2,6-nonadienal. In all systems and for all compounds a linear relationship between the initial concentration and the amount released was found (Table 1). The linear release curves for each compound were significantly different in the five systems, except for (E,E) -2,6-nonadienal where the slopes in the water system and in the 1% o/w emulsion were similar.

The slope of the curves may be considered as a measure of the partitioning of a given compound between the liquid phases and the gas phase under the dynamic sampling conditions. Theoretically, the slope would vary from zero to one, where higher values represent higher releases from the system. The slopes were dependent on both the volatile compounds and the model systems (Table 1).

The release of hexanal was dependent on the system in the following order of decreasing release: 10% o/w emulsion $> 1\%$ o/w emulsion $>$ oil + Tween-20 (Fig. 1). The analysis without addition of the volatile compounds showed formation of a small amount of hexanal during the preparation of the emulsions. Hexanal can be formed by oxidation of unsaturated fatty acids in the rapeseed oil from which hexanal is known to be a major product (Frankel, 1985). The higher value for the release from the 10% emulsion system compared with

Fig. 1. Concentration-dependent release curves for hexanal, nonanal and (E,E) -2,6-nonadienal in three oil/water systems. Sampling time was 10 min and the concentrations of the solution of volatiles were varied between 115 and 860 µg/l in the oil system and between 15 and 170 μ g/l in the other two systems. For visual clarity, the lines for the 1% o/w emulsion and 10% o/w emulsion have been extrapolated beyond the experimental range. \blacktriangle , 1% o/w; \blacksquare , 10% o/w; X, oil + Tween-20.

the 1% emulsion may be due to some oxidation of the rapeseed oil under the preparation of the emulsions. Correction for the blank runs did not change the values to any significant extent.

The release of nonanal and (E,E) -2,6-nonadienal was lower from the systems with a higher lipid content (Fig. 1) in the following order: 1% o/w emulsion $> 10\%$ o/w

emulsion $>$ oil + Tween-20, which agreed with the literature (Druaux & Voilley, 1997; Overbosch, Afterof & Haring, 1991). The degree of unsaturation did not influence the release order for the C_9 -aldehydes.

The effect of oil content on the release of the volatile compounds is illustrated in Fig. 2. The slopes of the release curves (Table 1) are shown as function of oil content for the systems: water, 1% o/w emulsion, 10% o/w emulsion and $oil + Tween-20$. The water system was not directly comparable with the other three systems, because the water system did not contain Tween-20. The fraction of volatiles released from the aqueous system for all aldehydes was in the range of 0.4 to 0.6 with a higher release for (E,E) -2,4-nonadienal. The release of the C_6 - aldehydes was dependent on the degree of unsaturation and decreased in the order hexanal $> (E)$ - 2 -hexenal > (E,E) -2,4-hexadienal (Fig. 2A). Hexanal and (E) -2-hexenal were released from the emulsion systems in much higher levels than from water and, even more, from the oil with Tween-20 system. For (E,E) -2,4hexadienal nearly equal levels were released from the aqueous and the emulsion systems but at a much lower level from the oil with Tween-20 system. The presence of oil had a substantial effect on the release of the C_9 aldehydes, which was lowered with increased oil content (Fig. 2B). The degree of unsaturation of the C_9 -aldehydes hardly affected the release from the different systems. However, the release pattern of (E,E) -2,4nonadienal differed from the other C_9 -aldehydes. As can be seen in Table 1, a fraction of less than 0.01 of the C₉aldehydes was released from the bulk oil and from the oil with Tween-20.

An indication of the hydrophobicity of a compound may be given by its $log P$ value, which describes the partitioning between water and octanol. For a number of the compounds studied, $log P$ values were calculated by the CODESSA software (Semichem, USA). For the C_6 -aldehydes the release order did not exactly parallel the calculated hydrophobicity of these compounds. The $log P$ values of hexanal, (E)-2-hexenal and (E,E)-2,4hexadienal, 1.24, 1.32, and 1.06, respectively, would lead to a prediction of the lowest release from pure oil of (E)- 2-hexenal having the highest hydrophobicity, and the lowest release from water of (E,E) -2,4-hexadienal having the lowest of the three $log P$ values. An explanation for the found differences could be that the P -value calculations assume one-phase systems, whereas the results reported here describe the situation in two-phase systems, including an emulsifier.

The lower release from the aqueous system in comparison to the emulsions, which was observed for the C_6 -aldehydes, was not expected. The presence of Tween-20 may have stimulated the release from the emulsified systems. However, in the oil system with Tween-20, a reduction in release of aldehydes was observed compared to the pure oil system (Table 1). A decreased

^a Diacetyl has not been included in the results due to breakthrough to the second trap.

^b The slope was calculated by regression analysis on the concentration-dependent release curves. The slope represents the amount of compound released from the system as function of the initial amount of compound added the system. The slopes differed significantly in a covariance analysis at $P < 0.05$, except for (E,E) -nonadienal.

 c r^2 , correlation coefficient for the release curves.

Fig. 2. Fraction released of C_6 -aldehydes (A) and C_9 -aldehydes (B) as function of the oil amount in the systems. The fraction released represents the slope of the concentration-dependent release curve for the given compound (Table 1). Sampling time was 10 min and the concentrations of the solution of volatiles were varied between 115 and 860 μ g/l in the oil system and between 15 and 170 μ g/l in the other systems: (A) \blacksquare , hexanal; \blacktriangle , (E)-2-hexenal; \blacksquare , (E,E)-2,4-hexadienal; (B) \blacklozenge , nonanal; X, (E)-2-nonenal; \blacktriangle , (Z)-6-nonenal; X, (E,E)-2,4nonadienal; $*, (E,E)$ -2,6-nonadienal; \bullet , (E,Z) -2,6-nonadienal.

release from emulsions has been attributed to the presence of an emulsifier (Land, 1979). Overbosch et al. (1991) stated that this is the case if the interface creates a barrier for diffusion from the water phase into the gas phase. Another explanation for the unexpected behaviour might be the occurrence of a different gas-liquid interfacial surface area when purging the emulsified systems in comparison to the aqueous system. Such an effect would support the more pronounced difference in release between the aqueous and emulsified systems for the C_6 -aldehydes. Addition of Tween-20 to water caused foam formation during dynamic headspace sampling. Therefore, this system was not included because of the extended surface area.

The slope of a concentration-dependent release curve $(Table 1)$ can be considered as a sort of partition coefficient of a volatile compound between the liquid system and the headspace in a dynamic system. The transfer of volatile compounds from the liquid to the gas phase will not reach equilibrium, which means that the partition coefficient calculated in the present study can not directly be compared to partition coefficients given in the literature. The partition coefficient of a volatile compound alone is insufficient to describe the phenomenon of release of volatiles from a system. The rate of mass transfer of a flavour compound across the different interfaces influences the release (Druaux $\&$ Voilley, 1997). It has been shown that the rate of transfer of diacetyl to the vapour phase in o/w emulsions was greater than in w/o emulsions (Salvador et al., 1994).

3.2. Release over time

The release curves presented in the previous section were all determined by purging the model systems for 10 min. The time-dependence of the volatile release in the different systems was also examined, which was done by exchanging the Tenax tubes after 30, 60, 90, 120, 240, 360, and 600 s. The release curves over time for diacetyl, hexanal, (E) -2-hexenal, (E,E) -2,4-hexadienal, nonanal, (Z) -6nonenal, (E,E) -2,6-nonadienal, (E) -2-nonenal, (E,Z) -2,6nonadienal, and (E,E)-2,4-nonadienal are shown in Fig. 3.

For all the studied volatile compounds, the fastest release was from the aqueous system and the slowest

Fig. 3. Time-dependent release curves of diacetyl and aldehydes from oil/water systems. The purge parameters were as follows: temperature 37°C, flow 340 ml/min. Tenax traps were exchanged after 30, 60, 90, 120, 240, 360, and 600 s. The initial concentrations for the aldehydes were about 70 μ g/l and for diacetyl around 160 μ g/l in the water and emulsion systems. In the oil systems concentrations were about 500 μ g/l for the aldehydes and for diacetyl around 1 mg/l. The fraction released was the amount of a volatile compound measured over the amount of volatile added to the system. \blacklozenge , water; \blacktriangle , 1% o/w; \blacktriangleright , 10% o/w; \ldots \blacktriangleright \ldots , oil + Tween-20; X, bulk oil.

release was from the oil (Fig. 3). An exception was diacetyl, which showed the slowest release from the 10% oil emulsion. In the bulk oil system a higher release of diacetyl was observed than from the water system. This was expected since the air-water partition coefficient of diacetyl is 3 times lower than the air-oil partition coefficient (Overbosch et al., 1991). Salvador et al. (1994) have described a similar tendency for the release of diacetyl, but found a larger difference between the systems than in the present study. This may be explained by the use of different concentrations of diacetyl and differences in the headspace sampling techniques.

Diacetyl and (E,E) -2,4-hexadienal had a similar linear progression over the whole time span showing that the release rates of these compounds were low in all five systems. Hexanal and (E) -2-hexenal showed exponential release curves in the aqueous and emulsion systems, whereas a linear progression was observed in the oil systems. (Z) -6-nonenal and (E) -2-nonenal had a linear time-dependent release curve in the 1% o/w emulsion. The release over time for the C_9 -aldehydes in the 10% o/w emulsion and in the oil systems were linear for the 10 minutes measured. The C₉-aldehydes showed an exponential release over time in the aqueous systems except for (E,Z) -2,6-nonadienal, for which the release from water was lower than for the other C_9 -aldehydes including (E,E) -2,6-nonadienal. The geometric isomers of 2,6-nonadienal have different physicochemical properties. Therefore, different solubilities in water and in oil may be expected, which in turn might affect the release rate from the aqueous system in favour of (E,E) -2,6nonadienal. The different geometric isomers have also been shown to possess different odour thresholds.

The concentration level in the time-dependent release experiment was one of the levels used in the concentration-dependent release experiment. The data show that a smaller (about $5-10\%$) amount was collected in the time-dependent release experiment. This is most likely due to a loss of volatiles in the few seconds without collection, that occurs at each switch-over of the seven Tenax tubes used for collection.

Exponential release curves were observed in the aqueous system for all the volatile compounds except for diacetyl and (E,E) -2,4-hexadienal. The equation for the exponential curve describes the depletion of the volatiles from the system. Harrison et al. (1997) have deduced a mathematical model for the flavour release from liquid emulsions. The Harrison et al. model contains parameters such as phase volumes of the emulsion and gas phases, surface area, droplet size and partition coefficients for the compound between the gas phase and the emulsion. We have used a sampling technique where the systems are mixed by the nitrogen flow. Therefore, a measurable surface area is not obtained and it is not possible to determine the partition coefficient between the gas phase and the system.

Fig. 4. Initial release rates for diacetyl and aldehydes in oil/water systems. The initial release rate was calculated from the linear part of the time-dependent release curves (Fig. 3). For the aqueous system and the 1% o/w emulsion, four time points equals the linear part of the curve and, for the 10% o/w emulsion and the oil systems, seven time points equals the linear part of the curve.

The time-dependent release curves in Fig. 3 were linear over the initial period of 2 min for most of the compounds. It is very important to consider the first part of the time release curves as this is the period during eating when flavours are released (Harrison et al., 1997). The initial linear part of the curves gives the release rate under the given flow conditions. Fig. 4 shows the initial release rates for the volatile compounds in the five model systems. The initial release rate curves are approximately linear with correlation coefficients between 0.900 and 0.999. The initial release rates appear to depend on the volatile compound and the system. The initial release rate of hexanal from the aqueous system under the given sampling conditions was faster than for the other compounds. Comparing the C_6 -aldehydes it is clearly seen from Fig. 4 that the initial release rate decreases both with the increased number of double bounds and with increased amount of oil in the model system. The slow release rate in the oil systems can be due to the higher viscosity and to the differences in polarity of the o/w systems. For the C_9 -aldehydes, there was a tendency of the release rate to depend on the unsaturation, except for the fast release rate of (E) -2-nonenal in the aqueous system. In the emulsion systems and the oil system the release rates for the C₉-aldehydes were very low. The volatile compounds are all oil soluble, which will decrease the release in these systems.

In conclusion, both the concentration- and the timedependent release curves showed that the release of aldehydes and diacetyl depended on the composition of the system, and for the aldehydes the chain length and the degree of unsaturation have influence on the release.

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