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Effect of Emulsion Properties on Release of Esters under Static Headspace, in Vivo, and Artificial Throat Conditions in Relation to Sensory Intensity

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The effects of oil content and droplet size distributions of dilute oil-in-water emulsions on release of four esters with different hydrophobicities were studied under in vivo, static headspace, and artificial throat conditions. The effect of oil content on orthonasal and retronasal perceived intensity of ethyl hexanoate was studied using a seven-person panel. With increasing oil content and with a higher hydrophobicity of the aroma compound, a stronger decrease in aroma release was found. This effect was stronger under static headspace conditions than under in vivo and artificial throat conditions, and the sensory intensity of ethyl hexanoate was perceived stronger orthonasally than retronasally. The lowest effective oil content was determined for all systems. Of the compounds tested, droplet size distribution only influenced the in vivo release of geranyl acetate. The artificial throat results correlated well with in vivo release, giving support to the assumption that a thin layer of liquid remaining in the throat after swallowing determines aroma release.

KEYWORDS: MCT; fat; lipid; APCI-MS; MS−Nose; effective oil content

INTRODUCTION

Oils are generally recognized as the nonvolatile food ingredient with the largest impact on aroma release when compared to proteins and polysaccharides. Understanding the impact of oil on aroma release is valuable for the optimization of increasingly popular low-fat products (*1*, *2*). Changes in oil content affect aroma release profiles but also change appearance and mouthfeel of the product. Reduction of the oil content results in a higher aroma release and perception and a lower persistence of the aroma compound, depending on its hydrophobicity (*3*). The flavor balance of an aroma mixture will be disturbed when the oil content is changed, given the variance in hydrophobicity of aroma compounds. The oil phase is a potential sink for hydrophobic molecules. Knowledge of aroma release from foods containing oils could therefore be applied to mask off-flavors (*4*).

Predicting release and perception of aroma compounds from food products that contain an oil phase has been the goal of several mathematical (*5*−*8*) and empirical (*9*) models. These studies demonstrated that the oil content of an emulsion and the hydrophobicity of the aroma compounds are key factors for predicting the release. Several other studies have also indicated this, using either analytical (*9*−*13*) or sensorial methods (*14*) or using both (*15*). Some authors (*6*, *10*, *12*, *15*) have compared the effect of oil content in liquid emulsions on in vivo aroma release or on sensory aroma perception with static headspace measurements. These studies have shown that the effect of oil content on in vivo aroma release or perception is smaller than expected from equilibrium headspace studies.

Recently, an artificial throat system has been developed that simulates in vivo aroma release (*16*). A thin liquid layer of product is formed in the human throat upon swallowing a liquid sample. Subsequently, exhaled air extracts aroma molecules from this thin layer into the breath. Buettner et al. visualized the formation of such a coating by videofluoroscopy when a volunteer swallowed viscous oral contrast medium (*17*). A mathematical model was developed recently on the basis of this principle (*18*). The results obtained in the artificial throat with oil-free products correlated well with in vivo measurements of liquid samples (*16*). This study aims to explain why the effect of oil content on in vivo aroma release or perception is smaller than expected from equilibrium headspace studies using this artificial throat.

To our knowledge, no detailed information about the minimal effective oil content under in vivo conditions is available, despite

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Table 1. Physicochemical Properties of the Esters

	P_{aw} (–)[a]	P_{ow} (–)	bp (°C)
ethyl acetate	0.00628	5.4	77.2
ethyl butanoate	0.0146	59	121
ethyl hexanoate	0.018	676	168
geranyl acetate	0.0037	12882	138

[a] Obtained from ref *21*.

the general acceptance of the importance of oil for aroma release. Carey and co-workers (*9*) have shown a significant effect of 0.025% (w/w) C8 triglyceride oil on release in static headspace experiments, but no systematic variation in oil content has been performed for in vivo aroma release measurements.

Oil droplet size distribution is another well-studied emulsion property possibly influencing aroma release. However, contradictory results have been reported. For static headspace, Carey et al. (*9*) found no effect, but an increased aroma retention with larger droplet diameters was reported by Van Ruth et al. (*13*). In another study, Van Ruth and co-workers reported an increase in aroma release with an increase in droplet diameter under the dynamic conditions of the artificial mouth (*19*). Rabe et al. found no effect of droplet diameter on aroma release under the dynamic conditions of their model system (*20*). To clarify this matter, we investigated the effect of three different droplet size distributions, under in vivo conditions, in the artificial throat and in static headspace experiments.

The aim of this paper was to explain the effect of oil-in-liquid systems on aroma release and perception in terms of the liquid film formed in the throat after swallowing and to determine the minimal effective oil concentrations. To this end, the effect of oil content and droplet size distribution on aroma release and perception was measured and compared under in vivo, static headspace, and artificial throat conditions and for orthonasal and retronasal perception.

MATERIALS AND METHODS

Materials. MCT oil (medium chain triglycerides, mainly consisting of octanoic and decanoic acid), gum arabic, and geranyl acetate (ester of acetic acid and a mixture of 3,7-dimethyl-2-trans, 6-octadien-1-ol [geranyl acetate, 67%] and 3,7-dimethyl-2-cis, 6 octadien-1-ol [neryl acetate, 33%]) were provided by Quest International (Naarden, The Netherlands). Ethyl acetate and ethyl butanoate were obtained from Fluka Chemie (Buchs, Switzerland). Sigma (Zwijndrecht, The Netherlands) supplied ethyl hexanoate. Ethanol (>99.9%) was purchased from J. T. Baker (Deventer, The Netherlands). Citric acid was obtained from Analar (Poole, U.K.). **Table 1** presents air−water and oil−water partition coefficients (P_{aw} and P_{ow}, respectively) and boiling points of the esters used. P_{ow} values of the esters used were calculated from their molecular structure by the log *P* software module of ACD/Labs (Toronto, Canada). All percentages given in this paper are based on weight/weight ratio.

Sample Preparation. MCT oil was slowly added to a final concentration of 5% to an aqueous solution of 5% gum arabic while stirring in a Cyclotron mixer (Kinematica AG, Luzern, Switzerland). The mixing continued for 5 min, yielding a crude emulsion. A Rannie 250 homogenizer (APV, Hendrik Ido Ambacht, The Netherlands) processed this crude emulsion at two regimes: single-valve at 30 bar and double-valve at 230 and 30 bar. The latter emulsion was processed three times at these conditions. This resulted in three emulsions with clearly different droplet size distributions: the crude emulsion (A), the low-energy-homogenized emulsion (B), and the high-energy-homogenized emulsion (C) with mean volume-based ($D_{(4,3)}$) oil droplet diameters of 10, 2.3, and 0.4 μm, and mean surface-based ($D_{(3,2)}$) oil droplet diameters of 4.3, 1.5, and 0.4 μm, respectively. A Mastersizer X laser diffractometer was used to determine the droplet size distribution (Malvern Instruments Ltd, Malvern, U.K.).

These three emulsions were mixed by overnight shaking in a bottle shaker at 4 °C and 50 rpm, with aqueous solutions of esters, water, and gum arabic solution, to produce a range of emulsions with oil contents of 0.00%, 0.01%, 0.02%, 0.05%, 0.10%, 0.20%, 0.50%, 1.0%, and 2.0%. The emulsion with an oil content of 5% was aromatized by adding a small amount of a concentrated solution of aroma compounds in ethanol (10−20 mg). Final concentrations of esters were 0.05 mg/L ethyl acetate, 0.01 mg/L ethyl butanoate, 0.01 mg/L ethyl hexanoate, and 0.1 mg/L geranyl acetate, for artificial throat and calibration measurements, and 1, 0.2, 0.2, and 1 mg/L, respectively, for in vivo aroma release and static headspace measurements. All final emulsions contained 0.05% ethanol, 0.125% citric acid, and 1% gum arabic, irrespective of the oil content. Emulsions with 2 and 5% oil, however, contained 2 and 5% gum arabic, respectively. All solutions were prepared using demineralized water. Solutions were stored at 4 °C and used within 1 week.

MS−Nose. Aroma compounds in the air releasing from the artificial throat, from the breath of panelists, or from the calibration were monitored by online sampling by an atmospheric pressure chemical ionization gas-phase analyzer (APCI-GPA) attached to a VG Quattro II mass spectrometer (MS−Nose; Micromass UK Ltd., Manchester, U.K.). Air was sampled (75 mL/min) through a capillary tube (0.53-mm i.d., heated to 100 °C). Source and probe temperatures were 80 °C. Compounds were ionized by a 3.0 kV discharge and monitored in selected ion mode (0.08 s dwell on each ion), in two independent sets (*m*/*z*-values and cone voltages used are given between brackets): ethyl acetate (*m*/*z* 89.00, 17 V) in one set and ethyl butanoate (*m*/*z* 89.00, 17 V), ethyl hexanoate (*m*/*z* 145.00, 18V), and geranyl acetate (*m*/*z* 137.00, 20 V) in the other. A spectrum of the daughter ions of the molecular ion of ethyl butanoate (*m*/*z* 117.00) was recorded by a second inline MS, to prove that the fragment of *m*/*z* 89.00 originated from ethyl butanoate. Argon was used as collision gas and the collision energy was set to 4.0 eV. For in vivo aroma release experiments, acetone release was measured in both sets at 58.80 *m*/*z* (19 V) as an indicator of the panelists' breathing pattern. The chosen *m*/*z* values were unique for each compound within each analysis. There was no difference in response between an aroma compound dissolved in a mixture and dissolved separately. Gum arabic and MCT oil showed some ionization at the combinations of *m*/*z* values and cone voltages used for the aroma compounds. To account for this, nonaromatized emulsions of all oil contents were assessed similarly to the aromatized ones, and the nonaromatized signal was subtracted from the signal of the aromatized emulsions. This was done for both the artificial throat and the in vivo measurements.

MS−Nose measurements were calibrated to quantify the results obtained under in vivo and artificial throat conditions. The method, described previously (*16*), is based on the determination of the area under the dynamic headspace release curve of the aroma compounds, with known aroma concentration, sample volume, and airflow.

Artificial Throat. In vivo aroma release was simulated by the artificial throat (*16*). This device consists of two vertical glass tubes. The MS−Nose sampling capillary samples air from the top of the upper tube. An essential part of the system is a rubber tube in the middle connecting the glass tubes that can be closed and opened by a clamp. At the start of the experiment, the clamp is closed and 4 mL of liquid is loaded above the clamp. After 10 s, the clamp is opened. The liquid pours down along the glass tubing. A thin liquid layer remains on the surface. Ten seconds after opening of the clamp, a stream of air (1.0 L/min) enters the tube and flows upward, where it can freely flow out of the system, while a small part of the air is sampled by the MS−Nose.

The inner glass surface was hydrophilized by rinsing the surface with sulfuric acid (95−98%), followed by rinsing abundantly with tap water. The glass kept its good wetting properties for over 50 measurements. These measurements were done in two replicates. The areas under the aroma release curves obtained were determined for the first minute of measurement.

Layer thickness was determined by weighing the separate pieces of the artificial throat (glass and rubber) before and 10 s after pouring a 4-mL aliquot of sample through. The syringe with sample and a collection bin below the artificial throat were also weighed before and

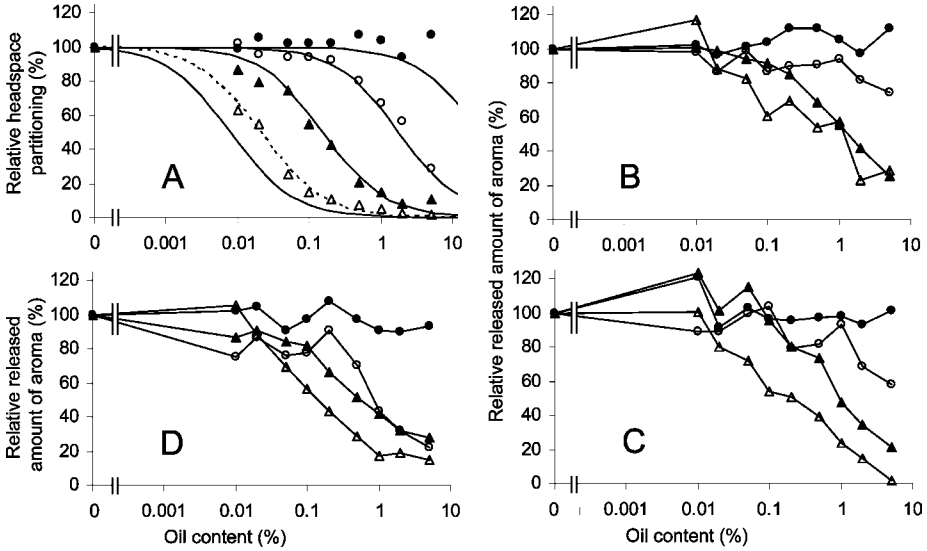

Figure 1. Relative HSGC peak areas (%) (**A**) and relative released amounts in vivo for panelists 1 and 2 (**B**, **C**, respectively) and in the artificial throat (**D**) of ethyl acetate (●), ethyl butanoate (○), ethyl hexanoate (▲), and geranyl acetate (△) at different oil contents (logarithmic scale), compared to partitioning (**A**) or released amount (**B**–**D**) at 0% oil. **A**: Solid lines represent the theoretical expected partitioning. The dotted line represents a fit for the geranyl acetate partitioning, with a modified P_{ow} value (5000). Panel **D** is discussed under Effect of Oil Content on Aroma Release in the Artificial Throat.

after, to obtain a complete mass balance. These measurements were done in six replicates.

In Vivo Aroma Release Measurements. Aroma release measurements in exhaled air were conducted using the MS−Nose according to a strict consumption protocol. This protocol has been developed for liquid samples (*22*) to control mouth movements, breathing, and swallowing, to reduce experimental error. The area under the first exhalation peak after swallowing was integrated and used for calculation of the amount of aroma released in this breath. Two panelists were trained and considered to be sufficiently trained because their averaged relative standard deviation of the area for all samples of a training session did not exceed 15%. All samples were assessed in five replicates.

Static Headspace Gas Chromatography (HSGC) Measurements. Equilibrium headspace aroma concentrations of esters (of an aliquot of 3 mL of solution in 10-mL headspace vials) were determined by GC. To this end, 1.0 mL of headspace was injected splitless on the column after 20 min of incubation at 30 °C. A GC-8000^{top} gas chromatograph (CE Instruments, Milan, Italy) was equipped with a CP-SIL 5 CB low-bleed column (44 m × 0.32 mm, film thickness 1.2 μm; Varian Chrompack, Bergen op Zoom, The Netherlands) and a flame ionization detector. The oven temperature was initially 40 °C for 2 min and then increased by 25 °C/min to 250 °C and was kept at 250 °C for 10 min. Inlet and detector temperatures were 250 and 270 °C, respectively. The headspace concentrations were expressed as peak areas in arbitrary units. Geranyl acetate was defined as the sum of neryl acetate and geranyl acetate (retention times 10.4 and 10.5 min). All samples were prepared and analyzed in triplicate.

Viscosity Measurements. An Ubbelohde viscometer (Schott Instruments GmbH, Mainz, Germany) was used to measure the kinematic viscosity (m^2/s) of the emulsions at 20 °C. The internal diameter of the capillary used (type I^d) was 0.63 mm. In combination with densities of the emulsions (determined by a Mettler-Paar DMA45 [Anton Paar GmbH, Graz, Austria] instrument at 20 °C), the dynamic viscosity (mPa·s) was calculated.

Sensory Rating. The intensity of ethyl hexanoate was tested for six emulsions containing 0%, 0.05%, 0.1%, 0.2%, 0.5%, and 1% MCT oil by a panel consisting of seven judges, all experienced with the procedure of sensory rating. The panel was familiarized to the odor of ethyl hexanoate. The panel was also familiarized to the background odor of gum arabic, which was present at the same concentration in every solution presented. Aliquots of 15-mL emulsion were presented in 20-mL glass bottles. Bottles were covered with aluminum foil to avoid any visual clues. All samples were assessed in duplicate and presented in a different random order for each panelist. All intensities were scored on a 0−10 scale. Fizz software (Biosystemes, Couternon, France) was used to acquire the data. In the orthonasal session, the panel evaluated the orthonasal intensity of 2 mg/L ethyl hexanoate by sniffing the headspace of the solutions. The orthonasal intensities of aqueous solutions with 0.4 and 1.6 mg/L were defined as anchor points in advance as 20% and 80% of the scale, respectively. In the retronasal session, the panelists took the sample (containing 10 mg/L ethyl hexanoate) in the mouth, while avoiding sniffing. First, they evaluated the fattiness mouthfeel. The fattiness mouthfeel of emulsions with 0.2% and 1% oil were defined as anchor points in advance as 20% and 80% of the scale, respectively. Next, they swallowed the entire sample and judged the retronasal ethyl hexanoate intensity during exhalation. Retronasal intensities of aqueous solutions with 2 and 8 mg/L were defined as anchor points in advance as 20% and 80% of the scale, respectively.

RESULTS AND DISCUSSION

Effect of Oil Content on Aroma Release under Static Headspace and in Vivo Conditions. The partitioning of the esters over air and emulsion phases is shown in **Figure 1A**. The data shown are measured by HSGC and calculated on the basis of the P_{aw} and P_{ow} values of the esters (**Table 1**) and the dimensions of the HSGC configuration. The calculated and measured data correlate well, except for geranyl acetate. The software used for estimation of the P_{ow}'s apparently overestimated the value of geranyl acetate. Esters with a higher hydrophobicity are more retained in the emulsion phase. The relatively hydrophilic ethyl acetate is not affected by the presence of oil from 0 to 5%, while the hydrophobic geranyl acetate remains almost completely in the emulsion phase at 5% oil. Ethyl butanoate and ethyl hexanoate show intermediate behavior.

Panels **B** and **C** of **Figure 1** show the effect of oil content on the amount of four esters released in the first breath after swallowing the emulsion for two panelists. The data of both panelists correlate well linearly ($R^2 = 0.86$). Similar to the HSGC results, the oil content does not influence the release of ethyl acetate under in vivo conditions. Geranyl acetate and ethyl hexanoate show a strong decrease in aroma release at higher oil contents, while ethyl butanoate shows intermediate behavior. However, the decrease in amount released as a function of oil content under in vivo conditions is less strong than the decrease

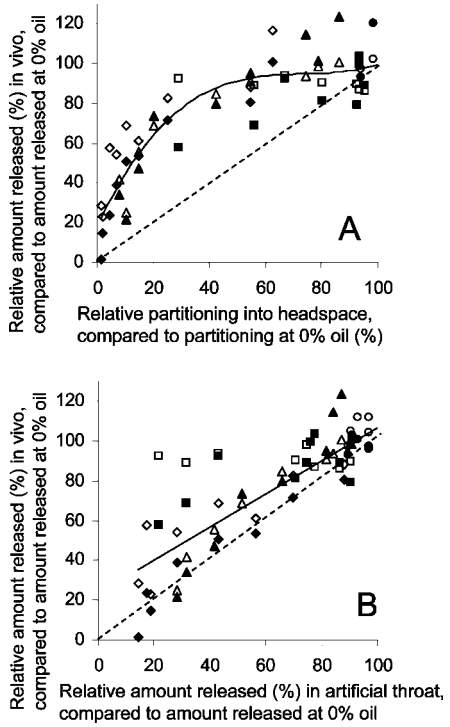

Figure 2. Correlation between relative released amount of ethyl acetate (●), ethyl butanoate (■), ethyl hexanoate (▲), and geranyl acetate (◆) under in vivo conditions (%) for two panelists (open and closed symbols represent panelist 1 and 2, respectively) and (**A**) the relative partitioning into headspace (HSGC) and (**B**) the relative amount released in the artificial throat. The solid lines represent a polynomial (**A**) or a linear (**B**) fit. The dashed line indicates perfect linear correlation.

Table 2. Averaged Perceived Orthonasal and Retronasal Intensities of Ethyl Hexanoate and Fattiness of Emulsions with Different Oil Contents Scored on a 0–10 Scale

emulsion oil content	0%	0.05%	0.1%	0.2%	0.5%	1.0%
orthonasal aroma intensity[a]	7.1^{A}	6.1^{AB}	5.8^{AB}	5.0^{BC}	3.5^{CD}	2.9^{D}
retronasal aroma intensity[a]	5.8^{A}	6.0^{A}	5.2^{AB}	4.4^{AB}	4.6^{AB}	3.8^{B}
fattiness mouthfeel[a]	2.7^{A}	4.3^{BC}	3.2^{AB}	3.8^{AB}	4.7^{AB}	5.8^{C}

[a] Different letters indicate significant difference (Fisher single-sided LSD test, $\alpha = 0.05$).

in partitioning under static headspace conditions, especially at low- and high-oil contents. This can be seen in **Figure 2A**, where the results obtained under in vivo conditions have been plotted against the HSGC results. A polynomial trend line was fitted to the data ($R^2 = 0.84$). No theoretical background could be given to support the choice of a polynomial trendline or to specify exactly the expected physical relationship between these static and dynamic processes.

Several authors observed before that the retention effect of oil on aroma release is smaller under in vivo conditions than under static headspace conditions (*6*, *10*, *12*, *15*). For example, the results obtained for release of three esters under static headspace and in vivo conditions by Doyen et al. (*12*) using C8 triglyceride oil match very closely with the results reported in this work.

Effect of Oil Content on Perception of Ethyl Hexanoate. A panel scored orthonasal and retronasal intensities of ethyl hexanoate and fattiness mouthfeel of emulsions containing a range of oil contents (**Table 2**). The aroma perception decreases with increasing oil content, during both orthonasal and retronasal evaluation (ANOVA: $p < 10E{-}8$ and $p = 0.013$, respectively). The effect is less strong at retronasal evaluation compared to orthonasal ($p = 0.11$, homogeneity-of-slopes model). A significant increase in perceived fattiness was found at higher oil concentrations (ANOVA, $p = 0.0005$). No significant panelist effect was found in any of the ANOVAs. The Levene test for homogeneity of variances was not significant for any factor, indicating homogeneity of variances throughout the datasets.

Orthonasal aroma intensity was assessed by sniffing from freshly opened bottles containing emulsions. This process is similar to HSGC analysis and the aroma release is governed by the partition coefficient. The aroma partitioning of ethyl hexanoate into the headspace decreases at higher oil contents (**Figure 1A**), and consequently, the orthonasal aroma perception decreases as well.

Retronasal perception and in vivo release of aroma compounds occur upon exhalation after swallowing when the aroma compounds release from the thin liquid layer in the throat and reach the olfactory epithelium (*16*). A large gradient in aroma concentration exists between the thin liquid film in the throat and the relatively large air flow that flows along it. This results in a strong driving force for aroma release. It is hypothesized that not only aroma compounds from the water phase will release into the air, but also aroma compounds from the oil phase (via the water phase). Consequently, this could explain why the effect of oil on aroma release is smaller under in vivo conditions than under static headspace conditions and smaller when judged retronasally as compared to orthonasally. To find proof for this assumption, the effect of oil content on aroma release was tested in an artificial throat.

Effect of Oil Content on Aroma Release in the Artificial Throat. The artificial throat simulates aroma release in the situation that a thin liquid layer is remaining in the throat after swallowing (*16*).

Figure 3 shows release curves of the esters from emulsions with different oil contents obtained from artificial throat measurements. An increase in oil content results in a lower maximum intensity (I_{max}) and more peak tailing at higher oil contents. The decrease in I_{max} and increase in peak tailing at higher oil contents are stronger for compounds with a higher hydrophobicity. The I_{max} of the ethyl butanoate curve at 0.5% oil is higher than the one at 0% oil. This is no systematic effect, but an example of experimental error in the data, which is generally less than 10%.

The P_{ow} values of the hydrophobic compounds used show that the concentration of an aroma compound in the oil phase of an emulsion is much higher than the concentration in the liquid phase (**Table 1**). When the oil content is increased, the aroma concentration in the oil phase decreases as a result of dilution, and consequently the concentration in the water phase decreases as a result of partitioning. The latter results in a smaller concentration gradient between the emulsion and air and consequently in a smaller driving force for release. Therefore, the release rate reduces significantly, which leads to low gas-phase concentrations and long release times. At a certain point, this release becomes too small to be analyzed accurately by the MS−Nose. Hence, peak areas cannot be compared directly among curves acquired at different oil contents.

An increase in release of geranyl acetate is observed for emulsions with higher oil contents (1−5%). This might be attributed to the increase observed in layer thickness of the emulsion remaining on the inside of the rubber tube of the artificial throat, after pouring the sample through. The increase in layer thickness is caused by an increase in emulsion viscosity (from 1.3 to 3.4 mPa·s for emulsions with 1 and 5% oil,

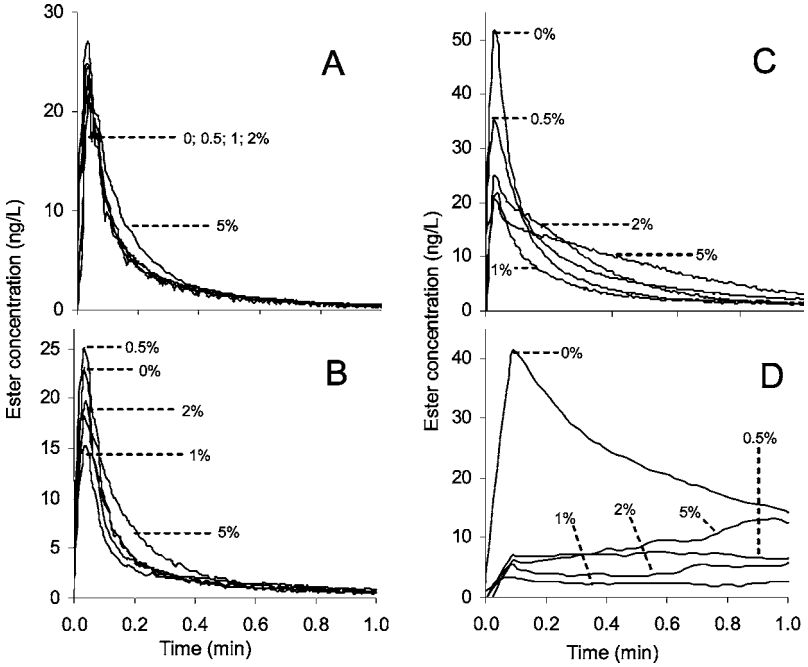

Figure 3. Release curves for ethyl acetate (**A**), ethyl butanoate (**B**), ethyl hexanoate (**C**), and geranyl acetate (**D**) of artificial throat measurements of emulsions with different oil contents.

respectively), possibly combined with an interaction between the apolar rubber surface and the emulsion surface, which has increased in hydrophobicity. No increase in layer thickness is observed for the hydrophilic glass tube. The layer thickness of the rubber tube increased from 0.045 to 0.11 mm, for emulsions with 1 and 5% oil, respectively. Consequently, a larger amount of aroma compounds will be available for release. A thicker layer also gives a higher diffusion time scale, which results in a slower release. However, these considerations do not explain why an increase in release for emulsions with higher oil contents (1−5%) is observed only for geranyl acetate and not for the other compounds.

The effects of oil content on release of several esters in the artificial throat are shown in **Figure 1D** and are comparable to the effect observed under in vivo conditions (shown in **Figure 1B**,**C**). The effect of oil content on ester release in the artificial throat is smaller than under static headspace conditions (**Figure 1A**). **Figure 2B** shows the correlation between the relative amount of ester released under in vivo conditions and the relative amount released in the artificial throat within 1 min. Although quite some scattering is present in the data, a linear correlation was observed between the results obtained under artificial throat and in vivo conditions ($R^2 = 0.75$). In vivo release measurements and static headspace results are less correlated at high- and low-oil contents. Therefore, artificial throat measurements have a higher predictive power for in vivo aroma release than static headspace measurements (**Figure 2**).

Smallest Effective Oil Content. The dataset acquired in this study allowed the determination of the smallest oil content that influences release or perception of aroma significantly ($\alpha = 0.05$), when compared to oil-free solutions, for the various systems used (**Table 3**). Lower effective oil contents are found for esters with a higher hydrophobicity. When the analysis is based on partition (HSGC and orthonasal perception), instead of release dynamics from a thin liquid layer (artificial throat, in vivo release, and retronasal perception), lower effective oil fractions are found as well. In ethyl hexanoate, the effective oil content of the artificial throat (0.2%), the in vivo aroma release measurements (0.5−1%), and the retronasal perception (1%) are all within the same range in this study, indicating the relevance of artificial throat and in vivo aroma release measurements for aroma perception.

Table 3. Lowest Oil Contents from Emulsions That Differ Significantly from 0% Oil Emulsions, for Various Analytical Techniques[a]

	ethyl acetate	ethyl butanoate	ethyl hexanoate	geranyl acetate
panelist 1	(>5%)[b]	(>5%)[b]	0.5%	0.1%
panelist 2	(>5%)[b]	(>5%)[b]	1%	0.05%
HS-GC	(>5%)[b]	0.5%	0.01%	0.01%
AT (1 min)	(>5%)[b]	0.5%	0.2%	0.05%
panel orthonasal	Nm[c]	Nm	0.2%	Nm
panel retronasal	Nm	Nm	1%	Nm
panel fattiness	Nm	Nm	0.5%	Nm

[a] All tested by single-sided *t* tests ($\alpha = 0.05$), except panel results (single-sided Fisher LSD test, to take the panelist effect into account). [b] Possible significant effect at higher oil content. [c] Not measured.

Effect of Droplet Size Distribution on Aroma Release. The effect of droplet size distribution on the release of ethyl butanoate, ethyl hexanoate, and geranyl acetate was tested at two oil contents (0.1% and 1%) under static headspace, in vivo, and artificial throat conditions. The release of ethyl acetate was not measured because an oil content of 1% did not influence its release at all. The HSGC and artificial throat measurements did not yield a significant effect of droplet size distribution (Fisher LSD-test, $\alpha = 0.05$, results not shown). No effect of droplet size on release in static headspace was observed by Carey and co-workers (*9*). The results of in vivo release of esters are shown in **Table 4**. A significant effect of droplet size distribution was found for geranyl acetate at an oil content of 0.1% under in vivo conditions. The release from the crude emulsion (**A**) was lower than from the emulsions homogenized at low and high energy (**B** and **C**, respectively). This was found similarly for both panelists.

The difference between in vivo measurements and artificial throat measurements for the effect of droplet size distribution on release of geranyl acetate may be related to a difference in time scales. The time scale of diffusion (τ) within an oil droplet

Table 4. Amounts of Ester Released (Nanograms) for both Panelists from Emulsions (A–C)[a] with Different Droplet Size Distributions

		panelist 1			panelist 2		
	% oil	A	B	C	A	B	C
$D_{(4,3)}$ (μm)		10	2.3	0.4	10	2.3	0.4
ethyl butanoate	0.1	0.9	0.9	0.8	0.6	0.6	0.7
	1.0	0.5	0.5	0.5	0.7	0.6	0.6
ethyl hexanoate	0.1	2.6	3.0	2.9	2.6	3.1	3.6
	1.0	1.1	1.4	1.4	1.6	2.1	1.9
geranyl acetate[b]	0.1	1.1[a]	2.5[b]	2.9[b]	1.0[a]	2.3[b]	2.9[b]
	1.0	0.3	0.5	0.6	0.7	0.8	0.8

[a] A, crude emulsion; B and C, low- and high-energy-homogenized emulsions, respectively. [b] Different letters indicate significant difference (Fisher single-sided LSD test, $\alpha = 0.01$).

is given by eq 1, in which d is the droplet diameter (m) and D is the diffusion coefficient (ranging from $1 \cdot 10^{-10}$ to $1 \cdot 10^{-9}$ m^2/s for small molecules in water, derived from the Hayduk−Minhas correlation (*23*)). Using the $D_{(4,3)}$ measured, this results in a τ in the order of roughly 1 s for the crude emulsion. For the low-energy, homogenized emulsion τ is approximately 0.05 s.

$$\tau = d^2/D \quad (1)$$

The experimental release time of geranyl acetate in the artificial throat is approximately 1 min, which is much larger than the diffusion time scale. However, the time scale of in vivo aroma release measurements is 3 s, as dictated by the breathing protocol, which is in the same range as the time scale of diffusion in the oil droplets. This may have a large effect on the initial release of highly hydrophobic compounds. The aroma concentration of less hydrophobic compounds in the water phase is probably too high to have their initial release influenced by diffusion processes in the oil droplets.

This effect is observed at an oil content of 0.1%, but not at 1%, while both have the same droplet size distribution. The amount of geranyl acetate released under in vivo conditions at 1% is much lower than at 0.1%. Therefore, the signal-to-noise ratio is lower at 1% oil content, and the effect of droplet size is not significant.

In literature, droplet size has been reported to increase, to decrease, as well as to have no effect on aroma release for different systems (*9*, *13*, *19*, *20*). The present results indicate that the effect of droplet size distribution on aroma release strongly depends on the hydrophobicity of the aroma compound, the emulsion characteristics, and the dynamics of the measurement. The effect of droplet size distribution on in vivo release of geranyl acetate shows the potential of the emulsion structure to influence the in vivo release of very hydrophobic compounds. Therefore, a relatively large change in release under in vivo conditions might be accomplished modulating the oil droplet size distribution, without changing the oil content.

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