Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Mass Spectrometry 239 (2004) 187–192
International Journal of Mass Spectrometry
www.elsevier.com/locate/ijms

Influence of mastication rate on dynamic flavour release analysed by combined model mouth/proton transfer reaction–mass spectrometry

Saskia M. van Ruth*, Katja Buhr

Department of Food and Nutritional Sciences, University College Cork, Western Road, Cork, Ireland

Received 14 April 2003; accepted 10 August 2004
Available online 25 September 2004

Abstract

The influence of mastication rate on the dynamic release of seven volatile flavour compounds from sunflower oil was evaluated by combined model mouth/proton transfer reaction–mass spectrometry (PTR–MS). Air/oil partition coefficients were measured by static headspace gas chromatography. The dynamic release of the seven volatile flavour compounds from sunflower oil was significantly affected by the compounds' hydrophobicity and the mastication rate employed in the model mouth. The more hydrophobic compounds were released at a higher rate than their hydrophilic counterparts. Increase in mastication rate increased the maximum concentration measured by 36% on average, and the time to reach this maximum by 35% on average. Mastication affected particularly the release of the hydrophilic compounds. The maximum concentration of the compounds correlated significantly with the compounds' air/oil partition coefficients. The initial release rates over the first 15 s were affected by the type of compound, but not by the mastication rate. During the course of release, the proportions of the hydrophilic compounds to the overall flavour mixture in air decreased. The contribution of the hydrophobic compounds increased. Higher mastication rates, however, increased the proportions of the hydrophilic compounds and decreased those of the hydrophobic compounds.

Keywords: Aroma release; Flavour; PTR–MS; Sunflower oil

1. Introduction

Flavour release and flavour perception are important issues for the food industry and consumers. Variation in the food matrix composition is a key factor that influences the binding and release of volatile flavour compounds [1]. However, it is well known that individual tasters often perceive different flavours from the same food, and some of this variation may be due to differences in release of flavour compounds in the mouth.

One of the oral physiological factors affecting volatile flavour release in the mouth is saliva. Saliva is secreted into the mouth by three major glands, which is under both sympathetic and parasympathetic control. The former has control over certain proteins released, whereas the latter controls the volume of saliva produced [2]. Salivary composition and flow rate are affected by the degree of hydration, body position, exposure to light, olfaction, smoking, (previous) stimulation, climatological circumstances, and circadian and circannual rhythms [3–4]. Therefore, they vary widely within and between subjects. It has been shown that hydration/dilution of food by saliva affect the partitioning of volatiles over food, saliva, and air phase [5].

Chewing behaviour varies also considerably among people. Mastication controls the extent of physical disruption and mixing of food. As a result, chewing behaviour and other conditions in the mouth can substantially alter the extent and the balance of the flavour released.

Volatile release in the mouth can be studied by analysis of exhaled air. This is the experimental approach giving data nearest to the site of perception. However, oral physiological variables are difficult to control. For this reason, mouth simulators have been developed [6]. The authors started the

* Corresponding author. Tel.: +353 21 4902496; fax: +353 21 4270244.
E-mail address: s.vanruth@ucc.ie (S.M. van Ruth).

1387-3806/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijms.2004.08.009

development of their model mouth ten years ago. This mouth simulator considers the sample volume, volume of the mouth, temperature, salivation, and mastication [7]. The latest version of the system is composed of a 70 ml glass sample flask and assembly, a plunger for mastication, two voltage controllers and two variable speed motors to give precise control of vertical and circular speed of the plunger, an externally circulating temperature controlled water bath connected to the cavity wall of the sample flask and a controlled gas supply [8].

The perception of flavour and texture of a food is not a static, non-changing experience during the course of eating. The overall perception of a food is based on initial impact, perception during chewing, and the perception of residual flavour (dynamics). In order to measure these dynamics real-time, fast analysis is required. Proton transfer reaction–mass spectrometry (PTR–MS) allows volatile flavour analysis with a high time resolution (<0.1 s per measurement). The advantage of PTR–MS over other direct MS techniques is that the generation of the primary H_3O^+ ions and the chemical ionisation of the volatile compounds are individually controlled and spatially and temporally separated processes.

In the present study, the model mouth was connected to a PTR–MS instrument. This combination was used to examine the influence of mastication rate on the dynamic release of seven volatile flavour compounds from sunflower oil. Air/oil partition coefficients were determined by static headspace gas chromatography to distinguish the effects of the type of compound and mastication on the thermodynamic component from those on the kinetic component of flavour release.

2. Experimental

2.1. Materials

Seven volatile flavour compounds were added to cold-pressed sunflower oil (Suma Wholefoods, Dean Clough, Halifax, UK) at a concentration of 0.001% (v/v) for each individual compound. The compounds, 2-butanone, ethyl acetate, diacetyl, hexanal, and 2-heptanone, were supplied by Aldrich (Steinheim, Germany). 3-Methyl-1-butanol was purchased from Fluka Chemie (Buchs, Switzerland) and ethyl butyrate from Merck (Hohenbrunn, Munich, Germany).

2.2. Static headspace analysis: equilibrium headspace gas chromatography

For equilibrium headspace gas chromatography, 2 ml of oil sample was transferred into a 10 ml headspace vial. Three replicate vials were prepared for each sample. 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 GC (Varian CP-3800; JVA Analytical Ltd.). One millilitre of headspace was injected onto the GC. The GC was equipped with an injector at 225 °C, a BPX5 capillary column (60 m length; 0.32 mm i.d.; 1.0 μm film thickness; helium carrier gas, 1.9 ml/min; SGE, Kiln Farm Milton Keynes, UK), and a flame ionisation detector at 275 °C. An initial oven temperature of −30 °C was used for 1 min, followed by a rate of 100 °C/min 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, further to 130 °C at 4 °C/min, and finally to 250 °C at 8 °C/min.

The time required for equilibration of the oil and air phase (6 min) was determined in preliminary studies. Partition coefficients of single compounds in oil were not significantly different from partition coefficients when seven compounds were added (Student's *t*-test, $P < 0.05$), which demonstrated that there were no interactions between the individual volatile flavour compounds at the concentrations used. Five concentrations of each of the compounds were analysed in triplicate for calibration, allowing quantification of the compounds in the air phase.

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

2.3. Dynamic headspace analysis: model mouth/PTR–MS analysis

The sample (10 ml) was placed in the model mouth. Two replicates of each sample were analysed. The model mouth analysis has been described previously [7]. Mastication rates were varied: 26 and 52 cycles/min. The headspace of the samples was analysed by PTR–MS according to the method described by Lindinger et al. [9]. The headspace was drawn from the model mouth at 100 ml/min by a vacuum pump, 15 ml/min of which was led through a heated transfer line into the PTR–MS for on-line analysis for 2 min. Data were collected for *m*/*z* 61 (ethyl acetate), 71 (3-methyl-1-butanol), 73 (2-butanone), 83 (hexanal), 87 (diacetyl), 115 (2-heptanone), and 117 (ethyl butyrate). The most abundant ions for the individual compounds were measured, which was determined by measuring the pure compounds individually. For most compounds, the parent mass plus 1, or as the parent mass plus 1 minus 18 (water) was measured. Dwell time was 0.2 s per mass, and with ions used for instrument monitoring this resulted in a cycle time of 2.2 s. Headspace concentrations were calculated as described by Lindinger et al. [9]. From the average release curves, the following parameters were taken: the maximum concentration measured (I_{max}), the time to reach this maximum concentration ($t_{I_{max}}$) and the average release rate between 0 and 15 s (initial release rate).

2.4. Statistical analysis

Data of the flavour measurements were subjected to multivariate analysis of variance (MANOVA) to determine significant effects of the type of compound and mastication rate. Differences between compounds were subsequently examined by least significant difference tests. Correlations were

Table 1
Experimental air/sunflower oil partition coefficients (K) of seven volatile flavour compounds and octanol–water partition coefficients ($\log P$ [17])

	$K \times 1000$	$\log P$
2-Butanone	4.8	0.29
Ethyl acetate	5.3	0.73
Diacetyl	4.9	0.80
3-Methyl-1-butanol	0.6	1.28
Hexanal	0.6	1.78
Ethyl butyrate	1.1	1.90
2-Heptanone	0.5	1.98
CV (%)[a]	5.3	–

[a] CV, average coefficient of variance.

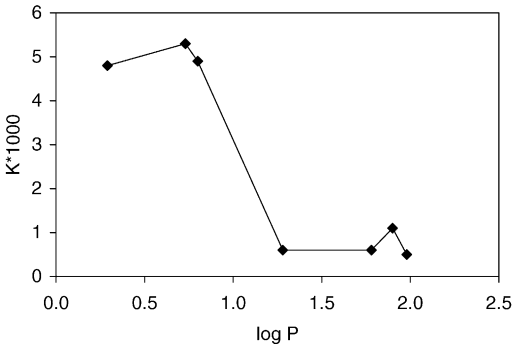

Fig. 1. Experimental air/oil partition coefficients (K) of seven volatile flavour compounds as a function of their hydrophobicity ($\log P$: octanol/water partition coefficients; [17]). The names of the compounds corresponding to the $\log P$ values are shown in Table 1.

determined by Pearson product moment correlation coefficients. The significance level was $P < 0.05$ throughout the study.

3. Results and discussion

3.1. Static headspace analysis

Seven volatile flavour compounds were selected on their physico-chemical properties. They are well-known flavour compounds with distinct odours: 2-butanone, ethereal; ethyl acetate, ethereal–fruity; diacetyl, buttery; 3-methyl-1-butanol, fruity–wine; hexanal, grassy; ethyl butyrate, fruity; and 2-heptanone, fruity–spicy [10]. The compounds were added to sunflower oil and their air/oil partition coefficients were determined using static headspace analysis (Table 1). Good agreement with data of other studies was obtained for the air/oil partition coefficient of hexanal [11,12]. Differences in air/oil partition coefficients indicate differences in the thermodynamic component of flavour release between the various compounds. Affinity for the oil phase is reflected by a relatively low air/oil partition coefficient. Fig. 1 shows the relationship between the hydrophobicity of the compounds and their air/oil partition coefficients. Generally, for the hydrophobic group (3-methyl-1-butanol, hexanal, ethyl butyrate, and 2-heptanone; $\log P > 1$), a relatively low partition coefficient was found, whereas for the hydrophilic group (2-butanone, ethyl acetate, and diacetyl; $\log P < 1$), a higher partition coefficient was observed. A reasonable correlation was found between $\log P$ and the air/oil partition coefficients ($R = -0.890$), which indicates that the larger, more hydrophobic compounds have lower air/oil partition coefficients, and therefore, a higher affinity for the oil phase. The hydrophobic, lipophilic flavour compounds are bound to the lipid molecules by weak, reversible Van der Waals forces, and unspecific hydrophobic interactions [13].

3.2. Dynamic headspace analysis: model mouth/PTR–MS analysis

The dynamic release of the seven volatile flavour compounds from sunflower oil was measured in the model mouth by PTR–MS. Two mastication rates were employed: 26 and 52 rpm. The release curves of the seven compounds are shown in Fig. 2. Large differences between the compounds were observed. The magnitude of release varied considerably between the compounds; with values below 1000 nl/l air for 2-heptanone, and values in the 30 000 nl/l air range for ethyl acetate. From the release curves, three parameters were calculated for the various compounds and conditions: I_{max}, $t_{I_{max}}$,

Table 2
Dynamic release of seven volatile flavour compounds from sunflower oil in the model mouth using two mastication rates (26 and 52 rpm): the maximum concentration measured during release (I_{max}), the time to reach I_{max} ($t_{I_{max}}$), and the release rate in the first 15 s (initial release rate)

	I_{max} (nl/l)			$t_{I_{max}}$ (s)			Initial release rate (ng/s)		
	26 rpm	52 rpm	Effect of higher mastication rate (%)	26 rpm	52 rpm	Effect of higher mastication rate (%)	26 rpm	52 rpm	Effect of higher mastication rate (%)
2-Butanone	12249	18943	55	24	35	46	16.3	14.8	−9
Ethyl acetate	19343	31010	60	24	35	46	26.5	28.3	7
Diacetyl	3236	5611	73	17	35	106	5.7	6.5	14
3-Methyl-1-butanol	1114	1343	21	33	35	6	1.0	0.9	−6
Hexanal	1043	1245	19	24	28	17	1.5	1.5	2
Ethyl butyrate	1757	2033	16	24	28	17	3.3	2.8	−14
2-Heptanone	707	765	8	33	35	6	0.9	0.8	−9
CV (%)[a]	42	43	–	70	12	–	–	–	–

[a] CV, average coefficient of variance.

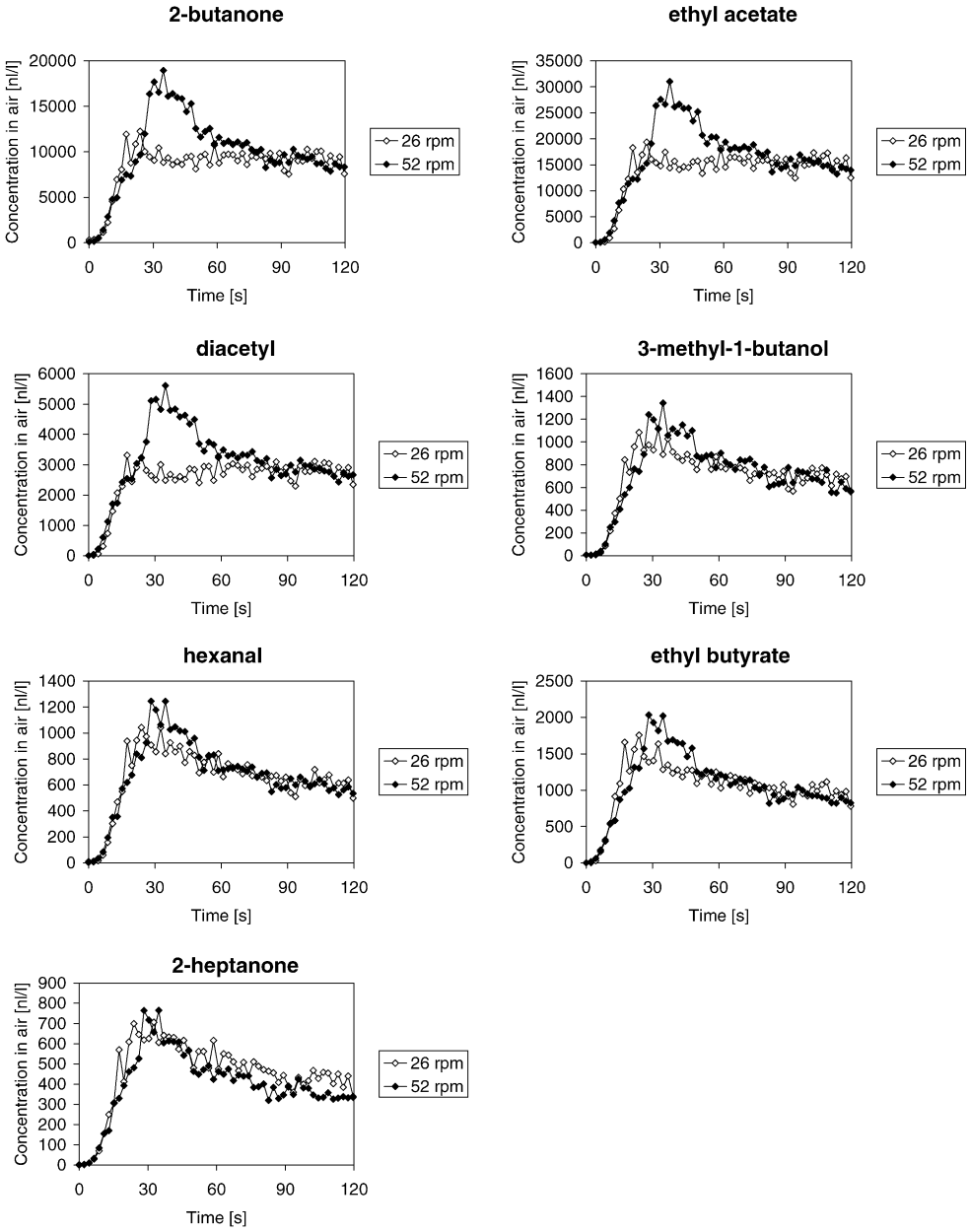

Fig. 2. Dynamic release of seven volatile flavour compounds from sunflower oil in the model mouth using two mastication rates measured by proton transfer reaction–mass spectrometry.

and the initial release rate (Table 2). The parameters are defined in Section 2.

3.2.1. I_{max}

The type of compound had a significant effect on I_{max} [$F(1, 28) = 13.555$, $P = 0.000$]. Between the compounds, significant differences were found between 2-butanone and all others except ethyl acetate as well as between ethyl acetate and all other compounds except 2-butanone. I_{max} was related to the hydrophobicity of the compounds (Fig. 3; $R = -0.735$), with lower I_{max} values for the more hydrophobic compounds. I_{max} correlated quite well with the air/oil partition coefficients; $R = 0.796$ for 26 rpm and $R = 0.809$ for the 52 rpm mastication rate.

The mastication rate had a significant effect on I_{max} [$F(6, 28) = 5.617$, $P = 0.033$]. A larger effect of mastication was shown for the lower molecular weight compounds; 2-butanone (increase I_{max} of 55%), ethyl acetate (60%), and diacetyl (73%) (Table 2, Figs. 2–3). The effect of mastication rate was related to the hydrophobicity of the compounds (Fig. 4). For the compounds with $\log P < 1$, the doubled mastication rate resulted in at least a 50% increase of I_{max}. For the more hydrophobic compounds ($\log P > 1$), this effect was between 10 and 20% only. Obviously, the release of the hy-

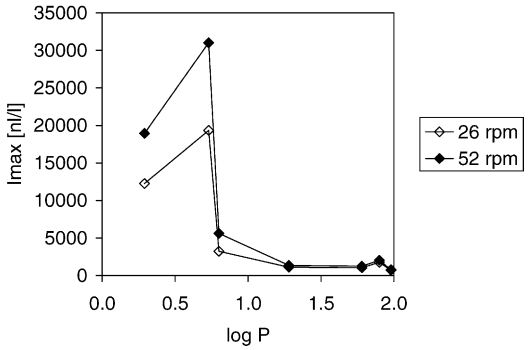

Fig. 3. Maximum concentrations (I_{max}) of seven volatile flavour compounds during dynamic release from sunflower oil in the model mouth using two mastication rates as a function of their hydrophobicity (log *P*: octanol/water partition coefficients; [17]). The names of the compounds corresponding to the log *P* values are shown in Table 1.

drophilic compounds is determined to a large extent by the kinetic component of flavour release.

Under the dynamic conditions used, the driving force for transfer of flavour compounds across the interface is the difference in flavour concentration between oil and air phase. The rate of the unidirectional diffusion from the oil to the air phase is determined by the concentration gradients as well as the mass transfer coefficients of the flavour compounds in each of the phases (Fick's law). Flavour compound diffusion is based on two mechanisms: molecular and eddy diffusion. Molecular diffusion is the random movement of the flavour molecules in the medium. Eddy diffusion relates to the transport of elements or eddies of the fluid from one location to another, carrying with them the dissolved flavour compounds. The rate of eddy diffusion is usually much higher than the rate of molecular diffusion [14]. In general, it is assumed that diffusion of flavour compounds in the gas phase is extremely rapid [15,16]. This assumption allows neglecting the concentration gradient in the gas phase, which implies that the concentration of the flavour compound at the oil side of the interface determines the concentration in the gas phase. The

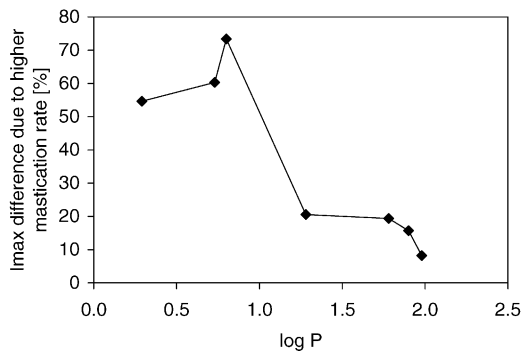

Fig. 4. Differences in maximum concentrations (I_{max}) of seven volatile flavour compounds during dynamic release from sunflower oil in the model mouth by increase of mastication rate as a function of the compounds' hydrophobicity (log *P*: octanol/water partition coefficients; [17]). The names of the compounds corresponding to the log *P* values are shown in Table 1.

rate of mass transfer in oil and air phase can be described as:

$$\frac{dM_o}{dt} = k_o\left(\frac{C_a}{K_i} - C_o\right)$$

where M_o is the total mass of flavour compound diffusing in the oil phase and k_o is the mass transfer coefficient in oil. C_a and C_o are the concentrations of the flavour in the air and oil phases, respectively. K_i is the air/oil partition coefficient at the interface. Principally, the concentration gradient depends on the depletion of the flavour compound at the interface. A high gas pressure and a low mass transfer coefficient of the flavour compound favour depletion at the oil side of the interface [14]. In the present study, especially the hydrophilic compounds had a high vapour pressure, as indicated by their relatively high air/oil partition coefficients (Table 1). This explains why the effect of mastication was larger for them, than for those compounds, which release rates were lower, and which were, therefore, not as much affected by depletion at the interface.

3.2.2. $t_{I_{max}}$

The time to reach the maximum concentration ($t_{I_{max}}$) is presented for the seven volatile flavour compounds in Table 2. The seven compounds did not differ significantly in $t_{I_{max}}$ [$F(6, 28) = 0.097$, $P = 0.995$). $t_{I_{max}}$ was higher for all compounds at the mastication rate of 52 rpm, varying from 6 to 106%. However, this effect was not significant [$F(1, 28) = 1.141$, $P = 0.303$), which is mainly due to the high coefficient of variance for the low mastication rate measurements.

3.2.3. Initial release rate

Generally, it is assumed that the rate of release is an important factor determining flavour perception. Liquids reside only briefly in the mouth under consumption conditions. Therefore, the linear release rate over the initial 15 s was calculated as the total amount of flavour released in 15 s, divided by 15 (Table 2). The compounds varied considerably in initial release rates. However, the mastication rate had only a small effect. This can also be seen in Fig. 2: the initial release curves of the various compounds are quite similar for both mastication rates. Apparently, in the initial phase, the flavour compounds are present in sufficient concentrations at the interface, and therefore, mass transfer is not a limiting factor yet. From about 25 s, mass transfer becomes rate limiting and the release at 26 rpm levels off (Fig. 2).

3.2.4. Proportions of the compounds

It not only changed the absolute concentrations of the compounds with time (Fig. 2), the proportions of the compounds, and therefore, the balance of the overall flavour, changed both with time and with mastication rate. The changes in proportions of 2-butanone and 2-heptanone are graphically presented in Fig. 5. The contribution of 2-butanone to the overall flavour decreased rapidly in the first 15 s from ca. 70 to 30% (v/v) of the whole flavour mixture in air. On the

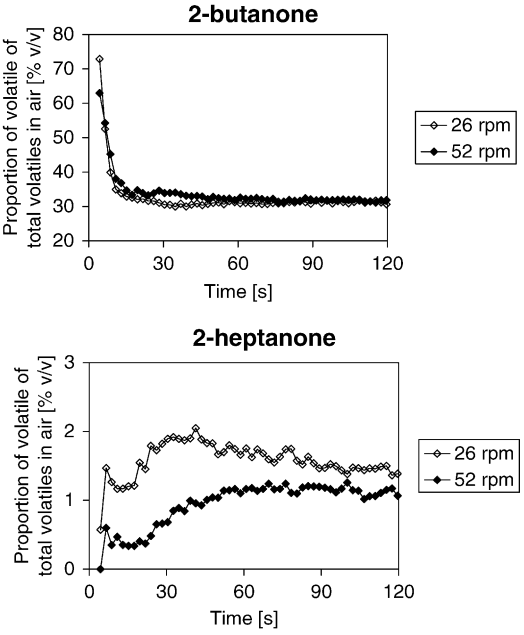

Fig. 5. Relative contribution of 2-butanone and 2-heptanone to the total concentration of seven volatile flavour compounds in air during the course of dynamic release from sunflower oil in the model mouth using two mastication rates.

other hand, the proportion of 2-heptanone increased more gradually: its concentration doubled within the first 60 s. The different compounds have different odours. In a mixture, it should be considered that the changes are of such a magnitude that it is possible that they have an effect on the overall flavour perception. To complicate this phenomenon even further, a higher mastication rate affected the proportions of the various compounds differently. It increased the proportion of hydrophilic compounds, like 2-butanone. However, it considerably decreased (ca. 50%) the contribution of hydrophobic compounds, such as 2-heptanone. Again, a swift in the flavour balance may result from these altered proportions.

4. Conclusions

The dynamic release of the seven volatile flavour compounds from sunflower oil was significantly affected by the type of compound and the mastication rate employed in the model mouth. Increase in mastication rate increased I_{max} and $t_{I_{max}}$, particularly those of the hydrophilic compounds. However, the initial release rate over the first 15 s was not affected by the rate of mastication. During the course of release, the proportions of the hydrophilic compounds to the overall flavour mixture in air decreased, whereas the proportions of the hydrophobic compounds increased. Higher mastication rates, however, increased the proportions of the hydrophilic compounds and decreased those of the hydrophobic compounds.

References

[1] E. Guichard, Food Rev. Int. 18 (2002) 49.
[2] L.M. Beidler, in: R.L. Doty (Ed.), Handbook of Olfaction and Gustation, Marcel Dekker Inc., New York, 1995, p. 503.
[3] C. Daws, J. Dent. Res. 66 (1987) 648.
[4] L. Wisniewski, L.H. Epstein, A.R. Caggiula, Physiol. Behav. 52 (1992) 21.
[5] S.M. van Ruth, J.P. Roozen, Eur. Food Res. Technol. 210 (2000) 258.
[6] J.R. Piggott, Biol. Mol. Eng. 17 (2001) 129.
[7] S.M. van Ruth, J.P. Roozen, J.L. Cozijnsen, in: H. Maarse, D.G. van der Heij (Eds.), Trends in Flavour Research, Elsevier, Amsterdam, 1994, p. 59.
[8] S.M. van Ruth, J.P. Roozen, Food Chem. 71 (2000) 339.
[9] W. Lindinger, A. Hansel, A. Jordan, Int. J. Mass Spectrom. Ion Process. 173 (1998) 191.
[10] S. Arctander, Perfume and Flavor Chemicals, Allured Publishing, Carol Stream, IL, 1994.
[11] G. Hall, J. Andersson, Lebensm. Wiss. Technol. 16 (1983) 362.
[12] R.G. Buttery, L.C. Ling, D.G. Guadagni, J. Agric. Food Chem. 17 (1969) 385.
[13] H. Plug, P. Haring, Food Qual. Pref. 5 (1993) 95.
[14] K.B. de Roos, in: D.D. Roberts, A.J. Taylor (Eds.), Flavor Release, American Chemical Society, Washington, 2000, p. 126.
[15] M. Harrison, B.P. Hills, J. Bakker, T. Clothier, J. Food Sci. 62 (1997) 653.
[16] M. Harrison, B.P. Hills, Int. J. Food Sci. Technol. 32 (1997) 1.
[17] D.R. Lide, CRC Handbook of Chemistry and Physics, CRC Press, Boca Raton, FL, 1997.