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INSTRUMENTALLY MEASURED RELEASE AND HUMAN PERCEPTION OF AROMA COMPOUNDS FROM FOODS AND MODEL SYSTEMS DIFFERING IN FAT CONTENT

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ACADEMIC DISSERTATION

To be presented, with the permission of the Faculty of Agriculture and Forestry of the University of Helsinki, for public criticism in the Walter-hall, Viikki on November 26th, 2004 at 12 o'clock noon.

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ABSTRACT

The overall aim of this thesis was to clarify the role of fat in the release of aroma compounds and their perception. The effect of matrix fat content on the volatility and perceived intensity of aroma compounds was studied in different model and real food systems. Special interest was placed on the temporal aspect of the release of aroma compounds as the literature concerning this is quite conflicting. The applicability of different sensory and instrumental methods in aroma research was investigated. The sensory evaluations focused on orthonasal or retronasal aroma, or comparisons of these. Instrumental methods included traditional static headspace gas chromatographic methods, one type of electronic nose (MGD-1), and *in vitro* and *in vivo* measurements with an atmospheric pressure chemical ionization mass spectrometer (APCI-MS). An additional aim in one of the sub-studies was to investigate the effects of emulsion parameters on the release of aroma compounds.

A hypothesized suppressing effect of fat on the volatility and perceived intensity of the model lipophilic aroma compound was generally observed with all the methods in all of the studies. Even low levels of fat (0.5% being the lowest level studied) had a significant suppressing effect, though this was more clearly seen using instrumental than sensory methods. The more polar compounds were in most cases not affected by the fat content of the matrix. The effects of other compositional or structural factors of the matrices studied (e.g. type of fat, emulsifier type, droplet size) were less important in the release of aroma compounds compared to the amount of fat.

In addition to the effect on volatility/intensity of aroma compounds, fat is often claimed to affect the temporal release of aroma compounds. The hypothesized effect of quicker release with shorter duration in lower fat samples appeared to be difficult to prove, especially with sensory methods. In some cases even the opposite effect was found, as the duration of aroma perception was found to be greatest in samples containing lower levels of fat. Yet the decrease in intensity after the maximum was in most cases steeper in the low-fat samples compared to fattier samples.

A novel tool for aroma research, MGD-1 was found to perform well in a study utilizing frozen samples, but its sensitiveness towards humidity made it incompetent in measurements of more humid samples. The retronasal and orthonasal measurements gave parallel results, yet retronasal aroma was rated to be more intense. As the eating process is so complex and individual variations in it are large, measurements on the release of aroma compounds are challenging. For example, APCI-MS measurements utilizing two different sampling methods gave substantially different results. Instrumental and sensory methods are to be considered to be more complementary than substitutive in the field of aroma research. Care should be taken when choosing appropriate method for each application as orthonasal and retronasal, static and dynamic, instrumental and sensory methods measure different aspects of aroma. Understanding the timing of aroma perception is an area that still needs further studies as the temporal properties of aroma compounds frequently cause flavor problems in, for example, reduced-fat products.

PREFACE

This study was carried out at the Department of Food Technology, University of Helsinki during the years 1999-2004. It was funded by National Technology Agency (Tekes) and the Finnish Graduate School Program 'Applied Bioscience – Bioengineering, Food & Nutrition, Environment' (ABS).

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Helsinki, October 2004

Sanna-Maija Miettinen

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original articles, which are referred to by their Roman numerals I-IV.

I Miettinen, S.-M., Piironen, V., Tuorila, H. & Hyvönen, L. 2002. Electronic and human nose in the detection of aroma differences between strawberry ice cream of varying fat content. Journal of Food Science 67(1): 425-430.

II Miettinen, S.-M., Tuorila, H., Piironen, V., Vehkalahti, K. and Hyvönen, L. 2002. Effect of emulsion characteristics on the release of aromas as detected by sensory evaluation, static headspace gas chromatography and electronic nose. Journal of Agricultural and Food Chemistry 50: 4232-4239.

III Miettinen, S.-M., Hyvönen, L., and Tuorila, H., 2003. The timing of the intensity perception of a polar vs. nonpolar aroma compound in the presence of added vegetable fat in milk. Journal of Agricultural and Food Chemistry 51: 5437-5443.

IV Miettinen, S.-M., Hyvönen, L., Linforth, R.S.T., Taylor, A.J., Tuorila H. Temporal aroma delivery from milk systems containing 0-5% added fat, observed by free choice profiling, time intensity, and APCI-MS techniques. Journal of Agricultural and Food Chemistry (in press)

RESEARCH INPUT AND AUTHORSHIP OF ARTICLES (I-IV)

Sanna-Maija Miettinen's dissertation is a summary of research reported in four (I-IV) appended articles. The research input and authorship of the articles are as follows:

I *Miettinen, S.-M., Piironen, V., Tuorila, H. & Hyvönen, L. 2002. Electronic and human nose in the detection of aroma differences between strawberry ice cream of varying fat content. Journal of Food Science, 67(1): 425-430.*

The planning of the study was carried out by Sanna-Maija Miettinen M.Sc., Prof. Lea Hyvönen, Prof. Vieno Piironen, and Prof. Hely Tuorila. The experimental study including empirical work and the preparation of the manuscript were carried out by Sanna-Maija Miettinen M.Sc. The study was supervised by Prof. Lea Hyvönen, Prof. Vieno Piironen, and Prof. Hely Tuorila and they also participated in the writing of the manuscript by giving their comments and suggestions.

II *Miettinen, S.-M., Tuorila, H., Piironen, V., Vehkalahti, K. and Hyvönen, L. 2002. Effect of emulsion characteristics on the release of aromas as detected by sensory evaluation, static headspace gas chromatography and electronic nose. Journal of Agricultural and Food Chemistry, 50: 4232-4239.*

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III *Miettinen, S.-M., Hyvönen, L. & Tuorila, H. Timing of intensity perception of a polar vs. nonpolar aroma compound in the presence of added vegetable fat in milk. Journal of Agricultural and Food Chemistry, 51: 5437-5443.*

The planning of the study was carried out by Sanna-Maija Miettinen M.Sc., Prof. Lea Hyvönen, and Prof. Hely Tuorila. The experimental study including empirical work and the preparation of the manuscript were carried out by. Sanna-Maija Miettinen M.Sc. The study was supervised by Prof. Lea Hyvönen and Prof. Hely Tuorila and they also participated in the writing of the manuscript by giving their comments and suggestions.

IV *Miettinen, S.-M., Hyvönen, L., Linforth, R.S.T., Taylor, A.J., Tuorila H. Temporal aroma delivery from milk systems at 0-5% fat levels observed by free choice profiling, time intensity and APCI-MS techniques. Journal of Agricultural and Food Chemistry (in press)*

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LIST OF ABBREVIATIONS

AEDA	Aroma extraction dilution analysis
APCI-MS	Atmospheric pressure chemical-ionization mass spectrometry
AUC	Area under curve
CHARM	Combined hedonic and response measurement
DAng	Decrease angle
DArea	Decrease area
DHS	Dynamic headspace
DUR	Duration
FCP	Free choice profiling
FTIR	Fourier transform infrared
GC	Gas chromatography
GC-O	Gas chromatography-olfactometry
HLB	Hydrophilic-lipophilic balance
HS	Headspace
IAng	Increase angle
IArea	Increase area
IMax	Maximum intensity
MS	Mass spectrometry
OAV	Odor active value
PCA	Principal component analysis
RAS	Retronasal aroma simulator
SDE	Simultaneous distillation and extraction
SEM	Standard error of the mean
SFE	Supercritical fluid extraction
SHS	Static headspace
SPME	Solid phase microextraction
TI	Time intensity
TMax	Time to maximum intensity

1. INTRODUCTION

The aroma of a food is of great importance in food acceptance and preference. Aroma perceived via the nostrils (orthonasal aroma), along with the appearance, gives the first impression of a food. After the food is placed in the mouth, the aroma compounds are transferred to the receptors via the pharynx (retronasal aroma). In everyday language retronasal aroma is often confused with taste perception (Rozin, 1982). For example, when strawberries are eaten, the taste components produce the perception of a sweet and sour taste, and by far the most characteristic sensation of strawberry flavor arises from volatile aroma compounds.

Typically, food aroma is an equilibrium mixture of perhaps dozens of aroma compounds. All aroma compounds are relatively small (< 400 Da), usually organic compounds (Landy et al., 1996). The chemical structures of aroma compounds however vary widely; they include acids, neutral compounds, sulfur and nitrogen compounds, alcohols, aldehydes, ketones, hydrocarbons, esters etc. There are also large differences in the volatility of aroma compounds, ranging from components with boiling points well below room temperature to compounds that are solid at room temperature (Parliment, 1997).

The release of aroma compounds from foods is determined by the partition coefficient between the air phase and food matrix and, in the retronasal case, by the partition coefficient between the water phase (saliva) and the food matrix. If an aroma compound is added to the water matrix in a closed system and allowed to reach equilibrium, it will distribute between the air and water phases according to its air-to-water partition coefficient P_{aw}:

$$P_{aw} = C_a/C_w,$$

where C_a and C_w are the concentrations (g/l) of the flavor compound in air and in water. In products containing a lipid phase, the aroma distributes between three phases; air, water, and lipid. The equilibrium distribution between the lipid and water phases is given by the oil-to-water partition coefficient P_{ow}:

$P_{ow} = C_o/C_w$,

where C_o and C_w are the concentrations of the aroma compound in oil and water (de Roos, 1997).

The eating process is, however, a dynamic process and equilibrium concentrations in the headspace of the mouth are not achieved. The release of aroma compounds in real eating situations is more determined by the rate of release (Baek et al., 1999) and the partition coefficients should be regarded as a maximum potential extent of release. The rate of aroma compound release is determined by the resistance to mass transport. The resistance to mass transport is a function of the texture (e.g. viscosity, emulsion droplet size, melting point of the lipid phase), the surface area of the food product, and the rate of surface renewal (de Roos, 1997).

Besides physiological factors, the amount and the rate at which aroma compounds are released depends on their retention in the food matrix. In addition to the physical factors (affecting resistance to mass transport) the degree of retention depends on interactions between aroma compounds and macrocomponents (lipids, carbohydrates, proteins), as well as interactions between aroma compounds and minor food constituents (e.g. tastants or other aroma compounds).

Of the major food constituents, fats have generally the greatest influence on aroma compound release (Rabe et al., 2003). Most aroma compounds are more lipophilic than hydrophilic (Kinsella, 1990), thus fats act as a solvents for aroma compounds reducing their volatility (Buttery et al., 1973). This means that generally the release is suppressed as the fat content of the matrix increases. Fats are also claimed to affect the temporal release of aroma compounds (de Roos, 1997). However, these effects depend on the physicochemical characteristics of the aroma compound; the volatility of more hydrophilic compounds is not similarly affected by the fat content of the matrix as the volatility of more lipophilic compounds. The different effect of fats on different aroma compounds makes the situation, when for example a recipe of a food product is changed, complicated as the optimum aroma very seldom consists of only a few character impact compounds, but rather a complex mixture of perhaps dozens of

compounds. Carbohydrates and proteins interact with aroma compounds differently from fats as they do not hold the same dissolving capability as lipids, rather they bind, adsorb, entrap, complex, and encapsulate aroma compounds (Chevance et al., 2000).

In addition to its role as an aroma carrier and release modulator, fat modifies the physical properties, like mouth feel, appearance, structure (e.g. texture and melting behavior), and heat transfer in foods (Marshall, 1990; Brauss et al., 1999a). From a nutritional point of view it is a very energy-rich constituent. As consumers have become more aware of the connection between a diet rich in saturated fats and several major chronic diseases (e.g. cardiovascular diseases), the demand for reduced fat or low fat foods has grown. However, consumers are not willing to sacrifice the flavor quality of their foods (Koeferli et al., 1996). Poor flavor quality, along with poor texture, are the major factors hindering the acceptance of reduced-fat foods (Giese, 1994; Mela, 1995; Graf & de Roos, 1996). This phenomenon is, however, quite food specific. Lloyd et al. (1995) found that for some foods fat reduction was easier to accept among consumers. However, as our understanding of the interactions of fats with aroma compounds, especially the temporal aspect, is still scant, the work of flavorists trying to produce reduced fat products with optimal sensory properties is still quite empirical.

The term aroma is used throughout this thesis to represent the olfactive component of flavor. The terms ortho- and retronasal refer to the two different routes that aroma compounds can be delivered to the olfactory epithelium in the nose.

2. LITERATURE REVIEW

2.1 THE EATING PROCESS AND AROMA PERCEPTION

2.1.1 Ortho- and retronasal routes for aroma perception

Before food is placed in the mouth, aroma is perceived orthonasally as volatile aroma components reach the olfactory epithelium via the nostrils (Fig. 1). The general requirements for odor perception, both ortho- and retronasal, are that the odor molecules reach the olfactory epithelium at a sufficient rate and concentration to allow penetration to the olfactory receptor neurons and activation of these receptors to develop central nervous system responses (Halpern, 2004). The orthonasal aroma is sensed either in a passive way (normal inhalation while food approaches the mouth) or by deliberately sniffing, the latter obviously producing a stronger sensation than the former (Laing, 1983).

After the food is placed in the mouth the retronasal perception of aromas via the oro- and nasopharynx (Fig. 1) also becomes available. The accessibility of the retronasal route for volatile compounds has been under a slight uncertainty: earlier a direct airway connection had been assumed (Buettner et al., 2001). And for example, early attempts to model the release of aroma compounds in the mouth applied a relatively free passage of volatiles from the oral to the nasal cavity. However, it has later been discovered that the physiology of the trachea is such that the velum with the posterior wall of the pharynx produces a closure capable of blocking the passage to the nasal cavity (Normand, et al. 2004).

Efforts have been recently made to understand the function of this velopharyngeal closure. In a study, in which 25mL helium was kept in the mouth for 3 min while breathing with closed lips, it was shown that no helium could be measured in the efflux from the nostrils unless vigorous mouth and tongue movements were made or “mouth air” was deliberately exhaled through the nose (Buettner & Schieberle, 2000b). This observation with gaseous helium might not reflect the real eating situation and in addition might be explained by learned control of the velopharyngeal closure (Halpern, 2004). Using aqueous ethyl butanoate, Buettner and Schieberle (2000b) found that with no tongue movements,

swallowing, or deliberate exhaling of mouth air through the nose, the recovered amount of ethyl butanoate (trapped in tenax for 1 min, eluted with diethyl ether and analyzed using high-resolution GC-MS) was insignificant compared to the amount recovered after swallowing (on average 100 times more was recovered). Based on these results, the mouth cavity was concluded to be a closed system for liquids unless swallowing or tongue movements that lower the base of the tongue occur, and that aroma is released mainly as bursts related to the swallowing event. Later Buettner et al. (2001) used videofluoroscopy and real-time magnetic resonance imaging to demonstrate physiological barriers that allow only intermittently access of volatiles to the nose during eating.

On the contrary, with various solid and liquid foods, Deibler et al. (2001) showed that aromas in the oral cavity will appear in the nasal cavity during exhalations with the lips closed. Hodgson et al. (2003) measured simultaneously mastication (using electromyography), swallowing (using a swallow button), and the release of aroma compounds (using an atmospheric pressure chemical-ionization mass spectrometer, APCI-MS) and found that during each chew there was a pulse of air pumped out of the mouth into the throat. In addition, the videofluoroscopy studies of Pearlman et al. (2000) showed that during breathing (exhalation or inhalation) and swallowing of liquid or paste matrices the connection between the oral and nasal cavities is usually open. In conclusion, a detailed understanding of the function of velopharyngeal closure is still partly missing, but it is likely that the passage to the nasal cavity for volatiles originating from solid foods is more readily open, due to the chewing action involved, than for volatiles originating from liquid foods.

The volatiles from liquid foods have access to the nasal cavity mainly after the swallowing action producing an aroma-rich “swallowing breath”. This “swallowing breath” has been considered to be the only source of aroma compounds in the nose cavity originating from the retronasal route (Land, 1994, Buettner et al., 2001). However, for example, a study by Linforth and Taylor (2000) showed the considerable persistence of aromas in the breath after the consumption of liquid samples, which does not support the concept of the swallowing breath as the only source of volatiles from the mouth to the nose.

The recent modeling studies of Normand et al. (2004) suggest that quantitatively most of the retronasal aroma of a liquid sample originates from the throat lining. In their model the kinetics of the release of aroma compounds during drinking is divided into three parts. First, the swallowing breath results in a small amount of aroma-rich air being transferred to the nose. Secondly, in the next few breaths, the release originates from the liquid film coating the throat. In the third phase the interaction with the mucosa must be considered - first aroma compounds diffuse from saliva to the air and mucosa, but after the concentration in the mucosa reaches equilibrium with the decreasing concentration in saliva, compounds are released from the mucosa to the saliva and air.

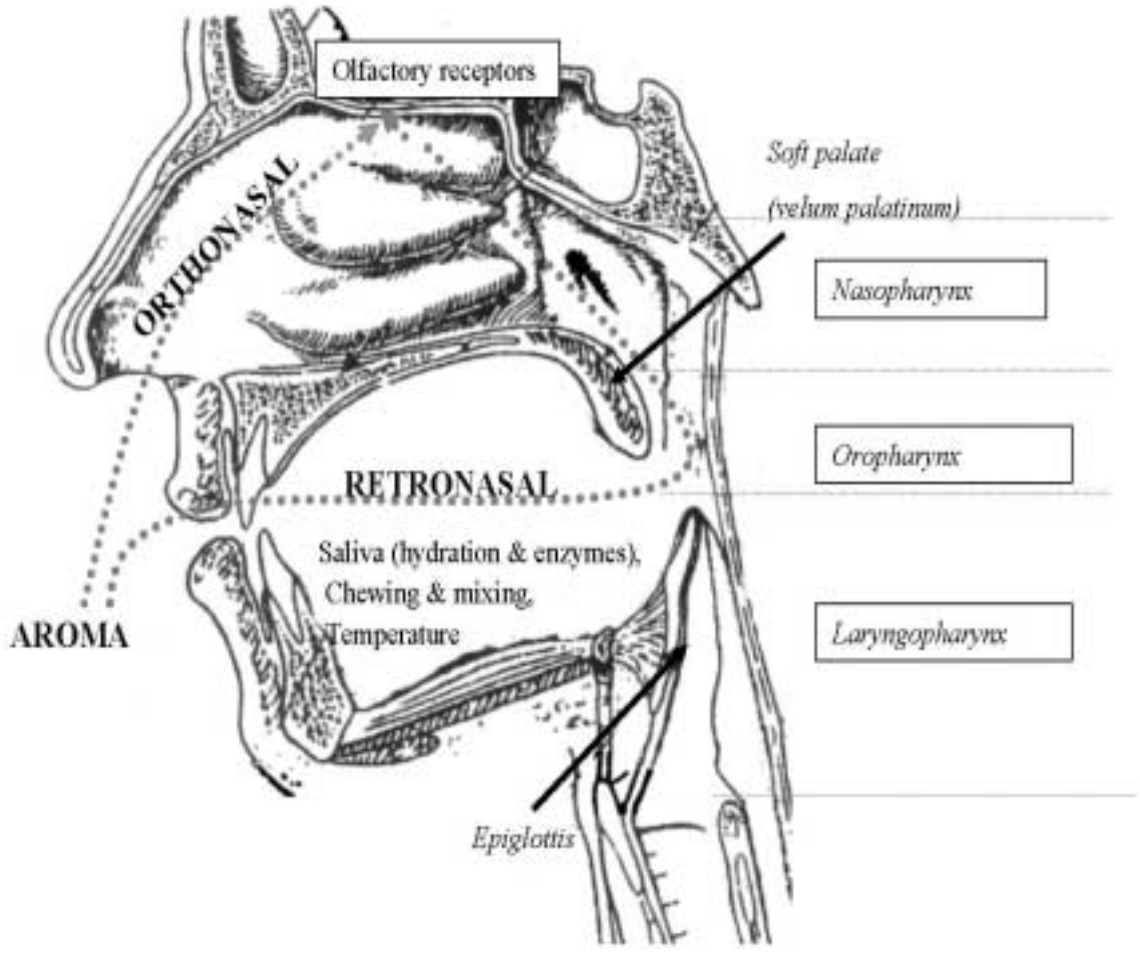

Fig. 1. Paramedian section of the human head showing the ortho- and retronasal routes of aroma perception.

In conclusion, the availability of a retronasal route for aroma perception is still somewhat uncertain. Similarly, the importance of the aroma portion that is adsorbed by the mucosa and its effect on retronasal aroma is not clear (Buettner & Schieberle, 2000a).

2.1.2 The quality and quantity of ortho- vs. retronasal aroma

The quality and quantity of ortho- and retronasal aroma are claimed to be different (Roberts & Acree, 1995; Heilmann & Hummel, 2004). These differences are suggested to arise from the events that food undergoes while it is in the mouth and from the fact that retronasal aroma is influenced by simultaneous taste/ chemosensory stimulation resulting in interactions at the receptor and/or cognitive levels. It is also likely that the efficiency with which the vapor phase is transferred to the olfactory epithelium is different via these two routes.

The literature on comparisons between the perception of ortho- vs. retronasal aroma is, however, contradictory. Some studies have found higher suprathreshold intensities for retronasal (Murphy & Cain, 1980; Garcia-Medina, 1981; Cometto-Muniz, 1981) and some for orthonasal aromas (Kuo et al., 1993), while others have found no differences between ortho- and retronasal aromas (Burdach et al., 1984; Marie et al., 1987). Aubry et al. (1999) found wine discrimination with ortho- and retronasal profilings to be fairly similar. Voirol and Daget (1989) found, using a meat flavoring stimulus, better concentration discrimination for sipping (retronasally) than for sniffing (orthonasally).

The above mentioned studies have used liquid retronasal stimulus, and thus conflicting results may arise from compound-specific interactions between odorants and tastants, and cognitive associations between particular odorants and tastants, both affecting retronasal perception (Pierce & Halpern, 1996). To compare purely olfactory responses via the ortho- and retronasal routes the stimulus should be presented into the oral cavity (for retronasal sensation) as a vapor. This has been done in some studies. Voirol and Daget (1986) used all three stimulus presentation methods (orthonasal, retronasal inhalation, and retronasal sipping) and found the lowest thresholds for the retronasal perception of vanillin and citral in liquid (sipping) compared to orthonasal or retronasal inhalation (the latter of these having the greatest threshold). Heilmann and Hummel (2004) found, using a novel technique for

retronasal stimulation based on odor release directly into the epipharynx above the soft palate, lower thresholds (for chocolate and lavender) for orthonasal compared to retronasal stimulation. In the same study, the intensity ratings for H_2S (but not of phenyl ethyl alcohol) were higher for orthonasal compared to retronasal evaluation. In an identification test the accuracy of retronasal identification of four different odorants was found to be somewhat less than that of orthonasal identification, even after retronasal training (Pierce & Halpern, 1996). Similarly, Rozin (1982) found that orthonasal stimulation performed better compared to retronasal stimulation (using oral stimulation) in identification tests.

In conclusion, most of the literature comparisons on ortho- and retronasal aromas have been done for intensity rather than quality differences. The obtained results are, however, too contradictory to allow a final conclusion to be made about which of these routes would produce a stronger sensation.

2.1.3 Physiological factors in the mouth cavity during eating

Retronasal aroma perception is affected by various events in the mouth during eating. In the mouth, food undergoes changes in structure, surface area, and composition as it is exposed to shearing and mixing caused by chewing, mixing with saliva and its components including enzymes, release/ retaining of compounds caused by adsorption/ (resorption/) diffusion/ binding to the saliva/ mucosa or to the air phase in the mouth (e.g. Burdach & Doty, 1987; Overbosch, et al., 1991; Roberts & Acree, 1995; Taylor, 1996; Buettner & Schieberle, 2000a; Buettner & Schieberle, 2000b; van Ruth & Roozen, 2000; Pionnier et al., 2004). In addition, the temperature of food changes, tending to become closer to the body temperature – the extent of temperature change depends on the original temperature of the food and the time that it is in the mouth before swallowing. The factors and events occurring in mouth during eating are summarized in Table 1.

Table 1. Physiological factors in the mouth during eating

1. Mastication	→	Increased surface area & mouth movements	→ Increased release of aroma compounds → Affects mouth feel
	→	Possible in-mouth generation of volatiles (enzymes e.g. originating from raw vegetables)	
2. Saliva	→	Interactions with saliva components (salts, enzymes)	→ Affects the release of aroma compounds
	→	Dilution	→ Possible phase inversion (saliva + temperature + shear) affects the release of aroma compounds, texture
	→	Hydration	→ Affects the release of aroma compounds and texture
3. Diffusion	→	Odorant and tastant release to saliva and air phase	→ Affects the release of aroma compounds
4. Binding	→	Odorant and tastant to the mucosa	→ Affects the release of aroma compounds (especially hydrophilic compounds are absorbed by the mucosa (Plug & Haring, 1994)
5. Temperature	→	Changes volatility of odorants	
	→	Melting (fats, gels)	→ Affects the release of aroma compounds and texture

The role of oral processing in aroma perception has been studied by de Wijk et al. (2003), who trained assessors to use five different techniques for eating food samples. They found that intensity ratings of all flavor attributes studied were influenced by the technique of eating; the technique utilizing the most complex movements gave the highest intensity ratings.

Individual differences in the eating process are large (van Ruth & Roozen, 2000; Pionnier et al., 2004; Geary et al., 2004). People vary in their breathing and chewing patterns, composition and amount of saliva, and volume of their oral cavities. In addition the moment and completeness of the velopharyngeal closure might vary between individuals, which affects the amounts of volatiles transferred to the nasal cavity (Buettner & Schieberle, 2000a). Further, the perceived sensation varies because of the great variation in sensitivity towards different volatile aroma components between people. For example a 25,000-fold difference in the detection limit of 2,4,6-trichloroanisole was found among 38 assessors tested (Suprenant & Butzke, 1996)

The type of food affects the significance of events occurring in the mouth while eating, as the possible changes are dependent on the length of the time food is kept in the mouth (Taylor, 1996). A beverage is hardly affected compared to solid foods that need to be thoroughly chewed before swallowing. Low-moisture solid foods, like biscuits, are prone to strong interactions with saliva components and to structural changes as they hydrate while eating. Foods that are of biological tissue (for example raw vegetables) release enzymes and substrates, which may generate aromas, while chewed in the mouth.

2.1.4 Cross-modal/ multi-modal interactions

One further complicating factor in aroma perception in humans is the fact that cross-modal/ multi-modal interactions occur, making, for example, an instrumental approach of measuring only the release of aroma compounds simplified in many cases. Some impressive examples of cross-modal interaction in flavor perception exist. Dalton et al. (2000) found a cross-modal summation of gustation and olfactory stimuli; the orthonasal threshold of benzaldehyde was significantly lowered by the presence of a subthreshold concentration of saccharin in the

mouth. Davidson et al. (1999) showed that the perceived mint flavor in chewing gum vanished along with the sugar even though the concentration of menthol (responsible for the mint flavor) in the breath was not decreased. Cook et al. (2004) found that when subjects were orally given sugar, water, strawberry aroma, and acid in a controlled way (using a multiple-channel flavor delivery system, McFlads), the removal of sugar led to a drastic decrease in the perception of strawberry flavor, whereas the removal of the actual strawberry flavoring (or the acid) had barely an effect on the perception of strawberry. The multisensory nature of perception was considered to be the reason for these results, not halo dumping. Halo dumping refers to a situation were assessors are provided with limited categories of attributes and thus when they experience a change in their perception, they may use inappropriate attribute if there is not appropriate attribute available.

In addition to psychophysical findings, neuroimaging and neurophysiological studies provide similar evidence suggesting cross-modal/ multi-modal interactions of taste and smell that are required to evoke flavor sensation (Rolls, 2002; Delwiche, 2004). Rolls (2002) showed in animal studies that neurons in the orbitofrontal taste cortex do not respond to only stimuli from one modality (taste), but can have sensitivity to other modalities as well. These neurons can be unimodal or multimodal. In a study, in which the texture of a product was modified a change was observed in the response of the neurons that respond to taste stimuli (Rolls, 2002). In addition, animal neurobiological studies have shown that there are specific neurons that require a taste and an aroma signal before they are activated (Rolls & Baylis, 1994).

Cross-modal interactions exist not only between gustation and olfaction, but also between other modalities. A number of studies have shown a decreased aroma/taste intensity as the viscosity of the food matrix is increased. In many cases these can be explained by a decreased diffusivity of aroma/taste compounds, by the reduced breakdown of the matrix while chewing etc. However, recent studies suggest there are also cross-modal interactions between taste/smell and texture perception. It has been shown in several studies that increased gel thickness did not significantly change the *in vivo* volatile concentrations in-nose (using the APCI-MS technique), although there were significant changes in the perceived odor and taste (Baek et al., 1999; Cook et al., 2003; Hollowood et al., 2002; Weel et al., 2002). In addition, irritants or temperature have shown cross-modal interactions with the

perception of tastes or smells (Delwiche, 2004). An example of interactions between color and taste/smell was shown in a study by Morrot et al. (2001), in which the odor of white wines colored red were described by enology students with descriptors normally related to red wine, while uncolored white wines tended to be described using white wine odor terms. However, the color and taste/smell interactions are always partly on a cognitive level as expectations always play a part in perception.

2.2 MEASURING AROMA COMPOUNDS AND PERCEPTION

Several factors make the release of aroma compounds measurements a challenging area of research. The versatility in the chemical structures of aroma compounds is large. The only generic characteristic of aroma compounds is that they are relatively small (molecular mass < 400 Da, Landy et al., 1996), volatile molecules. There are only a few so-called "character impact" compounds, which alone constitute a recognizable food aroma, but usually the aroma is a balanced mixture of perhaps dozens of volatile compounds. On the other hand, not all of the volatile components found in foods are of sensory importance. The concentrations of the aroma components in foods are usually very low, thus making them challenging to analyze, but exceptions occur. For example, the concentration of an important component of the grapefruit aroma, 1-p-menthene-8-thiol is 10^{-4} ppb (Fisher & Scott, 1997), whereas the aroma compound vanillin constitutes approximately 20% of the mass of the vanilla bean. The sensory properties of an aroma compound may change along with the concentration (for example *trans*-non-2-enal is described as woody, fatty or cucumber–like depending on its concentration, Fisher & Scott, 1997). However, it is not enough only to measure the concentrations of aroma compounds, the aroma activity and recognition/detection thresholds of the compound/compounds at issue should also be measured. A further complication arises from the fact that different compounds show different concentration – perceived intensity – relationships.

In foods, during cooking, storage or eating, the aroma compounds are prone to undergo chemical reactions that may alter their sensory properties (Fisher & Scott, 1997). Aroma compounds may interact with other aroma/taste compounds that may cause enhancement or inhibition of the aroma sensation. In addition, the release of aroma compounds is to a large

extent dependent on the interactions of aroma compounds and food macromolecules. And further if the volatility of a certain aroma compound is changed by a modification of the food macromolecules, this might cause changes in the volatility of some other aroma compounds, although they might not be directly affected by the macromolecule modification (Buettner & Schieberle, 2000a). All these changes in volatile profile are to be carefully taken into account for example when the recipe for a food product is changed, as a balanced mixture of components is a requirement for the right sensory perception of aroma.

In addition to empirical studies there have been numerous attempts to model the release of aroma compounds. Early models by McNulty and Karel (1973a,b,c) did not take into account the effect of the resistance to mass transfer. Later various models have been proposed by Overbosch (1986), de Roos & Wolswinkel (1994), Harrison et al. (1997), Harrison & Hills (1997), Roberts et al. (2003), and Rabe et al. (2004). However, it might be concluded that the eating process seems to be so complex that the different models have been mostly only moderately successful in predicting the release of aroma compounds *in vivo* and the work of flavorists is still in many cases very empirical.

In the following section techniques - instrumental, sensory, and combinations of these - for measuring the release of aroma compounds in foods are reviewed. Emphasis is placed on dynamic methods as eating is very much a dynamic process (section 2.1.3).

2.2.1 Instrumental techniques

Initially, the main focus in aroma research was to identify compounds that contribute to the aroma of a food product (Stephan et al., 2000). Much effort has been focused on establishing sensitive enough methods as aroma compounds are usually found only in trace amounts in foods. In the past, most instrumental analyses on aroma concentrated on the whole food products in a static situation without taking the dynamic nature of the real eating situation into account (Taylor, 1996). The instrumental analysis of aroma compounds consists of two phases – the separation and isolation phase (sampling) and the identification and quantification (analysis) phase. Two different approaches can be utilized for isolation of

aroma compounds from a sample matrix: techniques based on solubility or techniques based on volatility.

2.2.1.1 SAMPLING

Techniques based on solubility

Different extraction methods can be utilized in aroma isolation. The simplest, but quite tedious method for liquid-liquid extraction is the multiple-batch extraction using a separating funnel (Stevenson, et al., 1996), but more specialized equipment may be required depending on the solvents used. Extraction can be done using for example organic solvents, water, liquid CO_2, or supercritical CO_2. Solvent extraction using for example pentane, diethyl ether, or dichloromethane can be best applied to isolate volatiles from some nonfat foods (Stephan, et al., 2000). Solvent extraction with organic solvents is not as such applicable for fatty foods as fat is extracted as well and further purifying using for example liquid CO_2 or steam distillation is needed to separate aroma compounds and fats (Fisher & Scott, 1997). Liquid CO_2 has been used for extraction since the 1970's and it has the advantage of being nontoxic and inexpensive compared to many solvents (Parliment, 1997). The solvent properties of liquid CO_2 are similar to diethyl ether. More recent methods apply extraction with supercritical fluids (using for example CO_2), which allow gentle treatment of the sample (Stevenson, et al., 1996). The critical temperature of CO_2 is 31 °C so extraction can be made at slightly higher temperatures. Changing operational parameters such as pressure, temperature and co-solvents can modify the dissolving power of the supercritical phase.

Solid-phase extraction (SPE) is an alternative to liquid-liquid extractions (Junk & Richard, 1988). The advantage of SPE is its rapidity, however it should be noted that repeatability of analysis requires equilibrium state, thus the speed of the analysis has to be controlled in this respect. Disadvantages of the SPE method include high blank values, a large variation between the products of different manufacturers, and lot variation (Stevenson, et al., 1996). In addition, dialysis can sometimes also be used in aroma compound isolation (Stevenson, et al. 1996). Many extraction/isolation techniques produce fairly dilute aroma solutions and

some concentration may be required. This can be done by evaporation. This technique has the disadvantage that some compounds may be lost by co-distillation. Adsorption by charcoal, silica gel, alumina, porous polymers etc. can also be used as concentration techniques for dilute aroma solutions.

Techniques based on volatility

These techniques include traditional distillation methods and methods that collect samples from the gas phase of a closed, equilibrium system of a sample matrix and air.

The traditional distillation methods are steam distillation, and Likens and Nickerson distillation (simultaneous distillation and extraction SDE, Likens & Nickerson, 1964) (Fisher & Scott, 1997). As the formation of artifacts is a disadvantage in traditional distillation methods (Taylor, 1996), the milder forms, like different high vacuum distillation techniques are commonly applied.

If the purpose is to separate as many volatile compounds as possible from the matrix, different extraction techniques can be applied. As far as the portion of aroma that is available for orthonasal perception is concerned, the relationship with the real eating situation is clearer if the headspace of a sample is collected and analyzed. Headspace sampling can be either static (SHS) or dynamic (DHS), or it can be done using the solid-phase microextraction (SPME) technique.

In the SHS technique the sample material is placed in a sealed vial and the gas-phase and sample-phase analyte concentrates are allowed to reach equilibrium (sometimes heating at a certain temperature for a certain time period is used) (Stevenson et al., 1996). Static headspace sampling is usually done using injection with gastight syringes combined with cryofocusing or using an adsorptive fiber such as SPME or using headspace sorptive extraction (HSSE) (Zehentbauer et al., 2004). Static headspace sampling is rapid, easily automatized and reproducible. The problem with SHS sampling is that the concentrations of compounds in the HS are usually low. The use of dynamic headspace sampling enables the enrichment of volatiles. In the DHS technique an inert gas is swept over or through the

sample for a certain time period and the released volatiles are then trapped. The most common techniques for trapping are cryogenic trapping, adsorption on a desorbable sorbent bed (charcoal, Tenax etc.), on-column vapor traps, or whole-column cryotrapping (Stevenson et al., 1996). The disadvantage of the dynamic method is poor reproducibility due to the strong dependence of the yield of the volatiles on the velocity of the carrier gas and the selectivity of the adsorption and desorption processes (Zehentbauer, et al., 2004).

A relatively new sample extraction technique for aroma research is solid phase microextraction (SPME) (Arthur & Pawliszyn, 1990). SPME has been successfully used for extracting volatiles from, for example, orange juice (Steffen & Paliszyn, 1996), vanilla extracts (Sostaric et al., 2000), and many other food applications (Pillonel et al., 2002). In this technique a coated fiber (polymeric liquid coating) is introduced into the headspace of the sample (or in a direct extraction mode to the sample matrix). The volatiles are partitioned between the sample matrix, the air (in the headspace sampling mode) and the immobilized stationary phase. The amount of absorbed volatiles depends on many SPME parameters, such as the type of the fiber, sample volume, temperature and extraction time, salting, mode of extraction (headspace or direct extraction), desorption of analytes from the fiber and derivatisation (Wardencki, et al., 2004). The advantages of this technique are that it is rapid and solvent-free and it has in many cases a greater sensitivity compared to other conventional methods e.g. purge and trap (Chaintreau, et al., 1995). The sensitivity is however dependent on the detector used (Wardencki, et al., 2004). The amount of sample required is small and the costs of analysis are usually relatively low. The limitations of the SPME technique are the rather low recovery of analytes and the low precision of the determinations (Wardencki, et al., 2004). In addition, the quantification in SPME is considerably complicated by the fact that there is two equilibrium systems involved, one between the sample and air, and one between the stationary phase and air.

It must be emphasized that the instrumental techniques that measure the headspace are comparable only with sensory orthonasal aroma, unless some kind of model mouth system is used in sampling.

Model mouths

To approach the retronasal aroma using instrumental methods and to incorporate the effect of oral physiology on the release of aroma compounds *in vitro*, different kinds of model mouth systems have been developed for aroma sampling. These systems can be considered to be variants of dynamic headspace sampling, but with the aim of obtaining a volatile release that as closely as possible represents the release during the actual eating situation. Most systems developed provide temperature control at 37ºC, artificial saliva, stirring, agitation or simulated mastication. Nassl et al. (1995) used their artificial mouth system that simulated the process of chewing to study flavor release in liquid foods. Roberts et al. (1995) developed the Retronasal Aroma Simulator (RAS) and used it with direct transfer to a mass spectrometer via a suitable interface. Odake et al. (1998) have studied the release of aroma compounds in cream style dressings with a dynamic headspace mastication model. For solid foods, maceration is required and many systems have been proposed and used (e.g. Ingham et al., 1995a; Roberts & Acree, 1996; van Ruth et al., 1995a; van Ruth et al., 1995b). Later studies on the temporal release of aromas have also become possible along with the rapid gas-phase analysis techniques (van Ruth et al. 2000).

2.2.1.2 ANALYSIS

Gas Chromatographic methods

By far the most used actual analysis method in aroma research is gas chromatography with a variety of detectors. Different kinds of capillary columns are utilized, and often whole column cryogenics, cryogenic traps, or on-column injections are used in conjunction with further enhancement of resolution, especially for lower boiling point volatiles (Stevenson et al., 1996). The most common instrumental detectors for GC analyses are flame ionization (FID), thermal conductivity (TCD), electron capture (ECD), flame photometric (FPD), and photoionization (PID) varieties (Fisher & Scott, 1997). The “Hyphenated” GC techniques refer to combination techniques like GC-mass spectrometry (GC-MS), GC-Fourier transform infrared (GC-FTIR), and GC-atomic emission spectroscopy (GC-AES). These hyphenated

techniques are advantageous as they allow the identification and quantification of volatile compounds (Fisher & Scott, 1997). The most utilized method in aroma compound identification is GC-MS, as MS gives good sensitivity (Taylor, 1996). Different MS-techniques are also utilized in aroma research, and for example, the MS-MS technique has been applied to aroma analysis using a solid probe without any sample preparation (Stevenson et al., 1996).

Gas chromatography-olfactometry (GC-O, Acree & Barnard, 1994) is a technique in which the human nose is used as a detector. The GC effluent is split so that part of it (usually approximately 90%) is drawn into a sniffer port and the rest of the eluent is sent to another detector (e.g. FID). As a result an aromagram- a chromatogram, in which all odors perceived are indicated on a time basis, is produced. GC-O can be combined with methods such as AEDA (Aroma Extract Dilution Analysis; Ullrich & Grosch, 1987) or CHARM (Combined Hedonic and Response Measurement; Acree et al., 1984) to assess the impact of single odorants to the general aroma. In addition, some GC-O methods that do not involve dilution, like OSME (a time intensity approach for evaluating the significance of odor compounds in GC effluent) or the finger span method exist. Both of these are focused on intensity measurements. Another method to characterize the aroma compounds is the concept of Odor Active Value (OAV), which takes into account both thresholds and actual concentrations of the compound (Stephan et al., 2000).

Electronic noses

As a result of financial factors in aroma research, especially in the area of quality control, there have been attempts to partly substitute the human nose with some fast and easy-to-use analytical tools. There is a growing number of instruments available for the monitoring of odor compounds, usually called electronic noses. These instruments are usually based on non-specific gas-sensors based on conductive polymers, metal oxides, or surface acoustic waves utilizing the piezoelectric effect (Bartlett et al., 1997). Electronic noses measure the aroma components as a whole, unlike the traditional instruments that are more or less separation techniques. Thus, they resemble the human nose, but it must be pointed out that the totality that these instruments measure is not the total odor intensity, but rather only an

undefined measure of the total volatiles (Stephan et al., 2000). Some semi-specific metal oxides sensors do exist, but the ability to, for example, detect the character impact component of some aroma is still far away.

However, numerous recent studies have applied electronic noses to the field of aroma research. To mention a few, different kinds of electronic nose systems have been used, for example, for analysing off-flavors in milk (Marsili, 1999), chocolates and packaging materials (Werlein, 2001), off-flavors in olive oils (Aparicio, et al., 2000). Bleibaum et al. (2002) used an electronic nose and a tongue system to predict the sensory characteristics and their relationship to the quality of apple juices assessed by consumers. The future trends in the area of electronic noses seems to be in the use of sensors of biological origin, like proteins (Zorn & Berger, 2004) or the development of more sophisticated neural networks (Stephan et al., 2000).

In addition, there are also available other types of gas analyzers for volatile analysis that are not classified as electronic noses. One example of a technique that can be utilized in such analyzers is low-resolution Fourier Transform infrared (FT-IR) spectroscopy. FT-IR spectroscopy is based on specific rotational and vibration energy levels of different molecules (Jaakkola, et al., 1998, Larjava, et al., 1997). Wavelengths are separated by an interferometer and the detected IR-signal is Fourier transformed into the IR spectrum. The advantages of the method are high selectivity (as two compounds cannot have the same IR spectrum), speed, and rapid recovery between measurements. Both qualitative and quantitative analyses are possible using this method. The calibration phase requires a reference method, thus setting up a new method is slightly tedious. A low-resolution gas phase FT-IR analyzer has been used, for example, for analyzing strawberry (Hakala et al. 2001), and caraway (Ahro et al., 2001) volatiles, and rapeseed oil oxidation (Ahro et al., 2002).

2.2.2 Sensory techniques

The sensory approach for measuring the temporal release of aroma compounds uses the Time Intensity (TI) method. Using the TI method it is possible to obtain rate-related, duration, and intensity information simultaneously, this is not possible using traditional scaling methods

(Lawless & Heymann, 1998). The development of computerized TI data collection systems in the 1980's enabled the on-line collection of data required for efficient use of TI methodology. The TI method is applicable, for example, for studying the melting behavior of foods, for profiling the different pungent or astringent substances, or for profiling different low-energy sweeteners or chewing gums, or for adaptation studies, as well as for measuring the release of aroma compounds during eating (Lawless & Heymann, 1998). For example, the effects of fat on the flavor release using the TI method have been studied by Shamil et al. (1992) in dressings and cheeses, by Chung et al. (2003) in ice creams, by Mialon & Ebeler (1997) in model emulsions, by King et al. (2000) in yogurt, and by Brauss et al. (1999b) in biscuits.

The parameters which are usually calculated from the TI data included 'time to maximum' (TMax), 'maximum intensity' (IMax), 'duration' (Dur), 'area under the curve' (AUC), 'increase angle' (IAng), 'increase area' (IArea), 'decrease angle' (DAng), and 'decrease area' (DArea). These most common TI parameters are illustrated in Figure 2.

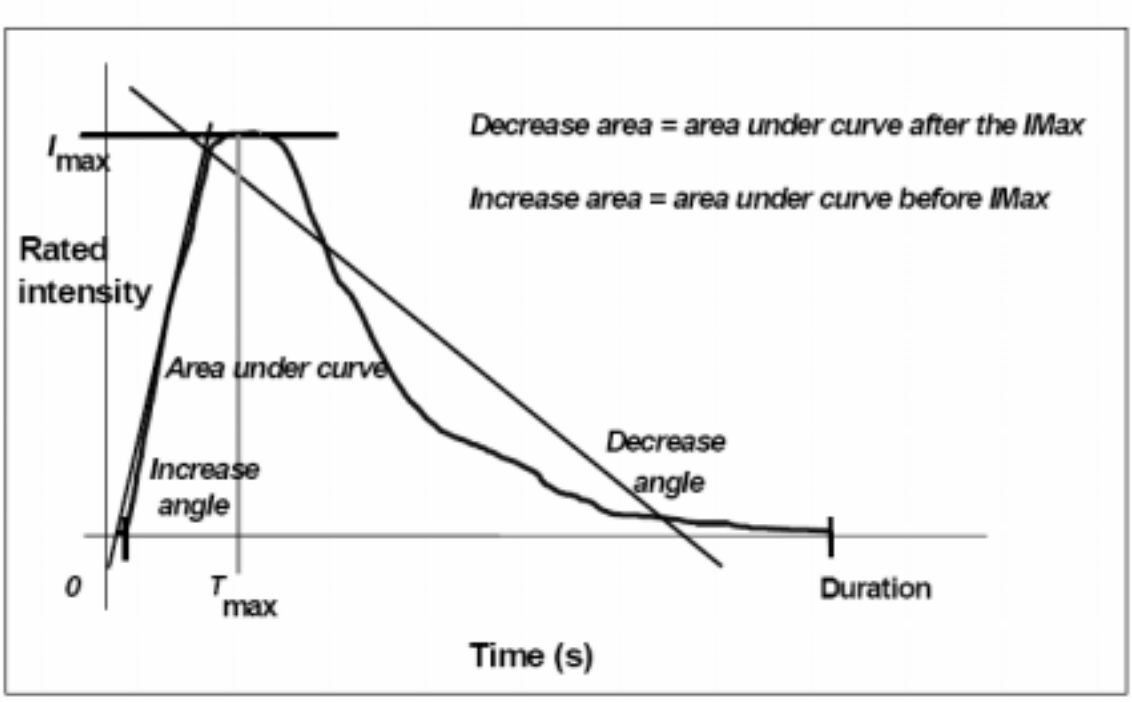

Fig. 2 Schematic representation of the most common TI parameters (TMax, IMax, Dur, AUC, IAng, IArea, Dang, and DArea).

The TI method is quite demanding for assessors and typically the variance obtained in results is large. This is partly due to the fact that assessors tend to have their own “signatures” for TI response based, of course, on pure physiological and perceptual differences, and also on differences in the use of a quite demanding time-related scale. The interpretation and analysis of the TI curves is still somewhat controversial. Some experimenters have utilized simple averaging of the curves obtained by different assessors, but others have proposed different kinds of data analysis methods to make the raw data more interpretable and to take better account of the assessors’ own signatures.

One option is to divide assessors into different groups based on similarities in their responses, and by using this procedure try to avoid irregular curves. Another option is to average the data both along the intensity axis and along the time axis. This latter procedure was first proposed by Overbosch et al. (1986) and later modified by Liu & MacFie (1990). The third option is to use Principal Component Analysis (PCA) or other multivariate methods to extract the major trends in a large data matrix, such as in the case of TI data.

Many TI studies have used expectoration in the evaluation procedure (e.g. Mialon & Ebeler 1997; Guinard et al., 2002). Several studies have shown that sensory responses obtained either by expectoration or swallowing of the samples correlate well (Kelly & Heymann, 1989; O’Mahony & Wong, 1989; Taylor & Pangborn, 1990). However, in a recent study by Buettner et al. (2002) it was found that over 90% of the aroma was detected (exhaled odorant measurement technique) immediately after swallowing of the sample (in the 'swallowing breath'), while prior to swallowing only a very small proportion of the volatiles reached the epithelium in the nose; the velum-tongue border effectively limited access to the nasal cavity. In our own pre-tests the aroma intensity was also rated higher when the samples were swallowed compared to the samples that were expectorated. This may be considered favorable with respect to detection of aroma differences between the samples.

2.2.3 Combinations of instrumental and sensory techniques

All the model mouth systems mentioned in the Instrumental sampling section (2.2.1.1) are yet still only models of the real eating situation: they help us understand the effects of processes that food undergoes while it is in mouth. To take one step closer to the eating process and to understand further the processes occurring in the mouth (interactions with mucosa and swallowing) systems have developed in which the sampling is done *in vivo*, either in the nose- or mouthspace.

The early methods incorporated trapping of volatiles from the nosespace to Tenax traps, followed by desorption and GC-MS analysis (Linforth & Taylor, 1993; Linforth et al., 1994; Ingham et al., 1995 a, b). Direct measurement of the breath (nose- or mouthspace) volatile concentration during eating has become possible along with the development of novel, online mass spectrometric techniques. The most common techniques utilize atmospheric pressure chemical ionization (APCI, Taylor et al., 2000) and proton transfer reaction (PTR, von Ruth et al., 2003). Both methods have limitations in sensitivity and selectivity, but yet they have greatly expanded our knowledge of the release of aroma compounds *in vivo*.

However, when comparing model mouths and these direct nose/mouthspace measurements, the different model mouth systems may often give a relatively good approximation of the release of aroma compounds, as individual variations in the oral parameters influencing release is great. In a verification study of a Retronasal aroma simulator (RAS) with APCI-MS it was found that this model mouth produced volatile profiles closely related to the profiles that were actually measured from panelists' breaths using APCI-MS (Deibler et al., 2001). Due to the individual variation in release often only few panelists with a large number of replications are used in, for example, APCI-MS measurements (Taylor & Hort, 2004).

The APCI-MS/PTR-MS techniques measure quite the same stimulus that actually reaches the epithelium. Yet there is still a difference in what is measured as the effect of mucin and odor-binding proteins located in the layer of mucus between nosespace air and odor receptors is not present in the instrumental techniques. In addition the cognitive level can not be taken into account in instrumental measurements.

2.3 THE EFFECTS OF FAT ON THE RELEASE OF AROMA COMPOUNDS AND THEIR PERCEPTION

Changing the fat content of a food matrix is known to affect the release of aroma compounds. Aroma compounds in general are rather lipophilic, but some more polar compounds exist. The effect of fat depends strongly on the lipophility of the aroma compound. The early studies of McNulty and Karel (1973a,b,c) already showed that the more lipophilic the compound, the greater was the retaining effect of oil on the release of *n*-alkanals. Later the effect of fat content of the matrix on the volatility and perception of aroma components has been studied widely and studies on the effects of the amount of fat are reviewed in the following sections (2.3.1 on intensity, and 2.3.2 on temporal release).

The effect of the type of the fat on the release of aroma compounds has received less attention, although the different nutritional impacts of different fat types are widely documented. An instrumental (SPME-GC-MS) study of Roberts et al. (2003) showed some effects of solid fat content of the matrix on the release of aroma compounds. Lee and Pangborn (1986) showed that the degree of unsaturation had an effect on the flavor properties of butter-like products. Hyvönen et al. (2003) found a slightly faster release of flavor from vegetable fat ice cream compared to its dairy fat counterpart.

Bulk fat is not usually found in foods, but rather it is present in the emulsified form. The interface may, as well as the actual fat component, impact on the release of aroma compounds. The impact depends on the emulsifier used, the amount of interface, droplet size, and the properties of the aroma component. Accounts in the literature concerning this are conflicting: some have found significant effects of emulsifier on the release of aroma compounds (Charles et al., 2000, Roberts et al., 2003) and others have found only a minor effect (Landy et al., 1996, Rabe et al., 2003).

2.3.1 The aroma intensities

Instrumental studies on the effects of lipids on volatility of aroma compounds have shown that very low fat levels can exert a significant effect. According to Hatchwell (1994) as little as 1-2% fat is enough to significantly affect the release of aroma compounds. This was seen for example in the study of Schierle-Keller et al. (1994) who found a very significant decrease in the headspace concentration of lipophilic compounds as the fat content was increased from 0% to 1%. Some studies have found effects even at a lower lipid level. Carey et al. (2002) studied the effect of hydrogenated coconut oil at levels of 0-2 g/kg in oil-in-water emulsion and found reduced static headspace concentrations of octanone and ethyl octanoate compared with the concentrations present in the aqueous matrix, while the concentration of ethyl butyrate, a more polar compound, was not affected. They found that as little as 0.25 g/kg fat reduced the headspace concentration of ethyl octanoate very significantly (approximately 20% headspace intensity compared with the level in the aqueous matrix). Roberts et al. (2003) found, using solid-phase microextraction and the GC-MS method, that as little as 0.02% milk fat in the matrix reduced the headspace concentration of a lipophilic aroma compound (limonene) by 50% compared to water. Seuvre et al. (2000) showed a significant retention (84% retained in the matrix) of 2-nonanone in the presence of 0.2% fat compared to an aqueous matrix using headspace-GC.

2.3.2 The temporal the release of aroma compounds

In addition to the effect on perceived intensities, fat is also believed to affect the temporal aspects of the release of aroma compounds. When the fat content of the matrix is decreased, the aroma compounds are claimed to be released at a faster rate (de Roos, 1997). The aroma of a reduced-fat product is often claimed to be harsh and unbalanced and to persist for a shorter time than the aroma of a full-fat product. A schematic representation of time vs. flavor intensity curves of full-fat and reduced-fat products in which the full-fat product has a delayed release curve with a decreased intensity compared to its reduced-fat counterpart is commonly shown (e.g. Bennett, 1991; Mattheis, 1995; de Roos, 1997).

These temporal effects of fat seem to be difficult to prove, especially with sensory methods (Brauss et al., 1999a). For example, Guinard et al. (2002) failed to observe a quicker release of garlic (but did in the case of pepper) in salad dressings upon fat reduction (0%, 6.75%, and 13.5% fat levels studied). Mialon & Ebeler (1997) failed to find an effect of fat on the temporal release of limonene (nonpolar) but the duration of a more polar compound, vanillin, was affected by the fat. However, Shamil et al. (1991/92) found, using the time intensity method, a quicker flavor release from reduced-fat foods compared with their full-fat counterparts (hard cheeses and salad creams). Using the instrumental (measuring the volatile composition of air from the nose with APCI-MS) method, Brauss et al. (1999a) found the release of lipophilic compounds to be faster and also at higher intensity levels in low-fat (0.2% fat) yoghurt than in yoghurts containing 3.5% or 10% fat. However, with the sensory TI method they failed to show this faster release.

Further conflicting observations are available for the effects of fat on the aroma duration. Rosin & Tuorila (1992) found a slightly shorter perception time for garlic and pepper flavor in fat-free mashed potatoes than in a counterpart containing 10% fat. Mialon & Ebeler (1997) similarly found that the duration of the retronasal aroma of vanillin (polar) increased with increased oil content of the emulsion (0%, 10%, or 50% oil). However, the oil content of the matrix had no influence on the temporal perception of limonene (nonpolar). Brauss et al. (1999) found with an instrumental method that lipophilic flavor compounds were less persistent in low-fat (0.2%) yoghurt than in yoghurts containing 3.5% or 10% fat, however using a sensory method they found no differences in the duration of flavor. In contrast to the above-mentioned studies, Shamil et al. (1991/92) found that the duration of flavor was greater in reduced-fat foods compared with their full-fat counterparts. Guinard et al. (2002) reported that with the removal of fat (0%, 6.75%, and 13.5% fat studied) the total duration of garlic and pepper flavor in salad dressing increased.

When examining the literature it is however clear that the hypothesised temporal effect is far from explained as the evidence is highly conflicting: some have found temporal effects for fat, but the findings might be contraindicative (Shamil et al., 1991/92, Rosin & Tuorila, 1992; Guinard et al., 2002 vs. Brauss et al., 1999a; Brauss et al., 1999b), and some have found no significant effects (the sensory results in Brauss et al., 1999a). According to Keast et al.

(2004) understanding the temporal profile of aroma perception is of great importance as the different temporal properties of aroma compounds cause flavor problems in products.

3 OBJECTIVES OF THE PRESENT STUDY

The overall aim of this study was to clarify the role of fat in the release of aroma compounds. The detailed aims were:

1. To study the effects of matrix on the release of aroma compounds by examining:

- the effects of the amount of fat on the intensity/volatility of aromas (I-IV) and on the temporal release of aroma compounds (III-IV). An additional aim in Studies III and IV was to investigate the amount of fat required for a significant effect on the release of aroma compounds.

- the effects of the type of fat on the release of aroma compounds (I)

- the effects of emulsion parameters on the release of aroma compounds (II)

2. To compare different techniques for measuring aroma compound release and their perception

- These studies apply different sensory and instrumental methods as an additional objective was to compare the applicability of different methods in aroma research.

4 MATERIALS AND METHODS

An overview of the materials and methods used is given in Table 2.

4.1 Samples

Base materials

The samples used were model systems except in Study I, where strawberry ice cream samples were used. The sample matrix in Study II was an oil-in-water emulsion, and in Studies III and IV milk based matrices were used. Samples were modified with regard to fat content (I-IV), fat type (I), or emulsion characteristics (droplet size, emulsifier type, II). The strawberry ice cream samples in Study I contained varying amounts (0%, 9%, or 18%) of dairy or vegetable fat. In Study II, model emulsions with rapeseed oil were prepared at fat levels of 5% or 50% using either modified potato starch or sucrose stearate emulsifier. These base emulsions were homogenized either at 100 bar or 300 bars of pressure to get emulsions with different droplet sizes. In this study pure water and oil were also used as sample matrices. In the case of milk based matrices in Studies III and IV, fat, either rapeseed oil (III) or cream (IV) was added to nonfat milk (UHT in Study IV). The added fat levels were 0%, 1%, 5% and, 10% (III) and 0%, 0.5%, and 5% (IV).

Aromatization

Strawberry ice creams were flavored with commercial strawberry flavoring during a normal ice cream manufacturing process. Studies II and III utilized a more simple aroma system: only two aromas, linalool and diacetyl, were used. These aromas were chosen to represent very different polarities to emphasize the strong dependence of the effect of fat on the physicochemical characteristics of aroma compounds. A simplistic aroma system was also beneficial due to the complexity of the other study parameters and the different methods applied. In the last study (IV) a more complex aroma system, containing 8 aroma compounds, was applied. The samples are described in more details in papers I-IV in the appendix.

Table 2. General designs of Studies I-IV

MATRIX	AROMA SYSTEM	METHODS	ORTHO/RETRO	AROMA DIFFERENCES MADE BY
I Ice Cream	Mixture	SH-GC, MGD-1, Sensory (R-index)	Orthonasal aroma	Amount of fat, type of fat
II O/W –emulsion	Linalool, diacetyl	SH-GC, MGD-1, Sensory (Intensity rating using single point-method)	Orthonasal aroma	Amount of fat, emulsifier, droplet size
III Milk	Linalool, diacetyl	Sensory (TI & Intensity rating using single point -method), SH-GC	Ortho- and retronasal	Amount of fat
IV Milk	Mixture	APCI-MS, Sensory (TI)	Retronasal aroma	Amount of fat

4.2 METHODS

General descriptions of the methods used are presented here. More detailed descriptions of the methods used can be found in papers I-IV in the appendix. All experimental conditions were chosen based on pretests.

4.2.1 Instrumental methods for the release of aroma compounds

Static headspace gas chromatography, SHS-GC

In Studies I-III the relative amounts of volatile components in the headspaces of the samples were measured using static-headspace gas chromatography. The columns used were an FFAP capillary column (25 m x 0.25 mm x 0.25 μm) in Study I and an NB54 (5%-phenyl-1%-vinyl methylpolysiloxane –phase) column (25m x 32μm) at 80 °C in Studies II- III. In Study I, a temperature program was used. Helium was used as the carrier gas and the detector in Studies I-III was FID (at 280°C in I and 250°C in II-III). All the studies utilized 22-mL vials and the amount of sample was 5g (I), 2mL (II), or 5mL (III). The samples were equilibrated for 20 minutes at 70 °C (I) or at 60 °C (II-III). The peak areas were measured as a result and triplicate samples were used. It was confirmed that the standard deviations of the peak areas were at a satisfactory level.

Electronic nose, MGD-1

In Studies I and II the release of aroma compounds was also measured with one type of electronic nose, MGD-1. The MGD-1 has six sensors, in which the detection is based on the ionization of gas molecules in a patented IMCELL^{TM} measurement cell. In the IMCELL^{TM}, an ^{241}Am (160 μCi) source is used for ionizing. The clusters formed through ion-molecule reactions are brought into different electrical fields perpendicular to the sample flow. The clusters hit different electrodes depending on their size and charge and are detected as the resulting current in the electrodes. In addition, the MGD-1 has one semiconductor metal oxide sensor, which is mainly used when the response of the electrodes needs to be further

confirmed. The operating principle of the MGD-1 has been described in detail elsewhere (Kotiaho et al., 1995; Tuovinen et al., 2000).

In Study I, 50g of the equilibrated ice cream samples in 300-mL Erlenmayer flasks were measured as triplicates. In Study II, 100-mL emulsion samples were equilibrated in 500-mL glass bottles and measured as triplicates. In Study II, the humidity of the sample air was kept between 30-40% by drawing the air via an Erlenmayer flask containing phosphorous (V) oxide. The cell temperature was 40 °C (I) or 35 °C (II). The airflow of the MGD-1 was adjusted to approximately 2.3 l/min (I) or 2.0 l/min (II).

As a result the slope, the area under the curve (measured for responses for 45 s, within the time the responses were stabilized), the maximum intensity and the slope of the descending part of the curve for each measurement channel (electrode) and for the sum of the channels were calculated in Study I. Based on the results of Study I, only the maximum responses of the sum of the channels were treated as measurement results in Study II.

Atmospheric pressure chemical ionization mass spectrometry, APCI-MS

In Study IV the release of aroma compounds was measured *in vitro* and *in vivo* using an atmospheric pressure ionization mass spectrometer. *In vitro* measurements were made by drawing air above the equilibrated sample (40-mL, 5 replicates) in a closed glass bottle (100-mL) into the ionization source via a heated transfer line. *In vivo* sampling was done directly from panelists' nosespaces (N = 5) using two different protocols, the sample (10-mL, 5 replicates) was either swallowed immediately after ingestion, or kept in the mouth for 15 minutes before swallowing. The latter protocol included making smooth mouth movements while the sample was in-mouth.

The molecules measured were *cis*-3-hexenol, ethyl butyrate (/iso-ethyl butyrate), and linalool. In the positive ion mode APCI-MS, *cis*-3-hexenol yielded the principal ion *m/z* 83, ethyl butyrate (/iso-ethyl butyrate) *m/z 117*, and linalool *m/z* 137. The measured gas phase aroma concentrations were calibrated by comparison against known standards, which were injected (1.5 µl/min via microsyringe using a syringe pump) at the beginning of each

analysis. The other four aroma compounds present in the flavoring were not measured due to the sensitivity problems. Of those four ethyl methyl phenylglysidate and undecalactone for example may have made some contribution to the perceived strawberry aroma, but they could not be quantified as the amounts of these compounds in the flavoring were too low in regard their relatively low volatilities.

4.2.2 Sensory methods

The sensory panels consisted of students and employees of the University of Helsinki. The panelists were mainly females and they were all between 25-52. Most of them had had previous experience of sensory evaluation. The fact that the majority of the assessors were females might be problematic, as recent studies have shown an enhanced sensitivity to odors with repeated exposure among females (Dalton et al., 2002). However, this gender imbalance was not considered to be crucial here, as the major consequence might have been that the sensory results might have been at an excessively sensitive level compared to instrumental ones, or that the sensory results could not be directly extrapolated to males. The performance of the sense of smell of the assessors was ensured using certain test aromas (in Study I) or using SOIT (the Scandinavian Odor Identification Test, Nordin et al., 1998) (in Studies II-IV).

In all studies the samples were presented in randomized order and marked with 3-digit codes. A red light was used to mask the subtle colour differences when required (i.e. Studies I, III-IV). Before actual evaluations, the assessors participated in practice sessions where they were familiarized with the sampling techniques and samples. All the evaluations were replicated.

Determination of R-indices

The determination of R-indices was used in Study I to evaluate the differences in the orthonasal aroma of ice creams. The R-index is the probability of distinguishing between two stimuli; reference and sample (O´Mahony 1992). The sample containing 9% dairy fat was treated as the reference sample. The scale used was:

A = the sample is certainly the same as the reference

A? = the sample is possibly the same as the reference
B? = the sample is possibly different from the reference and
B = the sample is certainly different from the reference.

Single point intensity ratings

Single point intensity ratings were used to evaluate the orthonasal aroma in Studies II and III. An unstructured 10-cm line scale (anchors: no aroma – very strong aroma) utilizing computerized data collection was used in Study II and the scale from 1 to 9 (1 = no aroma - 9 = very strong aroma) in Study III.

Free choice profiling (FCP)

FCP was used in Study IV to obtain raw data on the temporal release of aromas. Assessors (N=12) were asked to evaluate the intensity of their own attributes of the retronasal aroma of samples (10-mL in 80-mL cups) at two different time points; their first impression and the aftertaste. To block the influence of orthonasal aromas, nose clips were used while taking the sample into the mouth.

Time intensity measurements

A computerized TI method was used to evaluate the retronasal aroma in Studies III and IV. The assessors put the nose clip on (in Study IV only), placed the whole 10-mL sample in their mouths, removed the clip (in Study IV only) and simultaneously started evaluating the intensity of the attribute on a vertical TI scale (bottom = no aroma – top = strong aroma) making smooth mouth movements until instructed to swallow the sample (after 10 s). After swallowing the assessors were instructed to continue evaluating the aroma intensity while keeping their mouth closed, breathing through their nostrils and keeping their tongues still (in Study IV) or continuing the tongue movements (Study III). The total evaluation time was 90 s.

4.2.3 Other instrumental methods

Laser diffractometry was used in Study II to determine the average particle size of emulsions homogenized using different homogenization pressures. Particles were measured in a stirred cell system using deionized distilled water as the medium. The measurements were made with duplicated samples.

4.2.4 Data analyses

The R-index values were calculated from all the judges' data for each replicate using the formula outlined by O'Mahony (1986). The significance of the R-index values was tested according to Bi and O'Mahony (1995).

The individual TI curves were averaged across intensities at fixed times (each second) (III Fig 2). As expected the individual variation was large, and therefore the curves were also normalized along the time and intensity axes based on the method proposed by Overbosch et al. (1986) (shown in the upper right corners of II Figs 2 a, b). The parameters calculated from the TI data included 'time to maximum' (TMax), 'maximum intensity' (IMax), 'duration' (Dur), 'area under the curve' (AUC), 'increase angle' (IAng), 'increase area' (IArea), 'decrease angle' (DAng), and 'decrease area' (DArea). These are the most often used parameters in TI methods (Fig. 2).

Different types of analyses of variance were used to study the significance of effects of fat level (I- IV), fat type (I), aroma concentration (II) emulsifier type (II), emulsion droplet size (II), timing of evaluation (IV), assessors (II-IV) or, replication (III) and different interactions on the perceived intensity of aromas (II-III) or on the relative GC peak area of the components (I-II) or on the evaluations of different TI parameters (III-IV)

Principal component analysis (PCA) was performed to analyze the method performance (I-II) and the interrelationships of the various parameters calculated from the TI data (III).

In Study IV the Generalized Procrustes Analysis was used to analyze the FCP results (Senstools 2.3.28, OP&P Product Research BV, Utrecht, The Netherlands). The differences between matrices in the APCI-MS headspace results were tested with Student's t-test (paired). The parameters calculated from the APCI-MS nosespace data included "time to maximum" (TMax), "maximum intensity" (IMax), and "time to 75%, 50% or 25% of maximum intensity on the descending part of the curve" (T75%, T50%, T25%). Student's t-test (paired) was used to assess the significances of the differences in the values obtained for different matrices.

5 RESULTS

5.1 THE EFFECT OF MATRIX ON THE RELEASE OF AROMA COMPOUNDS AND THEIR PERCEPTION

5.1.1 The effect of fat on the intensity/volatility of aroma compounds

A suppressing effect of fat content on the intensity/volatility of rather lipophilic aroma compounds was generally observed with all methods in all studies. In Study I the headspace of the nonfat ice cream was very different from the headspaces of the fattier ice creams. This was clearly seen in the GC data (I: Table 1) and the effects of fat were in most cases in clear relation to the lipophility of the aroma compounds. Some differences in the GC results were also obtained for 9 or 18% fat (i.e. more 3-cis-hexenyl acetate, ethyl hexanoate, ethyl butanoate, methyl 2-methylbutanoate, and ethyl 2-methylbutanoate in the headspaces of 9% samples than in 18% samples). The sensory R-index values indicated a parallel effect of fat content on the perceived orthonasal aroma. The R-index for nonfat ice cream showed a clear difference for the orthonasal aroma compared to the 9% dairy ice cream (the reference sample). Also, the samples containing 18% fat, either vegetable or dairy, were differentiated from the reference, but not as clearly as the nonfat sample. The electronic nose, MGD-1 data were more complex and not easy to interpret. The only channels with a sufficient signal-to-noise ratio were channels 1 and 4. Channel 2 responded only to the nonfat sample and could therefore not be used in the comparisons. However, since only one sample had a response in channel 2 it is a significant factor in separating the samples. In the raw MGD-1 data the only

significantly different sample was the nonfat sample, although there were trends among the calculated parameters indicating that also the 9% and 18% samples might differ. The PCA revealed quite a clear separation of the samples based on the fat content of the matrix by instrumental methods used in Study I (I Figs 1,3).

The effects of fat on a simpler aroma and matrix systems were investigated in Studies II and III. The results on the effect of fat on the intensities of aroma compounds were parallel to those in Study I. The intensity/volatility of a rather non-polar aroma compound, linalool was suppressed in the presence of increasing fat (II Figs 1,4 and III Table 1, Fig 1). The more polar component, diacetyl was less dependent on the fat content of the matrix. A more complex aroma system utilized in Study IV gave parallel results. A similar suppressing effect of fat on the volatility of the non-polar aroma compound was obvious and the more polar components were more or less non-affected (IV Fig 3 a-c).

The amount of fat required for significant aroma suppression compared to a nonfat matrix was an additional aim in Studies III (0%, 1%, 5% and 10% fat) and IV (0%, 0.5%, and 5% fat). In Study III, the instrumental results showed that adding 1% fat to the matrix resulted in an approximately 50% decrease in the headspace concentration of linalool compared to the HS of nonfat sample. However, sensory results on the effect of 1% fat were not as convincing. Although the sample containing 1% fat was differentiated from the 0% sample in the orthonasal evaluation, the difference was not as clear as in the GC results. TI results showed a considerable change in the amount of retained aroma compounds between the 1% and 5% fat-containing matrices; the fat-free and 1% fat-containing matrices formed their own subgroup (IMax and area-related parameters). In Study IV, 0.5% fat was enough to significantly reduce the headspace/nosespace concentrations of linalool in APCI-MS measurements. Although there was a parallel trend in the TI results, it was not statistically significant.

The effect of fat type on the intensity and volatility of aromas was investigated in Study I. Only slight evidence for the effect of fat type on the volatility of aroma compounds was found. Using the sensory method, the sample containing 9% vegetable fat, could not be differentiated from the reference sample (9% dairy fat containing sample), however the 18%

vegetable fat sample differed more from the reference than the 18% dairy fat sample. There was also slight evidence, based on PCA, that MGD-1 might have been able to differentiate the samples based on fat type (I Fig 3).

5.1.2 The effect of fat on the temporal release of aroma compounds

The raw temporal data in Study IV was obtained using FCP at two different time points, “first impression” and “aftertaste”. The means over the two chosen general attributes (linalool-related attributes and the strawberry attribute) were calculated to examine whether any temporal effect was evident (IV Fig 2a for linalool and 2b for strawberry). Linalool was more strongly perceived in the ”aftertaste” than in the ”first impression”, however this trend was significant only in the samples containing 0.5% fat. The opposite effect was found in the case of strawberry; higher mean values were observed for the “first impression” than for the “aftertaste”. This trend was also only significant in the samples containing 0.5% fat. There was no indication that an increase in fat content caused a slower release of rather non-polar compounds (e.g. linalool) as the ”aftertaste” parameter did not change significantly. Similarly, no interaction of the fat content and timing was found in the strawberry evaluations.

The temporal aspects of the perception of aroma compounds were examined by the TI parameters TMax, Dur, IAng, DAng, and the area-related parameters. In Study III, the fat content of the matrix had no effect on the TMax of linalool. The Dur of linalool was affected by the fat content of the matrix. The duration of linalool aroma was shortest in the sample containing 10% fat; the sample containing 1% fat did not differ significantly from it, but samples with no fat or 5% fat did (III, Fig. 3a). The area-related parameters of linalool (III, fig. 3b) were affected by the fat content of the matrix. Increased fat content was related to decreased area parameters; generally two subgroups were observed, one with the nonfat and 1% fat samples and another with the two other samples that contained more fat. The parameters IAng and DAng were both affected by the fat content of the matrix. None of these parameters varied significantly for the diacetyl samples (III, Fig. 3d-f).

In Study IV the timing of the linalool perception was not greatly affected by the fat content of the matrix (See the normalized data in IV, Fig. 4a). Some significant effects on timing-related TI parameters were found (TI parameters in IV, Fig. 5a). These parameters appear to reflect significant differences in IMax values, rather than true timing effects. The duration of linalool perception was not significantly affected, but there was a slight trend of a shorter duration of perception in samples containing 5% fat compared to the less fatty samples (IV, Fig. 5a). The timing of overall strawberry aroma was more affected by the matrix fat content than the linalool aroma. Normalized data (IV, Fig. 4b) indicate that maximum intensity perception is delayed in samples containing 5% fat compared to samples containing less fat. However, DAng was greatest in the sample containing 5% fat, thus there were no differences in the duration of the strawberry aroma (TI parameters in IV, Fig. 5b). It must be noted however, that as in Studies III-IV the intensity perception did not revert to zero during the evaluation for some assessors, the term Dur has to be interpreted cautiously.

In APCI-MS measurements (IV) using the 15 s sampling protocol only one significant difference was found in parameters relating to the timing of release (TMax; T75%, T50% or T25%) in the case of the three aroma compounds measured: the TMax of *cis*-3-hexenol was significantly longer in the 0% fat containing sample compared to the 0.5% sample. This finding has no clear interpretation, and it may be irrelevant as the individual variance was large. The normalized intensity values for linalool (15 s sampling protocol) show that TMax seems to be slightly delayed in the 0% fat sample compared to the others, but the descending part of the curves are variable and there is no obvious difference (IV, Fig. 6b). The straight swallowing protocol gave more easily interpretable results. The time to maximum concentration was not affected significantly in the case of any of the aromas. However, here again, the standard deviations were considerable. The other time-related parameters of linalool release were affected by the fat content; linalool of the sample containing 0% fat persisted for a shorter time compared to fattier samples. The normalized concentration values for linalool (straight swallowing) are shown in Fig 6a (IV).

5.1.3 The effect of emulsion parameters on the intensity/ volatility of aroma compounds

The effects of emulsion characteristics were investigated in Study II. The effect of droplet size was observed in the case of linalool; The release was enhanced from small droplets resulting from a higher homogenization pressure (II P2 in Fig 2). The effect of the type of emulsifier on the release of aroma compounds was detected in the case of diacetyl: more aroma was released when the sucrose stearate emulsifier (II E2 in Fig 3) was used than when modified potato starch (E1) was used. The emulsifier type did not affect the release of linalool.

5.2 DIFFERENCES BETWEEN ORTHO- AND RETRONASAL AROMA

In Study III, the aroma differences in the samples due to the different fat content were obvious and similarly perceived both ortho- and retronasally. The retronasal IMax values were in general slightly higher than the corresponding orthonasal intensities (III Fig 1). This effect was pronounced in the evaluations of linalool samples.

6 DISCUSSION

6.1 THE EFFECT OF MATRIX ON THE RELEASE OF AROMA COMPOUNDS AND THEIR PERCEPTION

6.1.1 The effect of fat on the intensity/volatility of aroma compounds

The effects of fat on the intensity/volatility of aroma compounds were studied in a real food system, strawberry ice cream (Study I), and in model systems (Studies II-IV). The hypothesized suppressing effect of fat on the release of rather non-polar model compounds was pronounced in each study utilizing either static or dynamic methods. The results are in good agreement with previous studies as generally fat is claimed to suppress the release of aroma compounds.

The effect of fat on an aroma compound is strongly dependent on the physico-chemical nature of the aroma compound. The main factor affecting this dependency is the polarity of the aroma compound (Guyot et al., 1996; van Ruth et al., 2000). Early studies of McNulty and Karel (1973) showed that the more lipophilic the compound the greater the retaining effect of oil on the release of *n*-alkanals. Respectively, Guadagni et al. (1972) found higher odor thresholds in vegetable oil than in aqueous solutions. In addition, for instance, Delahunty et al. (1996) and Brauss et al. (1999a,b) have also shown the effect of fat on the volatilities of lipophilic compounds.

The present work consists of studies that utilized different kinds of aroma systems, either aroma mixtures which paralleled real life foods (I), a slightly more simplistic model (IV) or a very simple aroma model consisting of two aroma compounds, diacetyl and linalool (II-III). The approach in all the studies was to have control on which compounds are involved in order to be able to discuss the observed results of volatility/intensity in relation to the polarity of the compounds involved. In some earlier studies mixtures (for example, resoleum garlic oil, Guinard, et al., 2002) have been applied. Thus, in those cases interpretation of the results obtained was more difficult.

The lipophilic-hydrophilic nature of compounds can be described by calculating HLB values as was done in Study I. The GC peak area of a more lipophilic compound (3-*cis*-hexenyl acetate, HLB value 6.1) was larger in the headspace of samples containing no fat than in the headspace of samples with fat. Similarly, the effect of fat as a solvent for more non-polar aroma compounds was seen in Study II as with increasing fat content the release of linalool was decreased. Parallel observations were made for the orthonasal measurements of linalool in Study III and for the retronasal and APCI-MS measurements in Study IV.

The minor effects of fat content of the matrix on the less lipophilic compounds were expected as these compounds which are more readily soluble in the water phase, are not likely to be strongly affected by the rather small (a maximum of 10% in III and 5% in IV) fat content of the matrix. In Study I the area of a more hydrophilic compound (ethyl acetate, HLB value 8.0) was greatest in the headspace of the samples containing 18% fat compared to the samples containing less fat. The compounds with a hydrophilic-lipophilic nature between the

two extremes (ethyl acetate and 3-*cis*-hexenyl; the compound with the lowest HLB value 6.05, 3-*cis*-hexenol did not confirm this relation, but this could be due to the small amounts of this compound in the flavoring) had fairly even distributions between the different samples. However, only very polar compounds are claimed to behave in an opposite manner to non-polar compounds in respect to the fat content of the matrix (Roberts & Acree, 1995). For example, Schierle-Keller et al. (1994) found only a small effect due to fat reduction on the volatility of diacetyl and propanol in emulsions. Similarly Speirs (2001) found no notable effect of fat reduction on the intensities of the polar compounds isovaleric acid, maltol, and vanillin. Guyot et al. (1996) observed that the intensity of diacetyl was lowest in an emulsion containing 49% fat; in both more fat containing matrices (83 and 99% fat) and less fat containing matrices (0 and 15% fat) the odor was more intense.

In Studies II and III, a fairly polar aroma compound (diacetyl, log P = - 2.0) was utilized and it was seen that the effect of fat is not necessarily opposite to the effect of a non-polar compound. In Study II, diacetyl was slightly more retained in the aqueous than in the oil matrix. However, the release of diacetyl seemed to be slightly greater from emulsions containing 5% fat than that of those containing 50% fat. No significant effects of fat on diacetyl were observed in the sensory results in Study III. In the TI measurements in Study IV the perceived maximum intensity of the strawberry flavor (mainly due to the less lipophilic compounds) was slightly increased with an increasing fat content. Using the APCI-MS technique, the concentration of ethyl butyrate in the nosespace was found to be greatest in the 5% fat sample when using the straight swallowing technique, but not when using the 15s sampling protocol. The opposite effect was found for the concentration of 3-*cis*-hexenol.

It must be emphasized that the results obtained using SHS-GC, orthonasal intensity ratings, and *in vitro* APCI-MS measurements represent the static equilibrium state in which the maximum aroma, governed by the partition coefficients, is released. In a real eating situation the process is very different as discussed in the Literature review, and the release of aroma compounds is dependent on the mass transfer as well.

Instrumental studies on the effects of lipids on aroma volatility have shown that very low fat levels can exert a significant effect. Roberts et al. (2003) found that, using solid-phase

microextraction and the GC-MS method, as little as 0.02% milk fat in the matrix reduced the headspace concentration of a lipophilic aroma compound (limonene) by 50% compared to water. Seuvre et al. (2000) showed a significant retention (84% retained in the matrix) of 2-nonanone in the presence of 0.2% fat compared to an aqueous matrix using headspace-GC. The instrumental results in Study III showed that adding 1% fat to the matrix resulted in an approximately 50% decrease in the headspace concentration of linalool. However, the sensory results on the effect of 1% fat were not as convincing. Although the sample containing 1% fat was differentiated in the orthonasal evaluation, the difference was not as clear as in the GC results. TI results in Study III showed that there was a considerable change in the amount of retained aroma between the 1% and 5% fat-containing matrices; the fat-free and 1% fat-containing matrices formed their own subgroup (IMax and area-related parameters). Sensory results (III) do not strongly support the hypothesis that 1% fat in the matrix is enough to retain aroma compounds considerably. In Study IV, 0.5% fat was enough to significantly reduce the headspace/nosespace concentrations of linalool in APCI-MS measurements. Although there was a parallel trend in the TI results, it was not statistically significant. However, it must be noted that the TI method is not the optimum one if the interest of the experimenter is solely on the maximum intensities. In addition, it must be noted that the inherent fat content in the milks used in our studies (III-IV) was 0.004-0.08%, which might have been able to retain some of the aroma; thus the effect of fat might have had a more dramatic effect if the base level had been 0%.

The instrumental methods appeared to be more sensitive towards change in volatile concentration than the sensory methods. This was also seen in a study of the effects of matrix on the aroma perception of menthone and isoamyl acetate (Ebeler et al., 1988).For example a small decrease in diacetyl was observed in GC results in Study III along with the increased fat content, this was not observed when using the sensory methods. However, as the dynamics of different methods used are very different the observed differences are quite expected. The differences observed in the results between instrumental and sensory measurements may have also been partly due to the different temperatures used; because of the sensitivity problems the GC samples were equilibrated at 60 °C. Another factor may be that the concentrations of aromas were chosen to be of moderate intensity and thus

considerably above the odor threshold. At such intensity levels, the panelists who operate according to psychophysical laws and not on equal distances (Lawless & Heymann, 1998) may have found it difficult to distinguish between the samples. It must be noted that due to sensitivity problems, of the eight compounds in the flavoring mixture, only 4 (linalool, ethyl butyrate + ethyl isobutyrate, and cis-3-hexenol) were measured using the APCI-MS technique. Of those compounds not measured, especially ethyl methyl phenylglysidate and undecalactone may have affected the “strawberry flavor” attribute to some extent based on their sensory characteristics (fruity, sweet etc.) and this may have had a slight interfering effect. However, this was not considered crucial as ethyl butyrate was the main compound producing the strawberry note in the samples.

6.1.2 The effect of fat on the temporal release of aroma compounds

The FCP results in Study IV were considered as raw temporal data. Part of the changes in the overall aroma that were obvious in the FCP results could be due to the effect of fat on the temporal release of aroma compounds. However, in FCP there was no indication of changes in the temporal release of linalool, as no interaction between the time point and the fat level was found. In line with the hypothesis (a slower release in the more fatty samples), the "aftertaste" in the 5% sample should have been rated higher compared to the "first impression" than in the less fat containing sample. Neither were changes in the temporal release of strawberry flavor in FCP proved, as no interaction was found between the time point and the fat levels. Some temporal differences were found with other techniques.

To obtain information on the effects of fat on the temporal perception of aromas, the effects of fat on the parameters TMax, Dur, AUC, IAng, IArea, DAng, and DArea were analyzed from the TI data. Based on the literature, mainly the temporal release pattern of rather lipophilic linalool was hypothesized to be affected by the fat content of the matrix. There were no differences in TMax for linalool (Studies III-IV) for the various samples, this does not support the suggestion that the aroma of a product containing less fat is released more quickly than that of a product containing higher levels of fat (de Roos, 1997). Guinard et al. (2002) likewise failed to observe a quicker release of garlic (as they did in the case of pepper)

in salad dressings upon fat reduction (0%, 6.75%, and 13.5% fat levels studied). They suggested that the garlic flavor was possibly retained in the mouth and that the panelists perceived it even before the sample was placed in their mouths. It must be noted that they used resoleum garlic oil, which contains a mixture of aroma compounds; thus the results are not as easily interpretable as regards which compound had been affected by the fat content as in our study with single aroma compounds. TMax was likewise not affected by the fat content of the matrix (0%, 10%, and 50% fat levels studied) in the case of the nonpolar compound limonene in an oil-in-water emulsion (Mialon & Ebeler, 1997). However, Brauss et al. (1999a), using sensory and instrumental methods, observed a quicker release of nonpolar compounds in a low-fat sample compared with samples containing higher levels of fat. In Study IV the TI results showed that the perception of strawberry flavor was delayed in samples containing the most fat. These TI findings are not in line with this hypothesis, as fat was expected to affect more strongly the temporal release of the rather lipophilic compound, linalool, than it would ethyl butyrate (mainly responsible for the sensory "strawberry").

In addition to the more rapid release, the aroma of a product containing less fat has been suggested to persist for a shorter time than that of a product containing normal levels of fat (e.g. Matheis, 1995; Bennett, 1991). In contrast, the perception of linalool in the sample containing the greatest amount of fat (10%) lasted the shortest time in our Study III. A similar trend was seen also in Study IV. However, it must be noted that the decrease was steeper in lower fat samples than in samples containing more fat. The fat content of the matrix had no effect on the duration of diacetyl perception (Study III). Guinard et al. (2002) also obtained conflicting results; in their study of salad dressings, the total duration of garlic and pepper flavors increased with the removal of fat. Mialon & Ebeler (1997) found no significant differences in the duration of limonene flavor in matrices with different amounts of fat (0%, 10% or 50%), although there was a trend toward decreasing duration as the lipid concentration increased. In the case of the polar compound, vanillin, the perception tended to last longer with increasing amounts of fat in the matrix, this was suggested to be due to an increase in the mole fraction of the vanillin in the aqueous phase.

Guinard et al. (2002) offered a greater viscosity of samples containing more fat as an explanation for the shorter duration of flavors; the flavor was not totally released from the

viscous samples during the time the sample was in the mouth. In addition to fat content, the viscosity of the matrix affects the release of aroma compounds because the diffusivity in a viscous matrix is smaller than in a less viscous matrix. The milk samples used in Studies III-IV may have slightly different viscosities. However, they were all very fluid and stayed in the mouth for a reasonable time (10 s) before swallowing; thus viscosity differences appear to play a minor role in these samples. In a study (Wiet et al., 1993) with a fairly similar sample matrix (skim milk with varying levels of sunflower oil), it was stated that the oil exhibited only a little change in viscosity, although the highest level of oil in that study was as high as 18%.

When examining the results of the TI parameter Dur in our studies, it must be noted that the evaluation time was restricted to 90 s and that not all the evaluations reverted to zero intensity during this time period. If the evaluation time had not been restricted the differences may have been clearer. However, it was found in pretests that the panelists were more comfortable with a restricted time.

In Study III, the IAng and DAng values were larger in the linalool samples containing less fat (nonfat and 1% fat) than in those containing higher levels. This may suggest a quicker and shorter release of aroma, but when we examined the results more carefully it was clear that this occurred as the IMax values of samples containing less fat are larger than the IMax values of the other samples. The area-related parameters (AUC, IArea, DArea) reflect the same phenomena; the areas of the 2 fattier samples are smaller due to the smaller IMax values rather than temporal factors. Similarly, in Study IV the significant effects of fat on AUC, IArea, and IAngle reflect the effect on intensities rather than true timing effects.

The APCI-MS results on temporal release were different for the two sampling protocols applied. There were no temporal differences among linalool samples when using the 15 s protocol, however when the straight swallowing technique was used, the linalool release varied in relation to the fat content of the samples. Linalool persisted for a shorter time in the 0% sample, compared to the other samples, and the decrease was much steeper in samples

containing less fat than in the sample containing 5% fat. No significant temporal differences were observed for ethyl butyrate. When using the straight swallowing technique the results were in general easier to interpret, and the differences found were more significant. The length of the time the food is in the mouth is very likely to affect the changes that food is subject to in the mouth (Taylor, 1996). The aqueous samples in this study might not be as strongly affected as solid foods, but some changes may occur. The 15 s protocol is more prone to the physiological differences among assessors; the amount of aromas retained in their mucosa is different, and there are individual differences in the dilutions of the sample and in the interactions with the saliva (depending on the amount and composition of the saliva) which will affect the release of aroma compounds. In addition, the smooth mouth movements the assessors were instructed to make while keeping the sample in their mouths, might be quite different for different individuals, and this might affect the release of aroma compounds. It was hypothesised that the 15 s sampling protocol would give distinct differences for the samples as the aromas have more time to be released, but physiological factors seemed to mask the differences. In fact, the straight swallowing technique may be closer to the actual consumption of a liquid sample, and thus perhaps it is more relevant.

The results obtained for the temporal release of aroma compounds in the present work are not consistent with the prevailing claim for the quicker release of aroma compounds from low fat products. On examining the literature it is however clear that the hypothesised temporal effect is far from explained as the evidence is highly conflicting: some have found temporal effects for fat, but the findings might be contraindicative (Shamil et al., 1991/92, Rosin & Tuorila, 1992; Guinard et al., 2002 vs. Brauss et al., 1999a; Brauss et al., 1999b), and some have found no significant effects (the sensory results in Brauss et al., 1999a). The versatility of methods (sensory and instrumental) with the different matrices and aroma compounds and other design parameters utilized in different studies partly explain the conflicting findings.

6.1.3 The effect of emulsion parameters on the release of aroma compounds

Influence of emulsion droplet size on the release of aroma compounds

A reduced droplet size results in an increased total surface area of the droplets, which may increase binding/entrapment of the volatiles at the interface assuming that the amount of emulsifier is sufficient to cover the smaller droplets formed (Jacobsen et al., 1999). On the other hand, the increased surface area available for volatilizing may enhance the release of more lipophilic compounds (Charles, et al., 2000). The effect of droplet size is likely to be very specific, depending on the nature of the aroma compound and the type and amount of the surface active agent used.

The smaller the droplet size the more intense was the perceived aroma of linalool, indicating that the increased surface area enhanced the volatilizing of this compound. This is in good accordance with the results of Charles et al. (2000). Droplet size had no significant effect on the release of diacetyl. Earlier studies have reported conflicting results. Charles et al. (2000) found that bigger droplets led to a greater release of polar compounds. Dubois (1994) found, using a model cheese with 11-22% calcium caseinate, that the headspace concentrations of diacetyl and allyl sulfide decreased when the surface area of oil droplets increased. However, in a model emulsion prepared with 1% calcium caseinate, the surface area had no effect on volatility, probably due to the poor coverage of proteins on the surfaces of fat globules. Considering the structure of an oil-in-water emulsion, where the polar compounds are likely to dissolve in the aqueous continuous phase, it seems logical that the size of the oil droplets would not have a major effect on the release of more polar compounds.

Influence of emulsifier type on the release of aroma compounds

Only in the case of diacetyl was a significant effect of the emulsifier type on the release of aroma compounds observed in the sensory evaluations. The perceived intensity of diacetyl was greater from emulsions containing sucrose stearate based emulsifier than for emulsions containing modified potato starch emulsifier. This effect was more pronounced in the emulsions containing 5% fat than in those containing 50% fat. These results could indicate

that potato starch based emulsifier binds diacetyl. However, this is not likely in the light of GC results, as the headspaces of the emulsions prepared with potato starch contained more diacetyl than those prepared with sucrose stearate. The effect was, however, pronounced in the samples containing the highest amount of aroma i.e. in samples that were not even included in sensory measurements. A possible explanation for the sensory results could be that the slight odor of sucrose stearate itself enhanced the perceived aroma of diacetyl. Although a criterion for the choice of emulsifiers was that they should be odorless, both possessed a slight odor. However, neither of the blank matrices was considered to have an odor resembling that of diacetyl or linalool. No detectable volatile compounds were observed in the GC analysis of blank emulsion containing sucrose stearate, whereas in the case of potato starch, there was an unidentified peak of matrix with a retention time (1.60 min) near to diacetyl's (1.97 min). However, the peaks were well separated and there is no indication that this compound could interfere with the sensory properties of diacetyl. Yet this unidentified peak may have imparted the slight odor to the matrix and thereby possibly enhanced the aroma intensity of diacetyl.

Droplet size was also influenced by the type of emulsifier, this complicates the interpretation. The emulsions prepared with sucrose stearate had smaller droplet sizes than those with potato starch. Thus, the possible effect of the type of emulsifier could originate from the differences in droplet sizes. However, this does not seem to be likely as the headspace concentrations of aromas were greater in emulsions containing modified potato starch, which also had greater droplet sizes than in those with sucrose stearate. However, when the effect of droplet size (within an emulsifier type) was studied no effect on the release of aroma compounds was observed. As the reduction in droplet size is almost equal whether the homogenization pressure was raised from 100 to 300 bars in the case of potato starch emulsion or if the emulsifier was substituted with sucrose stearate, the effect must stem from the type of emulsifier per se. In the case of sensory evaluation, the release of diacetyl was greater from emulsions prepared with sucrose stearate. As there was no indication that the droplet size affected the release of diacetyl, the effect should have arisen from the emulsifier type.

Compared with the effect of fat on the release of aroma compounds, the effects of droplet size or emulsifier type were very slight. Similarly, Wendin et al. (1999) found that variations

in fat content had a greater effect than homogenization on the sensory attributes of mayonnaise. Landy et al. (1996) suggested that the affinity of the volatile substances for the fat phase was too strong to allow the detection of differences in volatility either due to the nature of the surface active agent present or to the surface area of the oil-in-water interface.

6.2 DIFFERENCES BETWEEN ORTHO- AND RETRONASAL AROMA

Despite the significant role of retronasal aroma perception in real eating situations, it has gained much less research activity than orthonasal perception (Heilmann & Hummel, 2004). Most of the traditional instrumental measurements parallel to orthonasal aroma as well. In many cases the release of aroma compounds in real eating situations is the goal in aroma research, and thus these studies have been more focused on retronasal aroma, for example by developing different kinds of model mouth systems. In the present work the focus has moved from orthonasal measurements (Studies I-II) to retronasal ones (IV) and some comparisons of these two aroma perception routes were made in Study III.

The aromas perceived orthonasally and retronasally are claimed to be different quantitatively and qualitatively (Roberts & Acree, 1995). In Study III the retronasal IMax values were in general slightly higher than the corresponding orthonasal intensities, a finding supported by some earlier studies (Murphy & Cain, 1980; Garcia-Medina, 1981; Cometto-Muniz, 1981). In contrast, Kuo et al. (1993) reported higher intensities for orthonasal perception than for retronasal ones. Despite the slight differences in levels observed, the differences in the samples were obvious and similar in both methods. The comparisons of the results obtained in Study III on the intensity differences between orthonasal and retronasal perception are slightly complicated by the fact that retronasal aroma was evaluated using the TI method instead of a single point evaluation (like orthonasal aroma). The TI IMax and intensity ratings using a single point evaluation might be different and thus further conclusions based on Study III should be moderate. In addition, the comparison of retronasal and orthonasal aroma was done using sipping as the retronasal sampling method. Retronasal aroma perception is affected by the physiological events occurring in the mouth, thus direct comparisons are not realistic, however, they are more relevant when considering normal eating situations.

6.3 METHOD PERFORMANCE

The studies in this work utilized different kinds of methods for the measurement of the aroma compounds released. Some methods measure the static, equilibrium state and others measure the situation more closely related to the real dynamic eating situation.

Comparisons between instrumental and sensory data are somewhat complex: the human perception of aroma can hardly be fully described by instrumentally measuring the release of aroma compounds. Sensory and instrumental measurements are to be considered complementary to each other in many cases (Taylor, 1996). To get the best interpretation of the factors affecting flavor release and perception both kinds of methodologies should be applied. In many cases instrumental measurements can perhaps be utilized only when they are calibrated using the sensory importance of the compounds measured and on the other hand, the objective chemical analysis that instrumental measurements can provide, is sometimes required to better understand sensory results.

Some impressive examples of cross-modal interaction in flavor perception exist as reviewed in the Literature (2.1.4). In addition to psychophysical findings, neuroimaging and neurophysiological studies provide similar evidence suggesting cross-modal/ multi-modal interactions for taste and smell and various other modalities. Many aspects of the cross-modal interactions both on the receptor and neural/cognitive levels are yet to be revealed. However, it is clear that the cross-modal interactions are hardly measurable by instrumental methods.

The human orthonasal aroma perception may be more easily compared with instrumental measurements than retronasal perception, as the processes occurring in the mouth cavity can be ignored (and here no cross-modal interactions are assumed). In the studies of this work the comparisons of orthonasal aroma perception and GC measurements were complicated by the fact that the temperatures used in the analyses were not the same. Due to the sensitivity problems with the GC method the equilibration temperature was 60 °C whereas the sensory evaluations were done at room temperature. In other studies, Guyot et al. (1996), for example, used ten times greater concentrations of diacetyl and higher temperatures in instrumental measurements than in sensory measurements in order to get a significant

response. The volatiles released at 60 °C vs. at room temperature are likely to be quantitatively different and perhaps also qualitatively. In the present study, it remains unclear whether the difference in temperatures made some contribution to the conflicting results obtained for example for the effect of emulsifier type (Study II). However, as the sensory measurements were done at room temperature, this method could be considered to be more sensitive and more relevant to real life situations than the GC method. On the other hand, in the sensory measurements in Study II there was slight evidence for the synergistic effects of the matrix volatiles and aromas. With this in mind, an instrument that divides a sample into its individual components (like GC) can better determine the effect of conditions on certain aroma compound. However, if we are interested in the quality of the end product what is most relevant are the perceived aroma changes. Due to the large standard deviations in the sensory data, not many of the matrices showed significant differences in aroma intensities. In this sense the GC results were in general more reliable.

6.3.1 The electronic nose vs. more conventional methods

In Study I comparisons should be mainly made between the electronic nose (MGD-1) and sensory method. Due to the sensitivity problems in GC analysis, these two methods gave information about the aroma of the samples more closely related to a real eating situation, albeit a rather static one, whereas in the gas chromatographic conditions the volatiles released are not necessarily the same (neither quantitatively nor qualitatively) as in a real eating situation, because the sampling temperature was elevated. The gas chromatographic method in Study I was used mainly to test the performance of a more traditional instrumental method in detecting the differences between ice cream samples.

MGD-1 showed a fairly comparable separating capacity in Study I with strawberry ice cream aroma. There was even slight evidence that it is capable of detecting the effect of fat type. The semiconductor metal oxide sensor was not capable of separating the samples and thus was not used in either Studies I or II. In Study II, MGD-1 failed to compete with the more traditional methods. It was able to detect the increasing concentrations of linalool, though only in the cases of the highest concentrations. No connection was observed between the results (maximum response of the sum channels) and the amount of diacetyl in the sample

(increasing concentration) and the standard deviations were very large. Thus, the release of diacetyl could not be detected with MGD-1, although the response profile of diacetyl was different from that of linalool. The response seemed to be more related to the matrix than to its diacetyl content. However, it was not simply related to the fat content of the matrix. Although the responses of the water samples were higher than the responses of the oil samples, the emulsion samples did not follow this trend. No clear evidence was found using MGD-1 that droplet size could affect the release of aroma compounds. Neither was any effect of emulsifier type on the release of aroma compounds found.

In Study II the effect of the fat content of the matrix was observed with all the methods used, as shown in the PCA biplots for linalool. As neither of the instrumental methods found an effect of droplet size on the release of aroma compounds whereas the sensory method did, the latter might be considered to be the most sensitive method in this respect. The MGD-1 results did not show any effect of emulsifier, and thus it can be considered to be less sensitive in this respect than the two other methods used. However, no final conclusions concerning MGD-1 can be made based on this study, as the maximum response of the sum of the channels was taken as a result. Although there was no sign that more selectivity could have been found in a more careful study of the responses of individual channels, it might be worthwhile to further study the way data for the MGD-1 was treated. The MGD-1 was less sensitive than the other methods (SHS-GC and sensory intensity rating) in detecting the differences in the aromas of different matrices in Study II. In the case of linalool, it was able to detect only the highest concentrations in the right order. In the case of diacetyl, the device was less successful, as it was unable to distinguish between different concentrations. However, it must be emphasized that the MGD-1 electronic nose used in Studies I-II, is a fairly simplistic device, with only six non-specific electrodes as detectors, and thus its applicability is quite limited. The sensitivity towards humidity, which was seen in Study II, makes its applicability even more limited. In addition, the variability in its sensitivity for different volatile compounds is a further limiting factor for further possible aroma applications of MGD-1. Yet, especially the possible applications for on-line detection make it an attractive screening tool for aroma measurements for those applications where it is sufficiently sensitive and selective.

6.3.2. Instrumental vs. sensory methods

In Study II the rising concentrations of both aromas in all matrices were easily detected by the sensory evaluations. The intensities increased approximately as a logarithmic function of concentration as predicted by Fechner's law (Lawless & Heymann, 1998) (plots not shown here, R^2 -values varied from 0.81 to 0.99, except for one curve $R^2 = 0.57$). The sensitivity of the sensory method seemed to be better (lower concentrations detected) than in either of the instrumental methods used although no definitive conclusions can be made since the blank matrices were not included in the sample series. The fact that the lowest concentrations of either aroma did not clearly obey trends observed for the effect of fat supports the possibility that, at least for some assessors the lower concentrations were around the odor thresholds. Based on the literature (for diacetyl: Druax & Voilley, 1997; Land & Reynolds, 1981; Stahl, 1973; Tuorila et al., 1981, and for linalool: Stahl, 1973) the concentrations used were at the suprathreshold level. However individual variations in perception are great. For example, the group average of odor recognition threshold for diacetyl in aqueous solution reported by Lawless et al. (1994) was 0.005 mg/kg, but the mean individual thresholds varied by a factor of 256. With the GC method, two to six concentrations of linalool could be detected, the extreme cases being pure oil, where only the two highest concentrations were detected, and water in which all concentrations could be measured. The increasing diacetyl concentrations from 1.25 to 156.25 mg /kg could be detected in all the matrices and the concentration steps (coefficient of five) were easily detected. The two lowest concentration of diacetyl (0.05 and 0.25 mg /kg) were detected only in some matrices.

It must be noted that due to sensitivity problems only 4 (linalool, ethyl butyrate + ethyl isobutyrate, and cis-3-hexenol) out of 8 compounds of the flavoring mixture were measured using the APCI-MS technique. Of those compounds not measured, especially ethyl methyl phenylglysidate and undecalactone may have affected the "strawberry flavor" attribute to some extent based on their sensory characteristics (fruity, sweet etc.) and this may have had a slight interfering effect. However this was not considered crucial for this study as ethyl butyrate was the main compound producing the strawberry note to the samples. In addition, where aroma mixtures are concerned there is the possibility of mixture suppression effects being present. If the volatility and thus perceived intensity of one component is decreased,

some other component may become more readily perceived as the inhibition effect diminishes.

In the present studies some of the contradictory instrumental and sensory results might be due to some cross-modal interactions that have an influence on the sensory results. It could be hypothesized that in addition to the aroma compounds, fat itself acts as a stimulus. The type of interaction could be a texture-aroma interaction, it could also be an aroma-aroma interaction. The latter is based on accumulating evidence for a taste/olfactory component in fat, although it has widely been considered to be identified by its textural properties (Mattes, 2003). Neurophysiological studies by Rolls (2002) support the perception of fat based on both texture and taste/odor. The perception of fats may actually be dependent on the numerous modalities involved: textural, olfactory, nociceptive, thermal and gustatory (Verhagen et al., 2003). The clearest evidence of cross-modal interaction in our sensory results in Study IV would be if the perception of linalool had decreased less as a function of the fat content of the matrix than was expected based on instrumental results. This would have implied that the response for the increased fat content was added to the response for the aroma. However, this was not the case in our results as the perception of linalool was clearly reduced along with the increasing fat content of the matrix. It is possible that the contradictory results for the temporal release of linalool could be partly explained by cross-modal interactions.

The fact that it seemed to be easier to find significant differences in the release of aroma compounds with the instrumental (the straight swallowing technique in Study IV) than with the sensory method partly reflects the difficulties inherent in the time-intensity method. In spite of the training, the variation among the assessors was great. Most to the differences in the attributes and many of the interactions were due to differences between the assessors. Differences in the temporal release of aroma compounds patterns are difficult to measure with sensory methods (Cook, 2004). It was seen in our results that even though some effects that seemed to be related to the temporal release of aroma compounds were observed, when they were studied more carefully they appeared at least partially to reflect intensity differences. The question remains whether in a context where the intensities released are very different, it is possible to measure them as clearly as they can be measured with instrumental

methods. For humans the temporal differences might be masked by more obvious intensity differences. To obtain a clearer picture of pure temporal release, samples within an isointensive aroma system might be worth studying. However, this would require a tedious tailoring of individual samples to each assessor.

7. CONCLUSIONS

The effect of fat on the volatility/ perceived intensities of aromas was demonstrated in all of the studies of the present work. The effect of fat was pronounced in the case of more lipophilic aroma compounds and this was seen using both simpler aroma systems and more complex aroma mixtures, in real food and model systems, and using different kinds of instrumental and sensory methods. It must be noted that these studies in general used only one model compound to represent all the relatively lipophilic aroma compounds and thus the results are not generalizable to all lipophilic volatile compounds. The effects of other compositional or structural factors of matrix studied (e.g. type of fat, emulsion droplet size or emulsifier type) were less important factors in the release of aroma compounds than the amount of fat.

As little as 0.5% fat in the matrix significantly reduced the headspace concentration of a nonpolar aroma compound, linalool in milk. However, the reduced intensity of linalool was not as pronounced when it was examined with sensory methods.

The often-claimed temporal effects of fat on the release of aroma compounds turned out to be difficult to prove, especially with sensory TI methods. Some results obtained here were actually contradictory to the prevailing claim: as the matrix fat content was increased, the aroma of linalool persisted for a shorter time than in the nonfat sample. In other cases there was no significant effect on the temporal release of aroma compounds. For example no differences were observed in the rate of linalool release (time to maximum intensity) in matrices containing different amounts of fat. In Study IV the perception of strawberry flavor (mainly due to less lipophilic compounds) was found to be delayed in the fattiest sample. These effects (on duration and time to maximum intensity) were not expected since based on

the literature, with increasing amounts of fat, a longer duration and slower release for lipophilic compounds were hypothesized. Understanding the timing of the release of aroma compounds is an area that still needs further studies as the temporal properties of aroma compounds frequently cause flavor problems in for example reduced-fat products.

Based on the APCI-MS measurements, temporal effects were seen only with the straight swallowing sampling technique but this procedure was not used in the sensory measurements. The longer sampling protocol (used in the TI and partly in the APCI-MS) perhaps interfered with physiological factors and no temporal effects of fat on flavor release were obvious. The results obtained for the temporal release of the aroma compounds emphasize the fact that the eating process is a complex, dynamic event and further studies are still needed to completely understand the physiological aspects related to it.

The capability of the novel analytical tool, an electronic nose for detecting the effect of fat level on the aroma of the strawberry ice cream was comparable with the static headspace gas chromatographic method and sensory method (determination of R-indices). However, the performance of the device was less successful in the case of emulsions, probably because of its sensitivity towards humidity. This study shows that the applicability of MGD-1 is quite case-specific and the device is too imprecise for analytical aroma research. In general, there are various electronic nose systems available, and some of them are promising new complementary tools for aroma research. However, it is evident that such new tools should provide additional value (speed, on-line possibilities) for measurements in order to succeed. All in all, instrumental and sensory methods in aroma research are to be considered complementary. Care should be taken when choosing an appropriate method for each application as static and dynamic, instrumental and sensory, orthonasal and retronasal methods measure different aspects of the release of aroma compounds.

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