Release and perception of aroma compounds during consumption

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Abstract

This thesis evaluated and validated the MS-Nose as a tool to measure aroma release during food consumption. Subsequently, the MS-Nose was used to enhance understanding of the interaction between release and perception of aroma during consumption. Cross-modal interactions, intra and interpersonal variation, and the development of a more relevant *in vitro* testing device (the artificial throat) were investigated.

For aroma release measurements of liquids, a strict protocol was developed to reduce intra and interpersonal variation in mouth movements, breathing, and swallowing. With the use of this protocol it was shown that appropriate concentrations of both sweeteners and protein did not affect *in vivo* aroma release, despite their well-established effect on aroma release in static headspace and mouth model studies. Also for emulsions, the effect of oil content was smaller under *in vivo* conditions than under static headspace and mouth model conditions. These discrepancies indicate the necessity to perform *in vivo* aroma release measurements, rather than static headspace and mouth model studies to investigate the effect of food parameters on the release of aroma compounds during consumption.

An artificial throat simulating the consumption of liquid foods was developed and validated as an alternative for conventional model systems, which generally focus on simulating aroma release during oral processing. The results obtained with the artificial throat correlated linearly with *in vivo* aroma release measurements. The aroma release and perception of a range of gels with different gel hardnesses were evaluated and a cross-modal interaction was demonstrated: An increase in gel hardness decreased the perceived aroma intensity, while the *in vivo* aroma release was unaffected.

These findings have increased the understanding of aroma release and perception and will open the way to new approaches to modulate perceived aroma intensity.

Key words: MS-Nose, *in vivo* aroma release, aroma perception, mouth model, artificial throat, liquid protocol, sweeteners, reversible protein-aroma interactions, emulsions, oil content, droplet size distribution, gel hardness, texture, cross-modal interactions.

Contents

Abbreviations used

APCI	Atmospheric pressure chemical ionisation
C*	Critical overlap concentration
CSLM	Confocal scanning laser microscopy
D	Diffusion coefficient
d	Droplet diameter
$D_{(3,2)}$	Mean surface-based droplet diameters
$D_{(4,3)}$	Mean volume-based droplet diameter
FID	Flame ionisation detector
GC	Gas chromatography
GDL	Glucono-δ-lactone
GPA	Gas phase analyser
I_{max}	Maximum intensity
L_D	Layer thickness
MCT	Medium chain triglycerides
MS	Mass spectrometry
P_{aw}	Air-water partition coefficient
P_{ow}	Oil-water partition coefficient
PTR	Proton transfer reaction
RAS	Retronasal aroma simulator
SHGC	Static headspace gas chromatography
SIR	Selected ion recording
SOOM	Spit-off odorant measurements
TI	Time intensity
T_{max}	Maximum intensity
τ	Time scale of diffusion
WHC	Waterholding capacity

Chapter 1

General introduction

The sensory impression of a food product is an important factor for consumers to appreciate it. Therefore, many research efforts are dedicated worldwide to the sensory properties of food products. In this respect, the term 'flavour' is commonly used. Generally, this term refers to the combined perception of aroma, taste, and texture at the time of food consumption (Taylor, '96).
In recent years, mass-spectrometric techniques were developed to measure the release of aroma compounds in a person's breath, during consumption (Taylor *ea*, '96, Lindinger *ea*, '98a). The research on *in vivo* aroma release was boosted by the high sensitivity and time resolution of these new techniques, compared to earlier attempts. Many research groups started to work in this suddenly emerging discipline. Now it had become possible to study the link between the aroma release profile of a product and its aroma perception. Aroma perception was assumed to play a large role in the overall sensory perception of a product.

1.1 Perception of food products

Consumer perception of food products is a multidisciplinary issue. Not only product-related sensory properties as aroma, taste, texture, and appearance determine consumer preferences, also numerous other factors have been identified. The latter relate to the psychology and physiology of the consumer, and include the image, packaging, and price of the product, the social context of consumption, variety seeking behaviour (sensory specific satiety), combination of consumption with other products, time of the day, season of the year, and aversions or preferences acquired earlier (Sheperd, '95, Meiselman, '96). The perception of product-related sensory properties will be discussed below.

1.1.1 Aroma perception

Aroma compounds can reach the olfactory epithelium, located along the top of the nasal cavity, either orthonasally by sniffing or retronasally when they release from the food in the mouth or pharynx and are transported to the nasal cavity by the flow of breath (Linforth *ea*, '02). Aroma perception or smelling is the detection of volatile compounds at the olfactory epithelium. Three celtypes exist in the olfactory epithelium that play a role in aroma perception: the basal cells, olfactory sensory neurons, and supporting cells. The basal cells generate olfactory neurons throughout their life. The olfactory sensory neurons link the mucosal surface to the olfactory bulb of the brain. The supporting cells give structure to the epithelium tissue. The binding positions for aroma compounds are situated on an extensive capture system of cilia on the mucosal surface (Goodenough, '98). Olfactory perception begins with the transit of aroma compounds through the layer of mucus covering the olfactory epithelium. Odorant binding proteins (OBP's) carry hydrophobic odorants through the mucus to the receptors (Pelosi, '96). Binding of an aroma compound to a receptor protein activates specific G-proteins. Once activated, two mechanisms

exist for activated G-proteins to depolarise receptor cells. This can be achieved by stimulating either an olfactory-specific adenylate-cyclase, which results in the formation of cyclic AMP, or by stimulating phospholipase C, which converts the membrane lipid phosphadityl inositol biphosphate into inositol triphosphate (IP_3) and diacylglycerol. The cAMP and IP_3 open ion channels, resulting in a change of the membrane potential of the cell and thus giving an electrical signal to the brain (Bell, '96). Formation of spatial maps in the olfactory bulb and other brain structures provides information about the identity of aroma compounds. The perceived intensity of an aroma compound is probably a function of the responses of the different receptor cells (Chastrette *ea*, '98, Livermore *ea*, '98).

1.1.2 Taste perception

Tasting is the detection of non-volatile compounds by taste-receptor cells in the taste-buds located on the tongue and the back of the oral cavity. Five basic taste sensations are recognised: sweet, salt, bitter, sour and umami, the latter is often represented by the savoury taste of monosodium glutamate. A neural response is elicited when the taste compounds solubilise in the saliva and have reached the taste-receptor cells. Specific proteinaceous receptors exist for the detection of sweetness, bitterness and umami, which give cell depolarisation through various second messenger systems. Sourness and saltiness transduction is mediated via ion-selective channels that can depolarise the cells (Le Coutre, '03).

1.1.3 Texture and appearance perception

Texture can be defined as the sensory manifestation of the structural, mechanical, and surface properties of foods detected through the senses of vision, hearing, touch, kinesthetics (feedback from masticatory muscles during chewing) and somesthetics (tactile senses). Some textural attributes commonly used are hardness, cohesiveness, springiness, viscosity, and creaminess. The trigeminal sensations of cooling, astringency, and pungency are usually referred to as textural properties as well.
No single and specific receptors exist for texture, because texture is a multiparameter attribute. The main evaluation of texture occurs when the food is placed in the mouth and deformed by chewing action, manipulated by the tongue, and mixed with saliva (Szczesniak, '02).
The appearance of food, as registered by the visual system, raises an expectance of what the aroma, taste, and texture will be like, and influences the food perception accordingly (Cardello, '96).

1.2 Measurement of aroma perception

Measurement of aroma perception can be performed in a static or dynamic way. For static measurement or sensory rating, a person must time-average or integrate the perceived stimulus or focus on the maximum intensity in order to provide a single response value (i.e. a line score). If the time-dimension is of low importance, this method is a fast, simple, and straightforward way to collect sensory information. Time-Intensity measurement (TI) is a dynamic sensory evaluation that monitors sensory perception of a stimulus from onset through extinction. This way of measurement appreciates the dynamic nature of sensory stimuli and perception. Early workers had panelists drawing TI curves on paper, but nowadays the method is fully computerised (Cliff *ea*, '93, Dijksterhuis *ea*, '01).

There is still debate on how TI curves should be analysed. A common approach is to summarise the curves by extracting parameters as maximum intensity (I_{max}), time to maximum intensity (T_{max}), area under increasing phase, initial slope, and many more, and use these for further (multivariate) analysis. The raw curves can also be averaged in different ways, such as normalising (Overbosch *ea*, '86), weighted averaging based on principal components analysis (Van Buuren, '92), and fitting of polynomial and ordinary differential equations (Wendin *ea*, '03).

1.3 Cross-modal interactions

During consumption of food products, the brain receives a constant stream of information about the aroma, taste, texture, and appearance. Usually each sense is stimulated by a mixture of stimuli. Within one sense, the presence of a stimulus can influence the intensity of a second stimulus, as was shown for aroma by e.g. Bult and co-workers (Bult *ea*, '01), and for taste by e.g. Lawless (Lawless, '79). Interactions between the senses occur as well, and are generally known as cross-modal interactions. Taste and aroma are widely described to interact, and also the influence of texture on aroma and taste perception has been documented. Visual cues as product colour are also known to influence taste and aroma sensations (Francis, '95). Different possible levels of interaction between stimuli can be identified (Nahon *ea*, '96). Physical or chemical interactions occur within the product when ingredients influence the level of availability of stimuli. Interactions are theoretically also possible at the receptor level. However, taste, aroma, texture, and appearance are sensed by distinctly different systems, and interactions at this level are highly unlikely (Noble, '96).

Most cross-modal interactions are likely to occur at the perception level during neural processing (Nahon *ea*, '96). An increasing amount of neurological studies show that taste, somatosensory and aroma stimuli converge, as reviewed by Cerf-Ducastel and co-workers (Cerf-Ducastel *ea*, '01). Cross-modal integration of sensory information is considered to be widely spread in every day perception (Driver *ea*, '00). In a study with monkeys, Rolls and co-workers have shown that the orbitofrontal cortex is an important site in the brain for the convergence of the taste, aroma,

appearance, and texture of food. Many neurons were found with bimodal responses, responding for example to both taste and olfactory, or taste and visual stimuli (Rolls *ea*, '94).

1.3.1 Interactions between taste and aroma perception

Many examples of interaction between the perceptions of taste and aroma have been described in the literature (e.g. Nahon *ea*, '96, Noble, '96). The presence of aroma compounds can increase the apparent intensity of tastes. At the same time, taste compounds can increase the perceived intensity of aroma. These effects are especially evident when congruency exists between the taste and aroma characters. This is for instance the case with sweetness and strawberry aroma, or with saltiness and savoury aroma notes (Frank *ea*, '88).
A striking example of perceptual taste-aroma interaction was given by Davidson and co-workers, who studied release of saccharose in-mouth and release of menthone in the nose during chewing gum consumption. The perceived intensity of mint was followed by TI analysis. The decrease in mint perception closely followed the decrease of saccharose concentration in the mouth. However, the menthone concentration in the nose remained at the nearly the original level throughout the measurement (Davidson *ea*, '99).

1.3.2. Interactions between texture and taste or aroma perception

Studies focusing on perceptual interactions between texture on the one hand, and taste and/or aroma on the other, can be divided in two categories. They deal either with the texture of gels or with the viscosity of solutions.
Protein and carbohydrate gels have been used to study the effect of matrix properties (such as gel hardness, waterholding capacity, and microstructure) on the perception of aroma. An increase in gelling agent concentration causes a decrease in the perceived intensity of the aroma, as was shown by sensory rating (Clark, '92, Jaime *ea*, '93, Carr *ea*, '96) and by TI (Guinard *ea*, '95, Bakker *ea*, '96, Wilson *ea*, '97). The release of various aroma compounds from gelatin gels has been measured with *in vivo* aroma release measurements (Baek *ea*, '99, Linforth *ea*, '99). In these studies aroma release measurements and TI recordings were performed simultaneously. Linforth and co-workers compared T_{max} values (time of maximum intensity) of *in vivo* aroma release and sensory TI. When the maximum was reached fast, the sensory perception was found to lag behind the aroma release. When the maximum was reached after a longer period of time, the T_{max} of perception preceded the T_{max} of aroma release, due to a sensory adaptation effect (Linforth *ea*, '99). Baek and co-workers found a significant decrease in sensory I_{max} (maximum intensity) and an increase in sensory T_{max} as the gelatin concentration increased. No correlation between I_{max} of aroma release and gelatin concentration was found (Baek *ea*, '99).

In general, this trend was found for liquid systems as well: an increase in the viscosity of a solution decreases the perception of taste and aroma compounds during consumption of that solution (Pangborn *ea*, '74, Christensen, '80). In a study by Baines and Morris the viscosity of solutions was increased by increasing the guar gum concentration, and they noted a steep decrease in perception of sweetness and aroma intensity, at the c* concentration. This is the coil-overlap concentration, which marks the transition from a dilute solution of polymer coils free to move independently, to an entangled network at higher polymer concentrations. As a consequence, the viscosity shows a strong and abrupt increase beyond c*. At that time, this result was explained in terms of restricted replenishment of surface depletion of tastants and aroma compounds with increasing viscosity (Baines *ea*, '87). However, with the availability of methods to measure real-time aroma release *in vivo*, Hollowood and co-workers found that the release of aroma compounds from a strawberry aroma was independent of the viscosity, also above c* (Hollowood *ea*, '02). Similarly to Baines and Morris, the perception of strawberry aroma decreased at higher viscosities in the latter study. Similar results were also observed for savoury aroma's (Cook *ea*, '03b). The solutions in these studies contained tastants as well. The decrease in aroma perception was either a result of restricted release of the tastants at higher viscosities, in combination with a taste-aroma interaction, or a perceptual interaction between perceived viscosity and aroma. Firm conclusions could not be drawn until the recent findings by Cook and co-workers (Cook *ea*, '03a). They used the Kokini oral shear stress (Kokini, '85), which is an estimation of the shear stress in the mouth based on simple rheological measurements to predict the perception of sweetness and aroma intensity. A high predictive power of the Kokini oral shear stress was found for aroma and sweetness intensity, independently of the type of hydrocolloid used. This illustrates the perceptual interaction between taste/aroma and tactile senses.

1.4 Mechanisms of *in vivo* aroma release

In order to be smelled, aroma molecules must release from the food matrix and reach the olfactory epithelium. In case of orthonasal aroma perception, the headspace above the product is sniffed, typically after opening of a food package. The ratio between concentrations of the aroma compounds in the product and the sealed headspace above the product is given by the equilibrium air-product partition coefficient. The partition coefficient depends on the affinity of the aroma compound for the product phase and on the temperature (Taylor, '98).

In the retronasal situation, aroma release occurs from a food bolus present in the mouth or during and after swallowing in the throat. During oral processing, the food is subject to chewing, tongue manipulation, mixing with saliva, temperature changes, and contact with mucous membranes, which all can have an influence on the aroma release. Obviously, also the structure and composition of the product determine the actual aroma release. Under these non-equilibrium conditions, the air-product partition coefficient can be regarded as the driving force for aroma release. However, there are additional parameters affecting the release, such as diffusion in the

product and in the air, convection of the product and the air (breath flow), and mass transfer from product to air (Overbosch *ea*, '91, Plug *ea*, '94, Taylor, '02).

1.4.1 Product composition

Carbohydrates can change the volatility of aroma compounds, but the extent of this effect is carbohydrate and aroma compound dependent. The volatility of aroma compounds in water is lowered by addition of carbohydrates, as a result of non-specific molecular interactions (Godshall, '97). Mono- and disaccharides have a salting-out effect, by lowering the amount of free water (Voilley *ea*, '77). The competing effects of intermolecular attractions and salting-out in each specific case make it difficult to formulate a general theory of carbohydrate-aroma interactions. The effect of mono- and disaccharides at concentrations generally observed in the beverage industry (<15% w/v) on volatility of most aroma compounds is relatively small (< 20% difference). As volatility is predominantly important for aroma release under equilibrium conditions, the effect of 0-15% (w/v) mono- and disaccharides on *in vivo* aroma release is expected to be rather small (Godshall, '97).
The largest effect of most polysaccharides on release is a decrease in aroma release, often attributed to limiting aroma diffusion, rather than to interactions at the molecular level. This effect strongly depends on the dynamics of the measuring system (Godshall, '97).
In contrast, starch is a polymer well known for its aroma binding properties. Starch molecules can form helical structures. The inside of these helical structures form hydrophobic regions which can especially entrap hydrophobic volatiles forming inclusion complexes (Solms, '86). A similar effect appears for cyclodextrin, which is composed of six to eight glucopyranose rings and is able to include hydrophobic aroma compounds into its hydrophobic core (Qi *ea*, '95).
Proteins and aroma compounds can interact via relatively weak reversible physical adsorption through Van der Waals's or hydrophobic interactions, and strongly via covalent or ionic chemical binding (Fischer *ea*, '97). The subject of interactions between aroma compounds and proteins, and parameters affecting these interactions, has recently been reviewed (Guichard, '02). The main factors are the physicochemical properties of the aroma compound, temperature, ionic conditions, presence of ethanol, and the conformation of the food protein. Much research effort has been invested in the interactions between aroma compounds and β-lactoglobulin. This has revealed an increase of the binding constants with increasing carbon chain length for the binding of small molecules as aldehydes, ketones, esters and alcohols (Mills *ea*, '84, O'Neill *ea*, '87, Pelletier *ea*, '98, Andriot *ea*, '00, Guichard *ea*, '00, Reiners *ea*, '00).
Lipids have a large influence on aroma release when compared to proteins and polysaccharides (De Roos, '97). Even at low concentrations, the presence of a hydrophobic phase in the system can have a large influence, particularly for strongly hydrophobic aroma compounds. Addition of only 0.5% (v/v) miglyol (a triglyceride of caprylic and capric acids) to water induces a stronger decrease in volatility on 2-nonanone than addition of 3% β-lactoglobulin (Seuvre *ea*, '00).

Knowledge of the impact of lipids on aroma release is valuable for the formulation of increasingly popular low-fat products (Overbosch *ea*, '91, De Roos, '97). Reduction of the fat content results in a higher release and perception and less lingering of aroma compounds, depending on their hydrophobicity (Brauss *ea*, '99).
In many foods lipids are present as oil droplets dispersed in an aqueous phase. This is the most prevalent form of lipids in model food systems for studies of aroma release. The oil content of the emulsion and the hydrophobicity of the aroma compounds are key factors predicting the release (Haahr *ea*, '00, Doyen *ea*, '01, Carey *ea*, '02, Van Ruth *ea*, '02a, Malone *ea*, '03, Miettinen *ea*, '03, Roberts *ea*, '03a). The type of lipid can also have an effect on aroma release. Increase in solid fat content was found to increase the release (De Roos, '97, Roberts *ea*, '03b).

1.4.2 Physiological factors

Various physiological factors can influence the release of aroma compounds under *in vivo* conditions and the effects of these factors can differ between people. Foods can undergo temperature changes once introduced in the mouth. A higher temperature leads to a stronger partitioning of volatiles in the air phase at equilibrium conditions. This has been demonstrated previously for a range of aroma compounds (Roberts *ea*, '95, Deibler *ea*, '99). Apart from this, the food can undergo physical changes upon temperature changes in the mouth, for instance melting of gelatin gels (Harrison *ea*, '96), or ice cream (Chung *ea*, '03), which can increase aroma release.
The flow rate and composition of saliva can vary greatly within and among people. This is caused by the degree of hydration, body position, exposure to light, the smell of food, smoking, previous stimulation, and climatological circumstances (Dawes, '81). Possible effects of saliva on aroma release are dilution, interactions between aroma compounds and saliva constituents, and enzymatic activity. Aroma compounds can interact with salivary proteins, especially mucin, as has been shown by static headspace measurements (Friel *ea*, '01, Van Ruth *ea*, '01) and by the SOOM-technique (spit-off odorant measurements) (Buettner *ea*, '00b). Losses in the oral cavity have been observed for aldehydes and esters during 1 minute of contact time (Buettner *ea*, '00b). In addition, esters, thiols, and aldehydes were found to be prone to enzymatic conversion upon contact with saliva within a period of 10 minutes (Hussein *ea*, '83, Buettner, '02a, b). Presence of α-amylase is only relevant for *in vivo* aroma release in high starch systems (Van Ruth *ea*, '00b).
In a recent study, Linforth and co-workers investigated the effect of factors such as adsorption to epithelia of the mouth, nose, and pharynx, and dilution by saliva, on *in vivo* aroma release. It was demonstrated that the mass transfer from an aqueous solution in the mouth into exhaled air was a major factor affecting the actual released amount of aroma (Linforth *ea*, '02).
In the case of solid products, chewing efficiency can influence aroma release. Different chewing styles and large person-to-person variation were observed while chewing gels (Brown *ea*, '96, Wright *ea*, '03b). Pionnier and co-workers were able to partly relate interpersonal differences in

aroma release under *in vivo* conditions to differences in respiratory and masticatory parameters (Pionnier *ea*, '04).

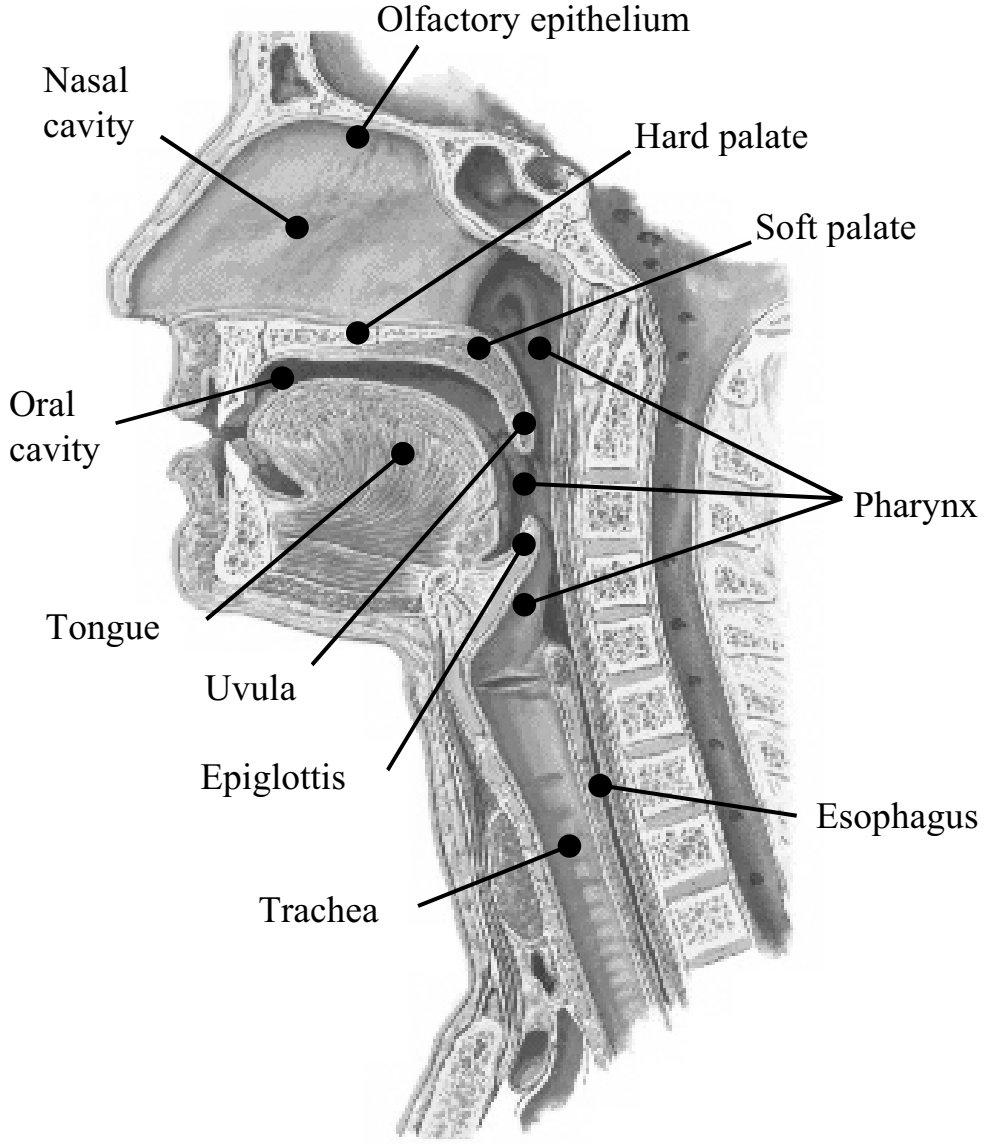

Figure 1.1. The human throat.

For liquid products, it has been shown that swallowing determines the *in vivo* aroma release rather than the preceding oral processing. This concept was first mentioned by De Roos and Wolfswinkel in 1994 and again later, in 1996, when Land introduced the 'swallow-breath' principle, which is 5-15 ml of air that is pumped into the nose retronasally as a result of swallowing. This plug of air has been in close masticatory contact with the food or drink in the mouth (De Roos *ea*, '94, Land, '96). This plug of air is important for aroma perception. It has become clear that the highest *in vivo* aroma release signal for liquids is generally found in the first exhalation after swallowing (Linforth *ea*, '00). It was shown that no air is transferred from the oral cavity to the nasal cavity as long as no opening of the barrier, formed by the tongue and the soft palate, occurs by swallowing or by vigorous tongue and mouth movements (Buettner *ea*, '00a). The existence of an anatomical barrier during the swallowing process has been proven by videofluoroscopy and real-time magnetic resonance imaging (Buettner *ea*, '01). In a recent study,

Hogson and co-workers combined synchronised measurements of mastication, swallowing, breath flow and aroma release and demonstrated that an average chew pumps a volume of 26 mL of air from the oral cavity into the throat. This is especially relevant for the situation when the food is not swallowed immediately. Nasal air flow and associated aroma release were not detected during swallowing, but air flow and release were obvious directly after the swallowing event. The volume of the retronasal pathway was calculated to be 48 or 72 mL (depending on the way of calculation) (Hogson *ea*, '03).

After swallowing, the majority of the sample disappears into the esophagus (Figure 1.1), but a thin layer of the liquid sample remains on the surface of the pharynx. Buettner and co-workers visualised the formation of such a coating by videofluoroscopy, when a volunteer swallowed viscous oral contrast medium (Buettner *ea*, '02). During the exhalation following a swallow, a steep gradient in aroma concentration exists between the thin liquid layer on the surface of the pharynx and the exhaled air that passes over this surface.

Persistence (lingering) of aroma compounds in the breath was defined as the ratio between the peak heights in the release signal of the first and second exhalations after swallowing and gives an impression to which extent compounds will linger in the breath. From an empirical model it was concluded that hydrophobicity and vapour pressure are the main predictors for *in vivo* persistence (Linforth *ea*, '00).

1.4.3 Modeling of *in vivo* aroma release

Despite the complex events taking place in the mouth during eating, several authors have attempted to predict aroma release by physical modelling of mouth conditions (Overbosch *ea*, '91). A physical model combined with semi-empirical relationships delivered workable models with predictive value for chewing gum (De Roos *ea*, '94). Other models were based on oil/water partition (McNulty, '87). Harrison and Hills published a series of articles, based on the penetration theory for interfacial mass transfer for different food systems (liquid emulsions, liquids containing aroma-binding macromolecules, gelatin gels) and with various physiological conditions incorporated (breathing, saliva flow), but these are yet to be validated (Harrison *ea*, '96, '97a, b, Harrison *ea*, '97c, Harrison, '98). A model based on this theory was able to predict *in vivo* persistence of aroma compounds quantitatively. However, empirical data were still needed to calculate the aroma concentration in the first exhalation after swallowing (Wright *ea*, '03a).

It is difficult to obtain values for the fundamental parameters that drive mass transfer *in vivo*. An approach that overcomes this limitation is given by QSPR modelling (Quantitative Structure Property Relationships), which assumes that physicochemical properties of the molecules are responsible for their behaviour. These models are easy to produce and generally have predictive power. However, their use is limited to the experimental conditions of the test set. Each different condition requires a new model (Taylor *ea*, '01b).

1.5 Measurement of *in vivo* aroma release

Static headspace gas chromatography (SHGC) is an appropriate technique for sensitive analysis of static headspace aroma compositions (Buttery *ea*, '69) and has proven its usefulness over the years. Various detection systems as flame ionisation (FID) or mass spectrometry (MS) are used. However, the method is not suitable for *in vivo* aroma release measurement. Aroma concentrations in food products are usually much lower than 10 ppm (Land, '78). From these already low concentrations only about 1% will release into the breath (Buettner *ea*, '00a) and thus will be available for detection. Real-time measurement of *in vivo* aroma release demands not only a high sensitivity, but also simultaneously a high resolution in the time course is required (> 1 data point/s) to accurately follow the aroma release within the time scale of a single breath. Other constraints of real-time *in vivo* methods are the disturbance of compounds present in the air such as carbon dioxide, oxygen, and water, and the desire to analyse more than one compound simultaneously.

1.5.1 Model systems to simulate *in vivo* aroma release

Model systems that mimic the release of volatiles from food products during consumption have the advantages of saving money and time spent on working with panelists, the omitting of palatability and safety matters, and a high reproducibility. In contrast to static headspace measurements, these model systems can also be used to study the time course of aroma release.
All model systems developed until now are designed to mimic the aroma release in the mouth. They are based on the shared principle of a certain amount of foodstuff (usually in liquid form), containing aroma compounds and other ingredients of interest (polysaccharides, proteins, lipids), that is put into a vessel and stirred or shaken in different ways (and heated to 37 ºC, in most cases) (Lee, '86, Van Ruth *ea*, '94, Naßl *ea*, '95, Roberts *ea*, '95, Van Ruth *ea*, '95, Elmore *ea*, '96, Roberts *ea*, '96, Bakker *ea*, '98, Springett *ea*, '99, Van Ruth *ea*, '00a, Van Ruth *ea*, '00b, Deibler *ea*, '01, Banavara *ea*, '02, Rabe *ea*, '02). Air is sampled from the headspace or nitrogen is purged through the liquid phase. The volatile compounds present in the stream of gas released from the model system are analysed in-line or batch-wise by a direct MS technique or by trapping the compounds on absorbing materials or cryogenically followed by GC-MS (Lee, '86, Van Ruth *ea*, '94, Naßl *ea*, '95, Roberts *ea*, '95, Elmore *ea*, '96, Bakker *ea*, '98, Springett *ea*, '99, Rabe *ea*, '02). All kinds of apparatus-related and product-related parameters have been studied using these model release systems. This has increased the insight in the process of volatile release in general (Van Ruth *ea*, '95, Roberts *ea*, '96, Van Ruth *ea*, '00a, Van Ruth *ea*, '00b, Deibler *ea*, '01, Banavara *ea*, '02). For solid products, when structural breakdown in the mouth is involved, the aroma release can be reproducibly studied in Van Ruth's model mouth system (Van Ruth *ea*, '94) or the retronasal aroma simulator (RAS) (Roberts *ea*, '95). In the mouth model, a plunger crushes

food samples by rotating and up-and-down movements in a glass vessel. The RAS is a modified blender.

1.5.2 Real-time *in vivo* breath analysis

Benoit described a direct mass-spectrometric method to analyse breath for medical applications as early as 1983 (Benoit, '83). In 1988, the technique was applied to aroma release (Soeting *ea*, '88) using an electron impact mass spectrometer. A membrane was used to exclude air and water from the electron impact source. Drawbacks of this approach were selective permeability of the membrane and a low sensitivity (25-250 ppm). A major impulse was given to the field when Taylor and Linforth successfully adapted an atmospheric pressure chemical ionisation (APCI) source for *in vivo* breath analysis. The ions formed are detected in a quadrupole mass spectrometer. This combination of APCI and MS detection used for *in vivo* breath analysis was called MS-Nose (Taylor *ea*, '96).

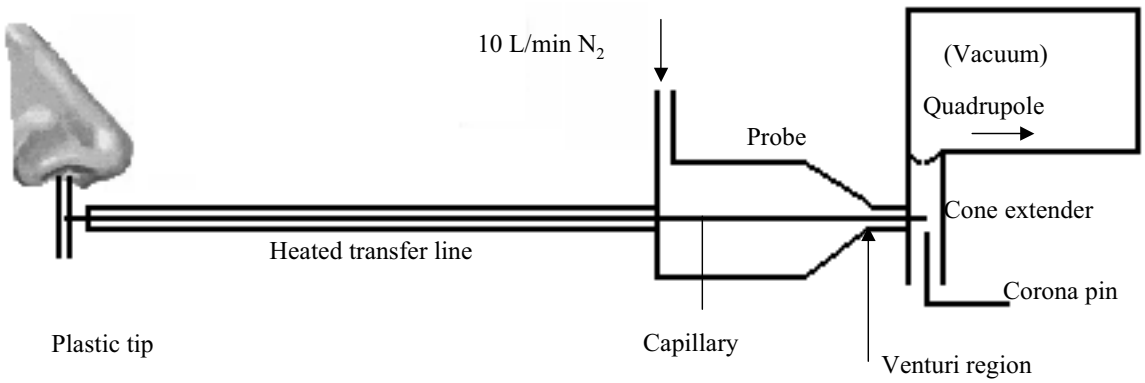

Figure 1.2. Experimental setup of MS-Nose measurements.

Figure 1.2 shows the experimental setup of the MS-Nose measurements. Volunteers put a small disposable plastic tube in one nostril, allowing them to breathe, drink, and eat normally. Air is sampled from the middle of this plastic tube through a deactivated fused silica capillary tube. Only a small part of the exhaled air (about 50 ml/min) is actually sampled. During exhalations the small plastic tube extension is filled with expired air, and the capillary only samples exhaled air. During inhalation, the tube is filled with laboratory air, which is then sampled into the capillary. In this way, the tidal flow of inhalation and exhalation is monitored and the APCI-MS traces show breath-by-breath volatile concentrations (Taylor *ea*, '00).

The lower limit of sensitivity is determined by the signal to noise ratio, whereas the upper limit occurs when all available charge is exhausted. The technique couples a high sensitivity to a high time resolution (multiple data points per second), at the expense of identification power. The APCI process involves the formation of an initial reactant ion by corona discharge, which can transfer its charge to any molecule with a higher proton affinity. Water molecules are present in air and are suitable as reactant ion. This is because their proton affinity is higher than the main

components of air, such as oxygen and nitrogen, and lower than most aroma compounds. APCI is a soft ionisation technique. Molecules are ionised but usually not fragmented under standard conditions. This is a major advantage when monitoring mixtures of compounds, when each compound can be monitored ideally by its molecular ion (Taylor *ea*, '00). If the ions of two or more simultaneously analysed aroma compounds have the same m/z value, unequivocal real-time identification can be achieved by focusing on unique fragment ions. A second MS coupled in-line with the APCI-MS configuration can be used to prove that the fragment monitored originates from a specific aroma compound.

An alternative soft ionisation technique is provided by proton transfer reaction (PTR-MS), in which reagent ions (water molecules) are formed under controlled conditions before mixing with the air sample containing the volatiles (Lindinger *ea*, '98a). This technique has been applied successfully for real-time *in vivo* aroma release measurements as well (Mayr *ea*, '03, Roberts *ea*, '03a).

The MS-Nose should not be confused with electronic noses. The latter ones are based on an array of sensors each having a partial specificity and thus producing an aroma fingerprint that can be identified by a pattern recognition system to identify the aroma through comparison with a reference library of previously obtained measurements of known samples (Strike *ea*, '99).

1.6 Aim and outline of the thesis

From the above it is clear that many groups have contributed to the field of aroma release in recent years. The results described in this thesis were published in the same time period. At the start of this project, the knowledge of *in vivo* aroma release was still very limited. Therefore, the aim at the beginning if this project was to develop appropriate methods for real-time *in vivo* aroma release measurements using the MS-Nose, and to elucidate the effects of product structure and composition and physiological consumer-related factors on release and perception of aroma.

Chapter 2 deals with the development and testing of a protocol for *in vivo* aroma release measurements with liquid products. The effect of sweeteners on aroma release is evaluated in this chapter as well. In chapter 3, this protocol and other analytical techniques are applied to study the effect of in-mouth conditions on the interactions between protein and aldehydes in aqueous solutions. Chapter 4 describes the development and validation of an artificial throat. This device simulates the events in the human throat after swallowing, which play a crucial role in the release of aroma compounds for liquid products. Chapter 5 shows the effect of emulsion properties as oil content and droplet size distribution on aroma release from liquid systems, studied by means of *in vivo* aroma release (using the protocol), artificial throat measurements and SHGC. Chapter 6 describes the effect of gel strength on release and perception of aroma compounds, measured by MS-Nose and TI, respectively. In this chapter special emphasis is placed upon cross-modal interactions. Chapter 7 contains a general discussion of the results obtained.

Chapter 2

A protocol for measurement of *in vivo* aroma release from beverages

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Abstract

The quality of *in vivo* aroma release measurements of beverages can be improved when a strict protocol is used to control variation in aroma release due to human factors. A trained panel was able to significantly discriminate between aqueous aroma solutions with a concentration difference as low as 17%. This protocol and headspace measurements have been applied to a lemon-lime type beverage with either bulk or low-calorie intense sweeteners. The ingredients examined did not influence aroma release at concentrations relevant to the beverage industry. Aroma release was only affected by very high concentrations of saccharose.

Introduction

In recent years, new mass-spectrometric methods for real-time analysis of aroma concentrations in exhaled air have been developed (Taylor *ea*, '96, Lindinger *ea*, '98a, Taylor *ea*, '00). Ever since, many *in vivo* aroma release experiments have been done with solid and semi-solid products like gels, vegetables, dairy products and chewing gums (Brauss *ea*, '98, Baek *ea*, '99, Brauss *ea*, '99, Davidson *ea*, '99, Linforth *ea*, '99, Taylor *ea*, '01a, Weel *ea*, '02). However, only a few studies have focused on liquid systems (Linforth *ea*, '00, Doyen *ea*, '01, Linforth *ea*, '02).

Solid foods reside in the mouth for a relatively long time (30 to 60 s). This period is sufficiently long to obtain an accurate release pattern during analysis. Liquids, however, are usually kept in the mouth for a much shorter period of time: people tend to swallow liquids quickly after having taken the product into their mouth, and aroma release takes place during this short time interval. Small irregularities in drinking patterns can, therefore, have a large effect on the amount of aroma released. These irregularities can originate from swallowing, depth of breath, jaw and tongue movements, saliva flow, interactions with mouth mucosa and so on.

It was reported that for liquids the highest aroma release signal is generally found in the first expiration after swallowing (Linforth *ea*, '00). No gas is transferred from the oral cavity to the nasal cavity as long as no opening of the barrier formed by the tongue and the soft palate occurs either by swallowing or by vigorous tongue and mouth movements (Buettner *ea*, '00a). The existence of such an anatomical barrier has been studied by the use of videofluoroscopy and real-time magnetic resonance imaging during the swallowing process (Buettner *ea*, '01).

The studies done on aroma release of liquid systems used an approach of swallowing followed by exhalation. The volatile concentration of the first peak in the release signal after swallowing, the so-called ‘swallow breath’, is taken as a measure for aroma release (Linforth *ea*, '00, Doyen *ea*, '01, Linforth *ea*, '02). In order to measure aroma release from liquid samples in an accurate and reproducible manner, it is necessary to control the human factors that cause irregularities and to take into account the large effect of swallowing. In this study a drinking protocol, based on a short residence time in the mouth, has been developed and evaluated. For this purpose, a non-alcoholic soft drink was used.

Non-alcoholic beverages are an important group of liquid food products. Intense sweeteners often replace conventional bulk sweeteners in soft drinks, such as saccharose and invert sugar, in order to reduce caloric intake and cariogenic activity. There has been much industrial and scientific interest in the replacement of bulk sweeteners by intense sweeteners (O'Brien Nabors *ea*, '91).

If the composition of a soft drink is changed, the aroma release profile might change as well due to changes in volatility of the aroma compounds, which consequently could affect the overall perception. In earlier studies possible interactions between bulk or intense sweeteners and aroma have been evaluated using static headspace analysis. Effects like pH and type and concentration of sweetener were reported to have different effects on a number of aroma compounds and

interactive effects between them appeared to be important as well (Nawar, '71, Voilley *ea*, '77, Land *ea*, '81, Le Thanh *ea*, '92, Nahon *ea*, '98a, Deibler *ea*, '99, Friel *ea*, '00, Hansson *ea*, '01a, Hansson *ea*, '01b). However, these results did not lead to a general conclusion about the interactions between sweeteners (conventional and intense) and aroma compounds and the effects these interactions have on aroma release. In addition, up to now, these interactions have not been studied by *in vivo* aroma release measurements. The aim of this study was, therefore, to test for differences in *in vivo* aroma release from a lemon-lime type beverage sweetened with either saccharose or a mixture of intense sweeteners, using a newly developed protocol.

Materials and Methods

Materials. Ethanol was obtained from J.T. Baker (Deventer, The Netherlands). Saccharose, glucose and citric acid were from Analar (Poole, UK). Fructose was purchased from Sigma (Zwijndrecht, The Netherlands). Merck (Schuchart, Germany) supplied sodium cyclamate and sodium saccharinate. Citral (3,7-dimethyl-2,6-octandienal), geranyl acetate (ester of acetic acid and a mixture of 3,7-dimethyl-2-trans, 6-octadien-1-ol [geranyl acetate] and 3,7-dimethyl-2-cis, 6-octadien-1-ol [neryl acetate]) and nonanal were obtained from Quest International B.V. (Naarden, The Netherlands).

Preparation of solutions. All aroma compounds were first dissolved in ethanol (100%). From these stock solutions, the desired amounts were added to demineralised water, introducing 0.1% w/w ethanol in all aqueous solutions. Aqueous solutions with an increasing concentration of a mixture of citral, geranyl acetate and nonanal were prepared for the determination of the minimal detectable concentration difference. The 4 solutions contained 1.5, 2.5, 2.9, and 3.0 ppm of each of the 3 aroma compounds. The concentration of the three aroma compounds in the lemon-lime type beverage was 3.0 ppm for each compound.

The following sweeteners were tested: saccharose (10% w/v), invert sugar (5% w/v fructose and 5% w/v glucose), and an intense sweetener mixture (0.09% w/v sodium cyclamate and 0.015% w/v sodium saccharinate). The sweetness of the intense sweetener mixture equalled the sweetness of 10% saccharose. A sample without any sweetener was also analysed. Each sweetener was tested in the presence and absence of 0.125% w/v citric acid.

Aroma solutions with high saccharose concentrations (0-60% w/v) were prepared with the concentration of the aroma compound calculated both on the total volume of the solutions ('total volume') and on the volume fraction of the water in solutions ('volume fraction corrected'). For the 'total volume' solutions, 3.0 mg each of citral, geranyl acetate and nonanal was added to 1 liter saccharose solution, irrespective of the saccharose concentration. In case of the 'volume-fraction corrected' samples, the added amount of aroma compounds was corrected for the saccharose volume fraction, in such a way that 3.0 mg of aroma compound was always added for every liter of pure water present. The concentrations used are listed in Table 2.1.

Table 2.1: 'total volume' and 'volume fraction corrected' concentrations of each aroma compound (citral, geranyl acetate and nonanal) in ppm for all saccharose concentrations, in the water phase and in the total saccharose solution.

	'total volume'		'volume fraction corrected'	
Saccharose % w/v	in water phase	in total solution	in water phase	in total solution
0	3.00	3.00	3.00	3.00
5	3.10	3.00	3.00	2.91
10	3.20	3.00	3.00	2.81
20	3.43	3.00	3.00	2.62
30	3.70	3.00	3.00	2.43
40	4.02	3.00	3.00	2.24
50	4.39	3.00	3.00	2.05
60	4.84	3.00	3.00	1.86

Static headspace gas chromatography (SHGC). The equilibrium headspace aroma concentrations (3 mL solution in 10 mL headspace vials) were determined by gas chromatography. The headspace aroma concentrations were allowed to reach equilibrium during at least 2 h of storage at ambient temperature. To this end 1.0 mL headspace was injected splitless on the column after 20 min of incubation at 30°C (samples were shaken during incubation). A GC-8000^{top} gas chromatograph (CE Instruments, Milan, Italy) was equipped with a CP-SIL 5 CB low bleed column (30 m × 0.25 mm, film thickness: 1.0 μm, Chrompack, Middelburg, The Netherlands) and a flame ionisation detector (FID). The oven temperature was initially 40°C for 2 min, then increasing by 25°C/min to 250°C and was kept at 250 °C for 10 min. Inlet and detector temperatures were 250 and 270°C, respectively. Gas flow rates were as follows: hydrogen, 35 mL/min; air 350 mL/min, make-up nitrogen, 30 mL/min. The headspace concentrations were expressed as peak areas in arbitrary units. Citral was defined as the sum of neral and geranial (retention times 10.5 and 10.7 min) and geranyl acetate was the combination of neryl acetate and geranyl acetate (retention times 11.3 and 11.5 min). All samples were analysed in triplicate.

***In vivo* measurement of aroma concentration.** Aroma concentrations in the breath of panelists were monitored by on-line sampling of part of the exhaled air by an Atmospheric Pressure Chemical Ionisation Gas Phase Analyser (APCI-GPA) attached to a VG Quattro II mass spectrometer (Micromass UK Ltd., Manchester, UK). By resting 1 nostril at a plastic tip, attached to an open pipe, the tidal flow of air from the nostril was allowed to pass back and forth through this pipe. In this way, the panelist can inhale and exhale freely, without experiencing hindrance from the air sampling. A small part of the breath in the pipe was sampled (75 mL/min) through a capillary tube (0.53 mm internal diameter, heated to 100°C) positioned at a right angle with the pipe, within the flow of breath. Because only a small part of the breath was sampled, the measurement was independent of the nostril used. The compounds were ionised by a 3.0 kV discharge. Source and probe temperature were 80°C. Citral, geranyl acetate, nonanal and acetone

were analysed in selected ion mode (0.08 s dwell on each ion), at m/z values of 95.0, 137.0, 143.0 and 58.8, respectively. The used cone voltages were 19 V for acetone and 20 V for the aroma compounds. Acetone was measured as an indicator of the panelists' breathing pattern.

Protocol for measurement of *in vivo* aroma release from beverages. The *in vivo* sampling started with taking 15 mL of a beverage in the mouth. This was immediately followed by nasal exhalation through the sampling unit, without any movement of the mouth for 3 s, indicated by a timer. In this way, the beginning of the measurement was marked in the acetone signal. Three seconds of inhalation followed, while every second a chewing movement was made. Then the entire sample was swallowed, followed by an exhalation of 3 s. The area of the resulting breath peak in the aroma signal was taken as a measure of *in vivo* aroma release. The panelists continued to breathe in and out for a further 20 s, with 3 s per breath and a chewing movement every second. All samples were assessed by 6 trained panelists in 5 (for the protocol testing) or 4 (for the lemon-lime type beverage) replicates.

Results and Discussion

Development of the protocol. Preliminary attempts to measure *in vivo* aroma release of liquid samples for approximately 1 min without strict instructions concerning breathing and swallowing, produced release curves with high variability (no further results shown). This is due to the natural short residence time of the liquid in the mouth. Because the highest aroma release of liquid samples is associated with swallowing (Buettner *ea*, '00a, Linforth *ea*, '00), strict control of the swallowing action is important to produce repeatable and reproducible aroma release data. We developed and tested the use of a protocol to measure volatile release from liquids, based on the high release measured directly after swallowing. This protocol is described in the Materials and Methods section.

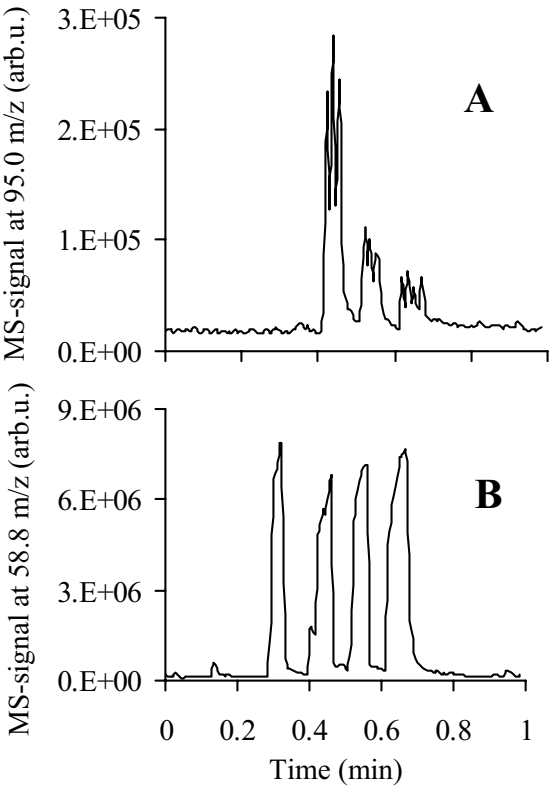

Figure 2.1. Example of raw data of a single *in vivo* measurement of release of citral (A) and acetone (B).

Persistence of aroma compounds in the breath was defined as the ratio between the peak heights in the release signal of the first and second exhalations after swallowing (Linforth *ea*, '00). To get an impression of the persistence of the aroma compounds used in the current study, the ratio of the peak area values of the first and second peak of all measurements of all panelists was averaged for the 3 compounds. Persistence values of 58%, 64%, and 38% were found for citral, geranyl acetate and nonanal, respectively. The persistence value found for nonanal is surprisingly high, in comparison with the results of Linforth and Taylor (Linforth *ea*, '00), who reported a persistence value of 6% for both hexanal and decanal. Despite the on-average relative high release in the second breath after swallowing for the compounds used, we have chosen to focus on the release in the first peak after swallowing in this study, because this peak still represents the highest release and because it is the first and the most important peak for aroma perception.

Training of the panelists. It requires only a few hours of practice, spread out over a few days, to train panelists in the use of this protocol. During the training sessions, the panelists received direct feedback about their produced aroma release curves on the PC-screen. This was found a valuable assistance in the learning process. The relative standard deviation of the values produced by a fully trained panelist is between 10 and 20%. This level did not decrease upon additional training, and was considered acceptable for the studies under consideration. To show the decrease in relative standard deviation of citral peak area during successive training sessions, the relative standard deviations of the results of the panelists are displayed in Table 2.2. The panelists 1,2 and 3 had already been familiarised with the technique during preceding experiments and less training

effort was required for this group. The panelists 4, 5, and 6 did not have any prior experience with *in vivo* aroma release measurements. An average untrained volunteer will produce data with a relative standard deviation of 20 to 30% (see Table 2.2). This value can increase further if no strict protocol is used and *in vivo* aroma release is calculated based on estimated release curves.

Table 2.2. Relative standard deviations of citral peak areas (%) of first breath after swallowing for 6 panelists in consecutive training sessions.

Training	Number of	Panelist ID					
Session	Samples	1.	2.	3.	4.	5.	6.
1	15	14	9	8	n.p.[1]	n.p.	n.p.
2	5	n.p.	n.p.	n.p.	n.c.[2]	30	23
3	5	n.p.	n.p.	n.p.	n.c.	25	16
4	20	13	8	11	36	19	21
5	20	13	9	24	28	20	23
6	16	16	12	19	14	16	22
7	16	10	8	20	21	10	17

[1] n.p. = not participated.

[2] n.c. = not calculated. No peak areas could be identified.

Evaluation of the protocol. Solutions containing a range of concentrations of citral, geranyl acetate and nonanal were assessed, in order to determine the significance of the differences in peak area between the different concentrations and to determine the minimal detectable difference. Averaging of the areas for all panelists produces a clear linear relationship between area of the peak in the aroma release signal of the first exhalation after swallowing and the concentration of the aroma compound in the solution, as shown by the linear regression lines in Figure 2.2.

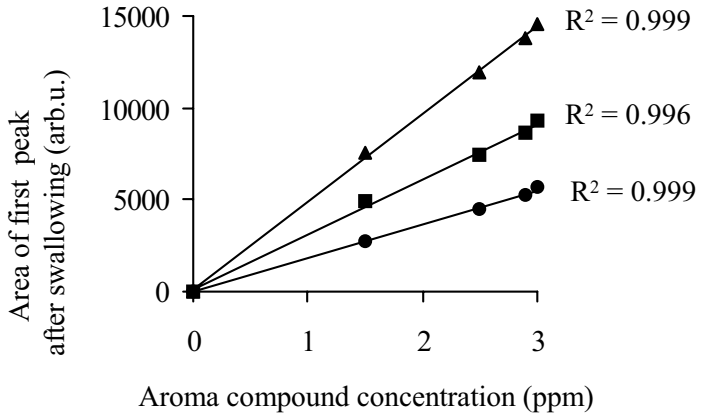

Figure 2.2. Effect of aroma concentration in the solution on area of first peak released after swallowing, averaged for all 6 panelists together (arb.u.) for citral (●), geranyl acetate (■) and nonanal (▲). The solid lines represent the best linear fits through the data points.

The significances of the differences in peak areas between the solutions containing different aroma concentrations were analysed (ANOVA). This has been done for various combinations of panelists, as listed in Table 2.3. Because 4 comparisons were made between different concentrations within the dataset of each aroma compound, all p-values listed in Table 2.3 have been multiplied by a Bonferroni correction factor of 4. The highest significance was obtained when the results of all the 6 panelists involved in the experiment were used. However, the use of the results of all panelists together violated the ANOVA assumption of homogeneity of variances, as was shown by significance of the Levene's test (p-value of 0.01 to 0.05 for a large part of the comparisons). This was caused by a difference in variance between the panelists, as is shown in Figure 2.3, in which the relative standard deviation has been averaged for each panelist for each aroma compound. It can clearly be seen that panelists 4, 5, and 6 have higher relative standard deviations than panelists 1, 2 and 3. If the significance of the concentration differences is calculated with only panelists 1, 2 and 3, the same significance is found as with all panelists involved, but now without violation of ANOVA assumptions. The panelists 1, 2, and 3 all have a fairly low and similar averaged relative standard deviation (Figure 2.3). Analysis with 2 or 3 panelists is preferable to analysis with only 1 panelist, since the former results in a higher significance of differences between solutions with different concentrations of aroma compounds (Table 2.3), and therefore increases the reliability of the results.

A remarkable observation that can be made with regard to Figure 2.3 concerns the differences between the aroma compounds. Despite the fact that the compounds were present in mixtures, and assessed simultaneously, the relative standard deviation varies greatly between the compounds and this effect varies per person. Especially for nonanal a higher relative standard deviation is found. This might be due to the higher volatility of this compound compared to citral and geranyl acetate.

Table 2.3. Significance of differences in peak area for various aroma concentration differences, expressed in p-values for all 6 panelists, for 2 groups of 3 panelists and for each panelist individually (ANOVA).

Concentration difference (%)	3	17	40	50	3	17	40	50	3	17	40	50
Panelists in analysis	Citral				Geranyl acetate				Nonanal			
1,2,3,4,5,6	0.75	0.00	0.00	0.00	1.18	0.01	0.00	0.00	2.18	0.14	0.00	0.00
1,2,3	3.91	0.00	0.00	0.00	2.74	0.00	0.00	0.00	2.52	0.29	0.00	0.00
4,5,6	0.63	0.07	0.00	0.00	0.80	0.22	0.03	0.00	2.85	1.00	0.51	0.09
1	3.57	0.10	0.00	0.00	3.54	0.09	0.00	0.00	2.09	0.78	0.02	0.00
2	0.01	0.01	0.06	0.00	0.20	0.05	0.13	0.00	1.79	0.43	0.82	0.03
3	0.20	1.14	0.00	0.00	0.28	1.73	0.00	0.00	3.35	3.22	0.00	0.01
4	3.39	1.01	0.04	0.01	3.32	1.46	0.73	0.16	2.17	2.99	0.98	1.47
5	1.61	1.65	0.24	0.12	1.99	1.85	0.36	0.23	1.23	1.78	0.34	0.14
6	0.96	0.15	0.06	0.01	1.21	0.50	0.59	0.09	3.26	1.11	3.80	1.03

It can be concluded that for this particular system the minimal detectable concentration difference is in between 3 and 17%, and a well-trained panelist should be able to produce data with a relative averaged standard deviation lower than 15%, depending on the aroma compound.

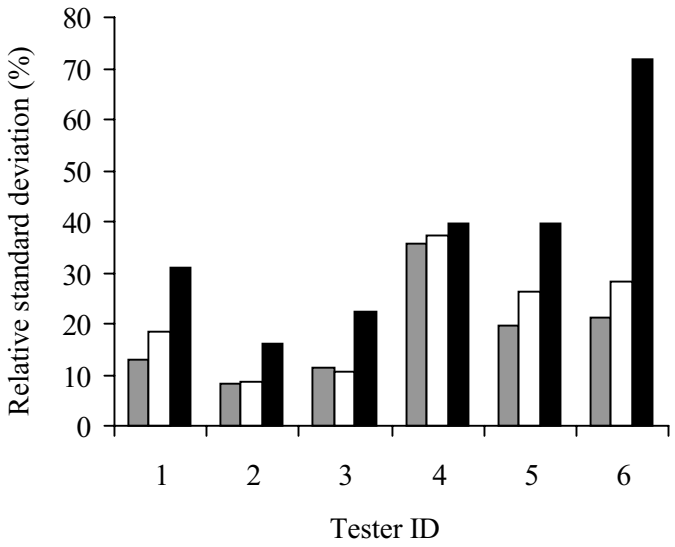

Figure 2.3. Relative standard deviations (%) of the *in vivo* release peak area after swallowing for panelists 1-6 of citral (gray bars), geranyl acetate (white bars) and nonanal (black bars). The relative standard deviations are averaged over all concentrations.

Application of the protocol to study interactions between ingredients in a lemon-lime type beverage. The food industry has a large interest in the effects of replacement of bulk sweeteners with intense sweeteners on aroma perception (O'Brien Nabors *ea*, '91). So far, the cause of the reported sensory changes in aroma perception due to this replacement is not clear (Von Sydow *ea*, '74, Larson-Powers *ea*, '78, Wiseman *ea*, '91, Nahon *ea*, '96, '98b). A possible cause for the reported sensory changes could be the change in aroma release. Therefore we have used our newly developed protocol for *in vivo* aroma release measurements of beverages to test the effect of sweetener type (bulk or intense) on aroma release in a lemon-lime type beverage.

The effect of citral, geranyl acetate, and nonanal on each other's release was tested by comparing solutions of single compounds with solutions of mixtures of 2 of them. This was done by static headspace analysis and by *in vivo* measurement of aroma release making use of the described protocol. For the analysis of the *in vivo* results, 3 panelists with relative standard deviations below 15% were selected. No significant differences were found for the results of either of the techniques (ANOVA, results not shown), suggesting that the presence of 3.0 ppm of one of the compounds does not influence the static headspace concentration or the *in vivo* aroma release of 3.0 ppm of one of the other compounds. Based on this knowledge, the 3 compounds were applied in a mixture in further experiments.

The effect of various types of sweeteners in presence or absence of citric acid on aroma release was studied. The sweetener type and citric acid did not influence the aroma release, in either the static or the *in vivo* measurement method (ANOVA). The *in vivo* data of the 3 panelists with the lowest relative standard deviations were used. As an example, the results of *in vivo* nonanal release and static headspace with and without sweeteners with and without citric acid are shown in Figure 2.4. The results for citral and geranyl acetate were similar (not shown). A slightly significant decrease of the release of citral was found in the presence of citric acid ($p = 0.048$ *in vivo*, $p = 0.037$ static headspace). This was caused by slow degradation of citral under acidic conditions, which was be confirmed by the observation of slow decrease in equilibrium headspace concentration of citral during storage of the samples.

Since the sweetener concentrations used here are the ones that are normally applied in beverages, and the protocol used here to assess *in vivo* aroma release is a sensitive and accurate method, it is concluded that substitution of bulk sweeteners by intense sweeteners has no profound effect on *in vivo* aroma release. In several studies of sensory perception of (fruity) aroma of sweetened solutions, the sweetener type has been shown to modify sensory aroma attributes (Von Sydow *ea*, '74, Larson-Powers *ea*, '78, Wiseman *ea*, '91, Nahon *ea*, '96, '98b). The study of Nahon and co-workers (1996) for example, clearly showed for a blackcurrant soft drink that changing the type of sweetener (from saccharose to equisweet mixtures of intense sweeteners) changes the sensory aroma attributes from a blackcurrant to a strawberry character. As is demonstrated in the present study, aroma release is not affected by changes in sweetener type and therefore it can be concluded that aroma release does not account for sensory differences between beverages sweetened with bulk or intense sweeteners. These perceptual differences are more likely to be explained as psychophysical effects, i.e. that the aroma perception changes, because of

differences in textural perception between solutions of saccharose and intense sweeteners, or because of interactions between the sensory perceptions of aroma and sweetness.

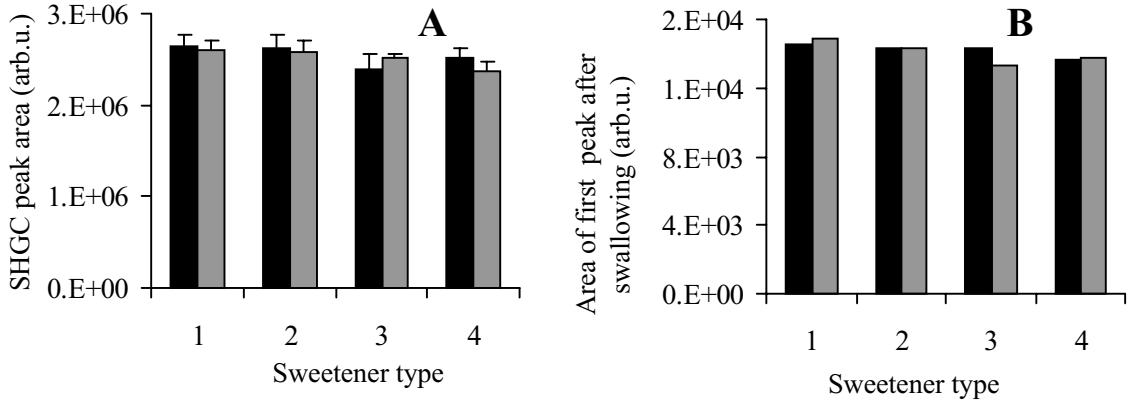

Figure 2.4. Averaged SHGC peak area (arb.u.) (A) and *in vivo* release peak area (arb.u) after swallowing averaged over the 3 best panelists (B) for nonanal solutions containing saccharose (1), invert sugar (2), intense sweetener mix (3) and no sweetener (4), with and without citric acid (black and gray bars, respectively). Error bars in GC peak area represent standard deviation.

Earlier studies have described an effect of saccharose on aroma release for high saccharose concentrations (up to 65% w/v) (Nawar, '71, Voilley *ea*, '77, Le Thanh *ea*, '92, Nahon *ea*, '98a, Friel *ea*, '00, Hansson *ea*, '01a). In order to compare these studies with our work, static headspace measurements of aroma compounds were performed with solutions with a range of saccharose concentrations, in which the concentration of aroma compound was corrected ('volume fraction corrected') or not corrected ('total volume') for the volume fraction of saccharose. The results of these experiments are shown in Figure 2.5. The data points in this figure indicate that the static headspace concentrations of the 'total volume' samples show a clear increase in static headspace concentration of all 3 compounds upon addition of saccharose. This is because the same amount of aroma compounds is put in a reduced volume of water; i.e. the mole fraction of the aroma compounds is increased (Voilley *ea*, '77). A higher concentration of aroma compounds in the water phase results in a higher headspace concentration if the partition coefficient is not changed, according to Henry's law. Once the aroma concentration was made proportional to the molar volume of water, in the case of the 'volume fraction corrected' samples, there is no longer a significant effect of saccharose on headspace concentration of citral and geranyl acetate. However, nonanal seems to interact with saccharose, because the headspace concentration decreases for the 'total volume' samples. These results illustrate how the direction of the effect (increase or decrease) is determined by the correction for added saccharose and by the type of aroma compound. Most authors do not correct their data for saccharose volume fraction, and results should, therefore, be compared carefully (Nawar, '71, Voilley *ea*, '77, Le Thanh *ea*, '92, Nahon *ea*, '98a, Friel *ea*, '00, Hansson *ea*, '01a).

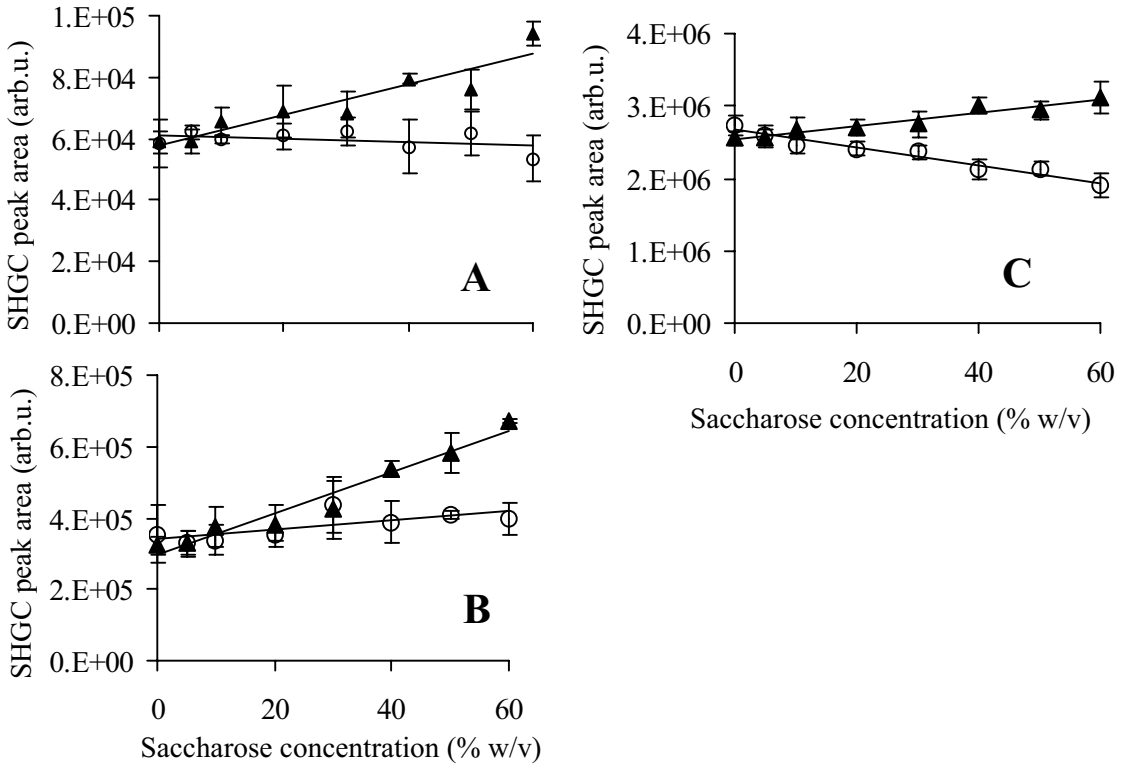

Figure 2.5. Averaged SHGC peak area (arb.u.) of citral (A), geranyl acetate (B) and nonanal (C), above solutions of different saccharose concentrations. Added amount of the aroma compounds, was either corrected (○) or not (▲) for the volume occupied by sucrose. Error bars represent standard deviations.

Conclusions

Use of a strict drinking protocol can greatly improve the reproducibility of *in vivo* aroma release measurements from beverages. Application of this protocol to a lemon-lime type beverage reveals that saccharose, invert sugar and a mixture of intense sweeteners, in the presence and absence of citric acid (all applied at concentrations relevant for beverages) has no significant effect on the *in vivo* aroma release. Saccharose might influence the aroma release, but only at concentrations far beyond relevance of application in beverages, and depending on the physicochemical properties of the aroma compounds.

Chapter 3

Effect of whey protein on the *in vivo* release of aldehydes

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Abstract

Retention of aldehydes by whey proteins in solutions buffered at a range of pH values was studied under static and dynamic headspace conditions and *in vivo* in exhaled air. Static headspace measurements showed a clear increase in retention in the presence of whey proteins for aldehydes with longer carbon chains and for buffer solutions with higher pH values. For *in vivo* aldehyde release measurements, these effects were much less pronounced. The presence of saliva or the binding of aldehydes to the surface of the oral cavity was not responsible for this effect. This difference can be explained by the highly dynamic conditions of *in vivo* aroma release of liquid products, due to the relatively large flow of air during exhalation. After swallowing, a thin film of aldehyde solution remains in the pharynx; subsequent exhalation will release both the free aldehydes present in this film, and those reversibly bound to the whey protein.

Introduction

Aroma quality, together with texture, taste, and appearance, is a consumer driver for the overall liking and acceptance of a food product. Aroma compounds are perceived when they are released from a food product and reach the olfactory region in the nasal cavity, either before or during consumption (Overbosch *ea*, '91). Measurement of real-time *in vivo* aroma release can be performed by new mass spectrometric methods that have been developed for this purpose based on atmospheric pressure chemical ionisation - mass spectrometry (APCI-MS) (Taylor *ea*, '96, Taylor *ea*, '00) and proton transfer reaction – MS (PTR-MS) (Lindinger *ea*, '98a).

Aroma compounds can interact with other food ingredients, and these interactions are thought to influence the amount of aroma compound available for release. This might affect the overall perception of a product (Guichard, '02). Much research effort has been invested in protein-aroma interactions, especially in the interactions between aroma compounds and β-lactoglobulin. The subject of interactions between aroma compounds and food ingredients, and parameters affecting these interactions, has recently been reviewed (Guichard, '02). Many studies have revealed an increase of binding constants with increasing carbon chain length for the binding of small molecules as aldehydes, ketones, esters and alcohols to β-lactoglobulin (Mills *ea*, '84, O'Neill *ea*, '87, Pelletier *ea*, '98, Andriot *ea*, '00, Guichard *ea*, '00, Reiners *ea*, '00), strongly suggesting hydrophobic interactions, which have a reversible character (Mills *ea*, '84). β-Lactoglobulin is reported to have 2 separate binding sites for hydrophobic ligands (Cho *ea*, '94, Narayan *ea*, '97, Wu *ea*, '99). While retinol (Cho *ea*, '94) and fatty acids (Wu *ea*, '99, Ragona *ea*, '00) are reported to bind in the central cavity of β-lactoglobulin, information about the exact binding side of other ligands is sometimes contradictory (Dufour *ea*, '90, Narayan *ea*, '98, Pelletier *ea*, '98, Sostmann *ea*, '98, Luebke *ea*, '99). A recent study of the binding sites of two aroma molecules, γ-decalactone and β-ionone, using nuclear magnetic resonance spectroscopy, demonstrated binding of the former compound into the central cavity, and binding to a groove near the outer surface of the protein of the latter (Luebke *ea*, '02).

The studies mentioned above, describing the interactions between β-lactoglobulin and various ligands, give no information about the behaviour of these interactions in the dynamic situation in the mouth during the consumption of a food. In an attempt to understand the dynamic conditions of food consumption, several mathematical models were developed (De Roos *ea*, '94, Harrison *ea*, '97b). The model developed by Harrison and Hills (Harrison *ea*, '97b) predicts dynamic volatile release from solutions containing aroma binding macromolecules. The model is based on first-order kinetics to describe the reversible binding between the aroma compound and the polymer and on the penetration theory of interfacial mass transfer to describe the aroma release across the liquid-gas interface. The latter has been shown to be the rate-limiting step for aroma release in most situations (Harrison *ea*, '97b). Andriot and co-workers (Andriot *ea*, '00) followed the initial release of volatiles from protein solutions by static headspace measurements after

different times of equilibration (15-2700 s). These experimental data were fitted to the model developed by Harrison and Hills (Harrison *ea*, '97b) and, in general, there was good agreement between experiment and theory (Andriot *ea*, '00). However, the model was not validated by in-mouth measurements. In addition, possible interactions between aroma compounds and saliva, and the effect of mucous membranes of the oral, nasal and pharyngeal cavities on aroma release were not taken into account.

These interactions between aroma compounds and saliva or mucous membranes have been investigated in other studies (Buettner *ea*, '00b, Friel *ea*, '01, Van Ruth *ea*, '01, Linforth *ea*, '02). Aldehydes and other aroma compounds can interact with salivary proteins, especially mucin, as shown by static headspace measurements (Friel *ea*, '01, Van Ruth *ea*, '01) and by the SOOM-technique (spit-off odorant measurements) (Buettner *ea*, '00b). Losses in the oral cavity were observed for aldehydes and esters during 1 min of contact time (Buettner *ea*, '00b). In addition, esters, thiols and aldehydes were found to be subject to enzymatic conversion upon contact with saliva within a period of 10 min (Hussein *ea*, '83, Buettner, '02a, b).

In a recent study, Linforth and co-workers (Linforth *ea*, '02) investigated the effect of factors such as absorption to epithelia of mouth, nose and pharynx, and dilution by saliva, on aroma release through the nose. It was demonstrated that the mass transfer from aqueous solution in the mouth into exhaled air was a major factor affecting the actual released amount of aroma. However, up to now, the effect of the in-mouth conditions on the interactions between aroma compounds and food proteins has not been studied.

Therefore, the aim of the present work was to reveal the relevance for aroma release of interactions between food proteins and aroma compounds under in-mouth conditions, by measuring and comparing the interactions between aldehydes and whey proteins under static and dynamic headspace conditions and under in-mouth conditions during consumption. During preparation of this chapter we became aware of comparable experiments performed by Le Guen and Vreeker (Le Guen *ea*, '03).

Materials and methods

Materials. Butanal was obtained from Fluka Chemie (Buchs, Switzerland). Hexanal, octanal and nonanal were obtained from Merck (Darmstadt, Germany). The purity of all aldehydes was higher than 98% (v/v). Buffers were prepared with citric acid (pH 3, 5 and 6), NaH_2PO_4 (pH 7) and glycine (pH 9). The pH was set with NaOH or HCl. Demineralised water was used. MCT (medium chain triglycerides) oil was provided by Quest International (Naarden, the Netherlands). Whey protein isolate (Bipro, JE 153-9-420) was obtained from Davisco Foods International Inc., Le Sueur, Minnesota. Specifications were as follows: pH 7.23; non-protein nitrogen 0.17%; ash 1.8%; lactose 0.34%; calcium 0.13%; fat 0.57%; total protein 93.39% (N × 6.38); α-lactalbumin 12.6%; bovine serum albumin 3.2%; immunoglobulin G 5.2%; β-lactoglobulin A 33.2% and β-lactoglobulin B 37.1%.

Artificial saliva was prepared in demineralised water according to van Ruth and co-workers (Van Ruth *ea*, '01) and consisted of $NaHCO_3$ (5.2 g/L), K_2HPO_4 (1.04 g/L), NaCl (0.88 g/L), KCl (0.24 g/L), $CaCl_2 \cdot 2H_2O$ (0.44 g/L) and 2.16 g/L porcine stomach mucin (Sigma, Steinheim, Germany). No α-amylase was added. The pH was set to 7.30.

Human saliva was a mixture of the saliva donated by 30 non-smoking individuals, who had not eaten for at least 1 h prior to donation. The pH was 7.30. After mixing, the human saliva was stored in small portions at -20ºC. The mucin concentration in mixed human saliva was determined by fluorimetric measurement of O-glycosylated reducing oligosaccharides, according to the method of Crowther and Wetmore (Crowther *ea*, '87).

The protein concentration in mixed human saliva was measured as total Kjeldahl-N. A conversion factor of 6.25 was used.

Preparation of solutions. The solutions used in this study consisted of 50 mM buffer, containing 5 ppm of each aldehyde, with and without 3% (w/v) whey protein isolate. The final ionic strengths of all solutions were standardised to 0.272 by addition of NaCl. Aldehydes were first dissolved in ethanol (100% v/v). This introduced 0.2% (w/v) ethanol into the final solutions. In order to dissolve the whey protein, the solutions were stirred for at least 3 h.

To test the effect of fat present in the protein isolate, 0.017% (w/v) of MCT oil was added to the aroma solutions of pH 3 and 7, described above. The oil/aroma solution was stirred for 3 h. Whey protein isolate (35 ppm) was used as an emulsifier. No creaming was observed.

Measurement of aroma release by APCI-MS. Aroma concentrations in the breath of panelists as well as in the mouth model system were monitored by on-line sampling of part of the exhaled air by the MS-Nose, an APCI gas phase analyser attached to a VG Quattro II mass spectrometer (Micromass UK Ltd., Manchester, U.K.). The compounds were ionised by a 3.0 kV discharge. Source and probe temperatures were 80°C. Butanal, hexanal, octanal, nonanal and acetone were analysed in selected ion mode (0.08 s dwell on each ion), at m/z values of 73.0, 101.0, 129.0, 143.0 and 58.8, respectively. The cone voltages used were 19 V for acetone and 21 V for the aldehydes. Acetone was measured as an indicator of the panelists' breathing pattern.

Measurement of *in vivo* aroma release in exhaled air. Aroma release measurements in exhaled air were conducted according to a strict protocol, developed for liquid samples (Weel *ea*, '03b) for which the panelists were trained. Panelists were considered to be sufficiently trained when their averaged relative standard deviation for all samples of a training session did not exceed 15%. In addition to this protocol, every measurement was preceded by rinsing the mouth with water followed by 30 s of drying the oral cavity by means of a hair dryer blowing unheated air. This procedure was followed in order to standardise the amount of saliva present in the mouth during the measurements. The *in vivo* sampling protocol started with taking 15 mL of solution in the mouth. This was followed immediately by nasal exhalation through the MS-Nose sampling unit, without any movement of the mouth for 3 s, indicated by a timer. In this way, the beginning of the measurement was marked in the acetone signal. Three seconds of inhalation followed, while every second a chewing movement was made. Then the entire sample was swallowed, followed by an exhalation of 3 s. The panelists continued to breathe in and out for another 20 s,

with 3 s per breath and a chewing movement every second. The panelists breathed in and out through a tube, from which continuously 80 mL/min of air was sampled directly into the APCI source. The area of the peak in the release signal corresponding to the first exhalation after swallowing was used as a measure of aroma release. Two panelists assessed all samples in 5 replicates.

Mouth model measurements. A mouth model system, developed by Van Ruth and co-workers (Van Ruth *ea*, '94), was used for dynamic headspace measurements. A volume of 2 mL of solution was 'chewed' by a plunger, making rotating and up-and-down movements, both at 1.25 Hz. The system was kept at 37ºC. The release of aldehydes from the mouth model was monitored for 4 min. Either human saliva, artificial saliva, or demineralised water was added to the samples in amounts proportional to the real-life situation. To determine this, the average saliva production of 5 persons who rinsed their mouth in triplicate with 15 mL of all the buffer solutions (Table 3.1), was measured. The saliva production was determined by weighing of expectorates. From inside the mouth model system, above the plunger and liquid-gas interface, 3 mL/min of air was sampled continuously into the APCI source. The maximum release signal was taken as a measure of dynamic aroma release. All samples were assessed in triplicate.

Table 3.1. Amount of saliva formed (± standard deviations) during 3 s of rinsing of 15 mL of aldehyde solution (buffered at a range of pH values) and pH values after expectoration. All values are averaged over five persons, who assessed the samples in triplicate.

Buffer	Set pH	Saliva formed (g)	pH after expectoration
Citric acid-NaOH	3.00	1.7 ± 0.5	2.93 ± 0.02
Citric acid-NaOH	5.00	1.1 ± 0.3	5.07 ± 0.02
Citric acid-NaOH	6.00	0.7 ± 0.2	6.23 ± 0.01
$NaHPO_4$-NaOH	7.00	0.9 ± 0.2	7.03 ± 0.00
Glycine-HCl	9.00	0.8 ± 0.3	8.88 ± 0.02

Spit-out experiments. Two panelists rinsed 15 mL of an aroma solution in the mouth in accordance with the protocol used during *in vivo* release measurements. Now, instead of swallowing the sample, it was spit out. Immediately after spitting out, freshly secreted saliva was collected from the same panelist and added to 15 mL of nonrinsed aroma solution, in the same amount as the averaged increase in weight of the corresponding spit-out sample. All samples were directly frozen (-20ºC), and defrosted only 20 min prior to analysis by static headspace gas chromatography (SHGC). For this purpose, the sample tray was coupled to a cooling water bath (-5°C). All samples were prepared and analysed in triplicate.

Static headspace gas chromatography (SHGC) measurements. The equilibrium headspace aroma concentrations of aldehydes (4 mL solution in 10 mL headspace vials) were determined by gas chromatography. To this end, 1.0 mL headspace was injected splitless on the column after 20 min of incubation at 30°C. A GC-8000^{top} gas chromatograph (CE Instruments, Milan, Italy) was equipped with a CP-SIL 5 CB low-bleed column (50 m × 0.32 mm, film thickness 1.2 µm; Varian

Chrompack, Bergen op Zoom, The Netherlands) and a FID. The oven temperature was initially 40°C for 2 min, then increased by 25°C/min to 250°C and was kept at 250°C for 10 min. Inlet and detector temperatures were 250 and 270°C, respectively. The headspace concentrations were expressed as peak areas in arbitrary units. All samples were prepared and analysed in triplicate.

Results and discussion

Aldehyde-whey protein interactions under static headspace and *in vivo* conditions. Interactions between whey protein and a homologous series of aldehydes were studied both using SHGC as well as *in vivo* analysis. At the concentrations of whey protein isolate and aldehyde mixture used, aldehyde molecules and β-lactoglobulin monomer units were present in the ratio of approximately 1:6. The interactions were studied over a range of pH values. The static headspace results are shown in Figure 3.1A. This Figure shows the relative change in static headspace aldehyde concentration due to the presence of 3% (w/v) whey protein. An increasing retention of the aldehydes is found with increasing length of the aldehyde carbon chain. This effect has been observed previously for aldehydes and other aliphatic compounds, and a hydrophobic interaction between protein and aldehyde has been proposed (Mills *ea*, '84, O'Neill *ea*, '87, Pelletier *ea*, '98, Guichard *ea*, '00, Reiners *ea*, '00). Also, a higher retention at higher pH is found; this has also been reported previously (Mills *ea*, '84, Jouenne *ea*, '00, Van Ruth *ea*, '02c). At a higher pH of the medium, the structure of β-lactoglobulin is more flexible, allowing a better accessibility of ligands to hydrophobic binding sites (Shimizu *ea*, '85).

The static headspace concentration was reduced (30-50%) for all aldehydes in the solution of pH 9, irrespective of the presence of whey protein. This reduction could be caused by the formation of aldol condensation products from two or more aldehydes, a reaction well known to occur at high pH (Geissman, '46). The calculation of retention due to presence of whey protein (as displayed in Figure 3.1) at pH 9 was based on the reduced headspace concentrations.

The whey protein isolate contained 0.57% (w/v) fat. This means that 0.017% (w/v) fat was present in the solutions of 3% (w/v) whey protein isolate. This hydrophobic phase might additionally affect the liquid-air partitioning of the aldehydes. This was tested with an aqueous emulsion of 0.017% MCT oil. A significant decrease in static headspace concentration was found for octanal (7 and 9%) and nonanal (25 and 27%) at pH 3 and 7, respectively (no further data shown). The presence of whey protein isolate (including the fat fraction), however, caused a decrease of 87 and 96% respectively for these two compounds (at pH 7). Therefore, compared to the effect of protein, the fat content of the whey protein isolate was considered to play only a minor role in the aldehyde retention.

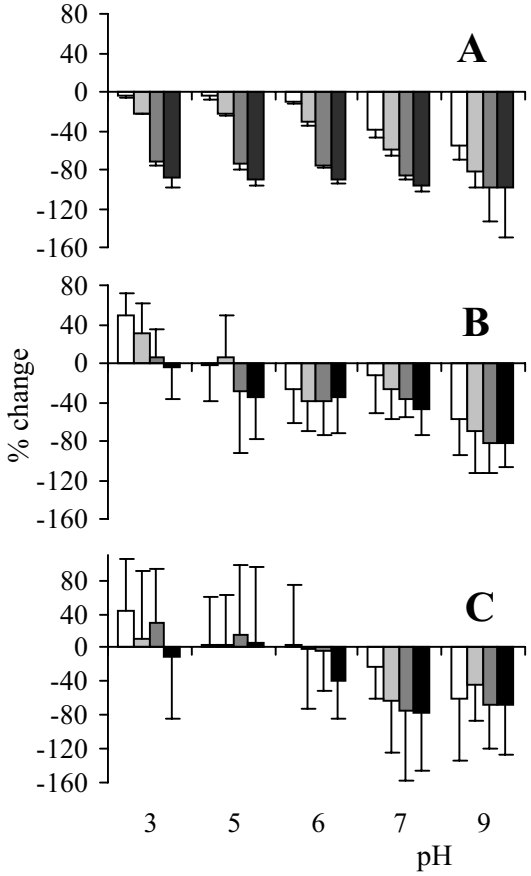

Figure 3.1. Relative change (%) in headspace concentrations (A) and *in vivo* aroma release of two panelists (B and C) of 5 ppm butanal (white bars), hexanal (light gray bars), octanal (dark gray bars) and nonanal (black bars) due to the presence of 3% whey protein at various pH values. Error bars represent standard deviations.

The results of the *in vivo* aroma release measurements in exhaled air for two panelists show (Figure 3.1B and C) a pattern that clearly differs from the static headspace data. In most cases, there is still retention of the aldehydes by the protein at pH values of 5-9. However, in contrast to the static headspace conditions, there is a much weaker effect of carbon chain length of the aldehydes on the extent of retention. At pH 3, there even seems to be an increase of release for all aldehydes, when whey-protein is added. Based on these experiments, it is clear that large differences exist between measurements in exhaled air and static headspace. The aldehyde-protein interactions seem to be less significant under dynamic *in vivo* conditions than under static headspace conditions. The averaged relative standard deviations of our trained panelists was below 15% in earlier investigations (Weel *ea*, '03b). In the current study, higher values (20-40%) were obtained, which was probably due to the influence of the very bad taste and smell of the solutions studied.

Subsequently, three possible hypotheses were studied to explain the observed differences in aroma-protein interactions between static headspace and exhaled air. Two possible explanations, the effect of saliva and the effect of mouth mucosa and epithelium, concern factors that are present under mouth conditions and absent under static headspace conditions. The third

explanation deals with the difference that exists between the physical conditions under which the aroma release takes place under static headspace and in-mouth conditions.

Influence of saliva on aldehyde-whey protein interactions. Preliminary SHGC measurements of hexanal solutions that had been rinsed in the mouth showed a decrease of hexanal headspace concentration (25-90%, depending on the test person, during hours of waiting time in the GC sample tray at ambient temperature). The decrease of aldehydes was accompanied by an increase of the same magnitude in hexanol headspace concentration, suggesting an enzymatic conversion. Moreover, a positive correlation was found between concentration of alcohol formed and waiting time in the GC sample tray before analysis (results not shown). Conversion of aroma compounds by saliva within 10 min has been described previously for esters and thiols by Buettner (Buettner, '02a). Hussein and co-workers (Hussein *ea*, '83) reported a reduction of benzaldehyde and cinnamaldehyde to their corresponding alcohols within 5 min, when solutions containing these compounds were rinsed in the mouth. The reduction of simple aldehydes to corresponding alcohols upon 10 min incubation with saliva was demonstrated recently (Buettner, '02b). Since we were interested in a shorter contact time between aldehyde-protein solutions and saliva, application of a mouth model directly coupled to APCI-MS seemed to be useful to exclude possible effects of longer contact times. In the mouth model used in this study it is possible to add saliva just before the start of the real time dynamic headspace measurement of the release of aldehydes into the headspace. In Figure 3.2, an example of a dynamic headspace measurement in the mouth model is shown for octanal. As the plunger starts plunging and rotating, a steep increase in release is observed. After 1 min a steady state is reached. In this situation, the rate of aldehyde release into the headspace is equal to the rate at which the aldehydes are sampled into the MS. After 3 min, a decrease in aldehyde release becomes visible, due to slow exhaustion of the aldehydes in the solution. In the 4 min during which the release of aldehydes from the mouth model was monitored, no differences were observed between samples with and without saliva.

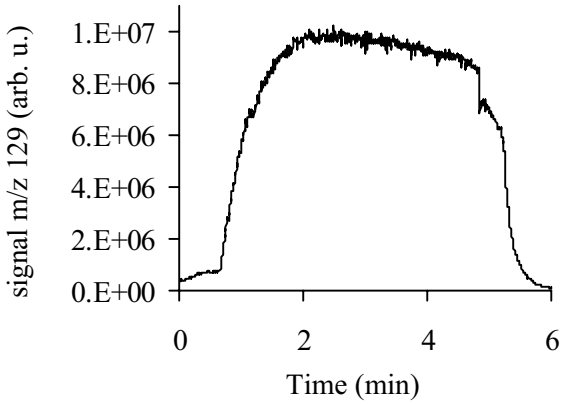

Figure 3.2. Raw data example of release of octanal from a 5 ppm solution in the mouth model.

The amount of water, human saliva or artificial saliva to be added to the mouth model was determined by measuring the increased weight of spit-out buffered aldehyde solutions after

rinsing these solutions in the mouth for 3 s. The amount of saliva produced varied slightly with pH (see Table 3.1). Solutions with lower pH provoke a stronger (more sour) taste sensation and this is known to cause higher saliva secretion rates (Dawes, '81). The pooled human saliva was analysed for its total protein and mucin protein content, which were 4 g/L and 1 g/L, respectively. This means that the pool of saliva contained 3 g/L of other proteins as α-amylase, proline-rich proteins, immunoglobulin, lysozyme, staterin, histatin, lactoferrin, and many other small protein fractions (Young *ea*, '81), which are not present in our artificial saliva. An advantage of using human saliva is that the effect of these fractions can be taken into account as well. However, the addition of artificial or pooled human saliva had no effect on the aldehyde-whey protein interactions, as shown in Figure 3.3, which represent aldehyde-whey protein interactions in the mouth model upon addition of water, artificial saliva, and human saliva. Moreover, no effect of addition of either artificial or human saliva on the dynamic headspace concentration of any aldehyde in the mouth model was found, irrespective of the presence of whey protein.

In contrast to these results, Van Ruth and co-workers (Van Ruth *ea*, '01) and Friel and co-workers (Friel *ea*, '01) reported a decrease in static headspace aldehyde concentration upon addition of artificial saliva. They both used pig gastric mucin, at concentrations of 2.16 g/L and 2 g/L, respectively. The interaction between aldehydes and artificial saliva was tested without any dilution. In our study, artificial or human saliva was added to the mouth model in the same weight ratio (saliva : aldehyde solution) as human saliva was formed under *in vivo* conditions (Table 3.1), which resulted in a more than 10-fold lower mucin concentration compared to Van Ruth and co-workers (Van Ruth *ea*, '01) and Friel and co-workers (Friel *ea*, '01). This might be the reason that no effect of saliva was found in our experiments. In an additional experiment, the mucin concentration was increased by a factor 10. A decrease in dynamic headspace aldehyde concentration was found (data not shown). These results confirm the importance of choosing a representative mucin concentration. The magnitude of retention was comparable to the results of Van Ruth and co-workers (Van Ruth *ea*, '01). Friel and co-workers (Friel *ea*, '01) reported a higher retention for aldehydes. However, it is difficult to compare the results in detail, because of variation in experimental conditions used.

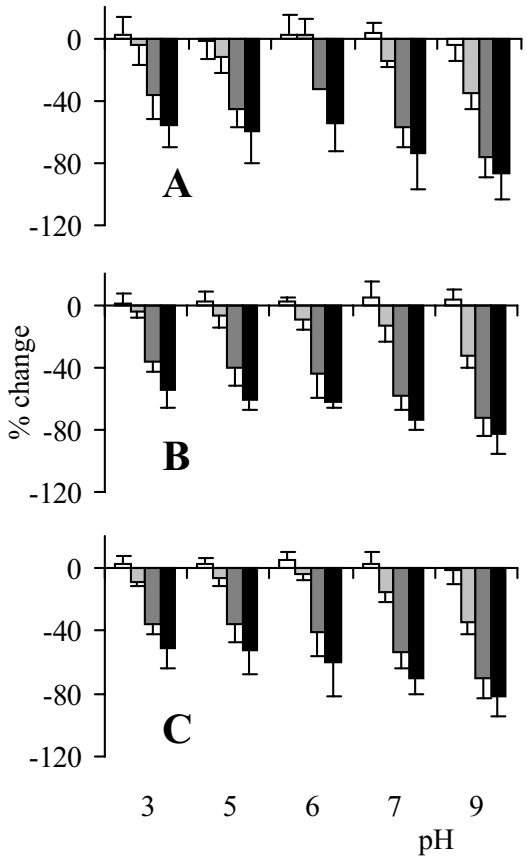

Figure 3.3. Relative change (%) in mouth model release signal with addition of water (A), artificial saliva (B) and human saliva (C) of 5 ppm butanal (white bars), hexanal (light gray bars), octanal (dark gray bars) and nonanal (black bars) due to the presence of 3% whey protein at various pH values. Error bars represent standard deviations.

Effect of mucosa and epithelial tissue on aldehyde-whey protein interactions. As an excretion product, saliva can be obtained easily by expectoration and tested *in vitro*. However, the effect of the inside surface of the mouth is more difficult to study since isolation of this material is less simple. Nevertheless, the effect of the mouth coating could be studied by comparing headspace concentrations above aldehyde solutions that were rinsed in the mouth with those that were not. To account for the effect of saliva, the mouth was dried in advance. Furthermore, freshly formed saliva, sampled immediately after expectoration, was added to the nonrinsed sample in the same amount as the saliva, which was secreted into the rinsed sample during rinsing in the mouth. Because the saliva was collected directly afterward, its composition closely resembles that of the saliva secreted during the actual rinsing. Therefore, both the rinsed and the nonrinsed samples are assumed to have had a similar interaction with saliva. Two differences remained between rinsed and nonrinsed solutions: the contact of the rinsed sample with the coating of the mouth and a minimal loss of aroma molecules due to a short exposure to the air flow during chewing. The former is the subject of this experiment and the latter will be ignored because the actual released amount during 3 s of rinsing is extremely low. This was concluded from an additional experiment. *In vivo* aroma release measurements of 15 mL aqueous solutions containing 10 ppm

hexanal were performed using the same protocol. The total released amount of hexanal in exhaled air was quantified and determined to be less than 1%. Buettner and Schieberle (Buettner *ea*, '00b) also showed for aldehyde solutions that the release during the first 5 s of mastication was minimal by using SOOM.

Table 3.2. Static headspace concentrations (GC area, arbitrary units, divided by 10^5) of aldehyde solutions at various pH values, before and after rinsing the solution in the mouth. Data were collected in triplicate by 2 panelists.

		Without prot.		With protein				Without prot.		With protein	
	pH	Before	After	Before	After		pH	Before	After	Before	After
Butanal	3	8.1	7.5	8.3	7.1	Octanal	3	11.4	10.4	4.7	4.3
	5	8.0	7.6	7.1	6.8		5	13.0	11.6	4.3	4.2
	6	8.1	7.6	6.7	6.3		6	13.0	12.0	3.7	3.7
	7	8.1	7.5	5.1	5.0		7	12.9	11.8	2.2	2.2
	9	5.3	5.2	2.1	2.2		9	6.4	6.2	0.2	0.3
Hexanal	3	13.6	12.5	11.3	10.4	Nonanal	3	11.3	10.2	4.7	4.4
	5	13.8	12.8	11.1	10.4		5	12.5	11.5	4.5	4.4
	6	13.8	12.8	9.3	8.7		6	13.0	11.4	3.9	3.8
	7	14.1	12.6	6.3	6.1		7	12.9	11.6	2.3	2.4
	9	9.4	9.2	1.8	1.9		9	7.6	7.1	0.3	0.3

The static headspace concentrations of aldehydes, averaged over 2 panelists (whose values closely agreed), which were either rinsed in the mouth or not, are given in Table 3.2. It can be seen that, in both the presence and absence of whey protein, the differences between rinsed and nonrinsed samples are small. The overall averaged decrease due to rinsing is 7% (ANOVA, $p = 0.0002$). Furthermore, the retention effect of whey protein did not differ between rinsed and nonrinsed samples (ANOVA, $p = 0.65$). The effect of whey protein on aldehyde retention, and the aldehyde release itself, were not influenced by the contact with the oral cavity.

Because the surface of the oral cavity causes only a small retention of aldehydes during a short rinsing time, it is reasonable to assume that the surfaces of the pharynx and nasal cavity will not cause large aldehyde retention. Linforth and Taylor reported similar results (Linforth *ea*, '00). In their investigation of the persistence in the breath of a range of aroma compounds, a relatively low value was found for aldehydes.

In the protocol used in this study, neither saliva nor mouth coating can account for the large difference in interactions between aldehydes and whey protein under mouth conditions compared to static headspace conditions. Apparently, the difference is caused by another factor.

Effect of in-mouth conditions on aldehyde-whey protein interactions. An important difference between the *in vivo* and static headspace measurements is the way the measurements are performed. Static headspace data are the result of an equilibrium headspace concentration,

while *in vivo* aroma release data are obtained from a highly dynamic nonequilibrium release during a short time interval. Linforth and co-workers (Linforth *ea*, '02) have shown that the mass transfer from aqueous solutions in the mouth into exhaled air was a major factor affecting the actual released amount of aroma compounds.

To fully understand the results of the present study, a closer look into the protocol used for measurement of *in vivo* aroma release is necessary. The sample is swallowed, immediately followed by an exhalation. After swallowing, most of the sample disappears via the throat into the esophagus. However, a small fraction remains as a thin film, coating the inner wall of the pharynx. This process was visualised by videofluoroscopy and real time magnetic resonance imaging (Buettner *ea*, '01). The aroma compounds present in this thin film are exposed to a relatively large air flow (1.5–2.0 L/min) during the subsequent exhalation. To illustrate this effect, a panelist swallowed 15 mL of 5 ppm octanal solution, and exhaled continuously for 15 s afterward. The release of octanal and acetone as breath indicator is shown in Figure 3.4. Octanal is released in a sharp peak at the beginning of the exhalation. Apparently, the octanal present in the thin film coating the pharynx is exhausted quickly. In this respect, the *in vivo* release process clearly differs from the release during SHGC and mouth model measurements. SHGC measurements involve the analysis of a small part of the equilibrated headspace air. In the mouth model system, a relatively large volume of aroma solution is exposed to a relatively small air flow, compared to the *in vivo* situation. Our hypothesis for the *in vivo* aroma release process is that not only will all free aldehydes in the film be released into the exhaled air, but also that all the aldehydes that are reversibly bound to the whey protein will release. This would explain why the presence of whey protein does not cause retention of aldehydes under *in vivo* conditions. The results also agree with the model of Harrison and Hills (Harrison *ea*, '97b), where fast partitioning between bound and unbound states of aroma compounds plays an important role in the similar release of aroma compounds from samples with and without aroma-binding macromolecules during initial stages of release. More experiments are currently being conducted in our laboratory to test this hypothesis.

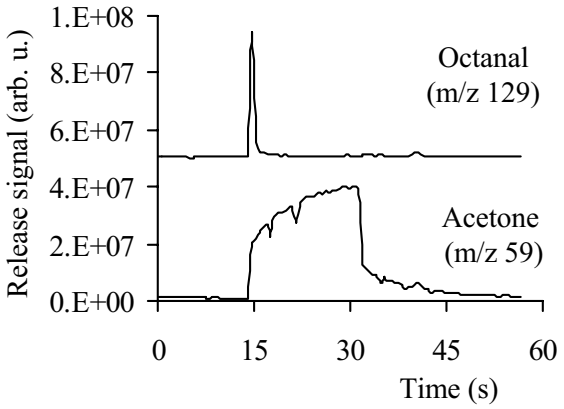

Figure 3.4. Raw data example of *in vivo* release of octanal during 15 s of continuous exhalation, directly after swallowing 15 mL of a 5 ppm octanal solution. Acetone is displayed as a breath indicator.

Chapter 4

A new device to simulate swallowing and *in vivo* aroma release in the throat from liquid and semi-liquid food systems

This chapter is submitted for publication by the authors K.G.C. Weel, A.E.M. Boelrijk, J.J. Burger, M. Verschueren, H. Gruppen, A.G.J. Voragen, and G. Smit.

Abstract

This chapter describes a novel device to simulate *in vivo* aroma release from liquids. This artificial throat simulates the act of swallowing followed by exhalation and shows aroma release curves that are similar in shape to *in vivo* release profiles. Liquids are poured down a tube, and a thin liquid film remains at the inner wall of the tube. Subsequently, aroma compounds release from this film into a stream of air flowing through this tube, which is analysed by MS-Nose analysis. The effects of air flow rate, contact time with glass surface, presence of saliva and addition of whey protein, as well as volume, concentration, temperature, and viscosity of the liquid have been studied and compared with aroma release measurements *in vivo*. A high level of agreement was found. These results confirm the importance of swallowing for aroma release of liquids, as mentioned in literature, and the usefulness of the new mimicking device.

Introduction

In the last decades, a lot of research has been dedicated to the development of model systems that should mimic the release of volatiles from food products during consumption. The advantages of having such devices are the possibility to save money and time spent on working with panelists, the omitting of palatability and safety matters, and a high reproducibility.
All model systems developed until now are designed to mimic the aroma release in the mouth and are based on the shared principle of a certain amount of foodstuff (usually in liquid form) containing aroma compounds and other ingredients of interest (polysaccharides, proteins, lipids) that is put into a vessel and stirred or shaken in different ways (and heated to 37ºC, in most cases) (Lee, '86, Van Ruth *ea*, '94, Naßl *ea*, '95, Roberts *ea*, '95, Van Ruth *ea*, '95, Elmore *ea*, '96, Roberts *ea*, '96, Bakker *ea*, '98, Springett *ea*, '99, Van Ruth *ea*, '00a, Van Ruth *ea*, '00b, Deibler *ea*, '01, Banavara *ea*, '02, Rabe *ea*, '02). Air is sampled from the headspace or nitrogen is purged through the liquid phase. The volatile compounds present in the stream of gas released from the model system are analysed in-line or batch-wise by a direct mass spectrometry (MS) technique or by trapping the compounds on absorbing materials followed by gas chromatography-mass spectrometry (GC-MS) (Lee, '86, Van Ruth *ea*, '94, Naßl *ea*, '95, Roberts *ea*, '95, Elmore *ea*, '96, Bakker *ea*, '98, Springett *ea*, '99, Rabe *ea*, '02). All kinds of apparatus-related and product-related parameters have been studied using these model systems, which has increased insight in the process of volatile release in general (Van Ruth *ea*, '95, Roberts *ea*, '96, Van Ruth *ea*, '00a, Van Ruth *ea*, '00b, Deibler *ea*, '01, Banavara *ea*, '02).
For solid products, when structural breakdown in the mouth is involved, the aroma release can be reproducibly studied in Van Ruth's model mouth system (Van Ruth *ea*, '94) or the retronasal aroma simulator (RAS) (Roberts *ea*, '95). A computerised apparatus developed to simulate dynamic aroma release from liquids by Rabe and co-workers (Rabe *ea*, '02), focuses on simulation of events taking place in the mouth. The aroma release from liquid products, which are swallowed directly after intake, is determined by swallowing rather than by the preceding oral processing. This concept was first mentioned by De Roos and Wolfswinkel in 1994 (De Roos *ea*, '94) and again later, in 1996, when Land introduced the 'swallow-breath' principle, which is 5-15 ml of air that is pushed through the nose immediately after swallowing and which had been in close masticatory contact with the food or drink in the mouth (Land, '96). This plug of air is important for aroma perception. Since the emergence of direct MS methods for sufficiently sensitive and fast real-time analysis of volatiles in human breath (Taylor *ea*, '96, Lindinger *ea*, '98a, Taylor *ea*, '00), it has become clear that the highest *in vivo* aroma release signal for liquids is generally found in the first exhalation after swallowing (Linforth *ea*, '00). It was shown that no gas is transferred from the oral cavity to the nasal cavity as long as no opening of the barrier formed by the tongue and the soft palate occurs by swallowing or by vigorous tongue and mouth movements (Buettner *ea*, '00a). The existence of an anatomical barrier during the swallowing

process has been proven by videofluoroscopy and real-time magnetic resonance imaging (Buettner *ea*, '01). A protocol was developed in our laboratory, that increased reproducibility of *in vivo* aroma release measurements of liquids by control of breathing and swallowing and thus reconfirming the importance of swallowing for release (Weel *ea*, '03b). In a recent study, Hogson and co-workers (Hogson *ea*, '03) combined synchronised measurements of mastication, swallowing, breath flow and aroma release and demonstrated that an average chew displaces a volume of 26 mL of air from the oral cavity into the throat (which is especially relevant to the situation when the food is not swallowed immediately). Nasal air flow and associated aroma release were not detected during swallowing, but air flow and release were obvious directly after the swallowing event. The volume of the retronasal pathway was calculated to be 48 or 72 mL (depending on the type of calculation).

After swallowing, the majority of the sample disappears into the esophagus, but a thin layer of the liquid sample remains on the surface of the pharynx. Buettner and co-workers (Buettner *ea*, '02) visualised the formation of such a coating by videofluoroscopy, when a volunteer swallowed viscous oral contrast medium. During the exhalation following the swallowing, a steep gradient in aroma concentration exists between the thin liquid layer on the surface of the pharynx and the exhaled air that passes over this surface. It has been suggested that the major part of the aroma compounds present in this thin liquid layer coating the throat will release almost instantaneously during this exhalation, due to the large surface area : volume ratio (Weel *ea*, '03a). A model system that aims to simulate the dynamic conditions of *in vivo* aroma release of liquids should therefore be able to simulate this process. Chewing induced mixing and temperature changes of the sample during oral processing, are only marginally important for aroma release from liquids, because of the short residence time in the mouth and because the release is determined by the events taking place after swallowing. Thus, a model system is needed in which a thin layer of liquid is exposed to a relatively large flow rate, in order to approach the dynamic *in vivo* release conditions. This chapter describes the development, characterisation and comparison with *in vivo* release of such an artificial throat.

Materials and methods

Materials. Citral (3,7-dimethyl-2,6-octandienal) and geranyl acetate (ester of acetic acid and a mixture of 3,7-dimethyl-2-trans, 6-octadien-1-ol [geranyl acetate] and 3,7-dimethyl-2-cis, 6-octadien-1-ol [neryl acetate]) were obtained from Quest International B.V. (Naarden, The Netherlands). Butanal was obtained from Fluka Chemie (Buchs, Switzerland). Hexanal, octanal and nonanal were obtained from Merck (Darmstadt, Germany). A buffer was prepared with NaH_2PO_4. The pH was set with HCl. κ-Carrageenan (extracted from Irish moss, batch 398961/1 40202) was obtained from Fluka Chemie (Buchs, Switzerland). All solutions were prepared using demineralised water.

Whey protein isolate (Bipro, JE 153-9-420) was obtained from Davisco Foods International Inc., Le Sueur, Minnesota. Specifications were as follows: pH 7.23; non-protein nitrogen 0.17%; ash 1.8%; lactose 0.34%; calcium 0.13%; fat 0.57%; total protein 93.39% (N × 6.38); α-lactalbumin 12.6%; bovine serum albumin 3.2%; immunoglobulin G 5.2%; β-lactoglobulin A 33.2% and β-lactoglobulin B 37.1%.

Artificial saliva was prepared in demineralised water according to Van Ruth and co-workers (24) and consisted of $NaHCO_3$ (5.2 g/L), K_2HPO_4 (1.04 g/L), NaCl (0.88 g/L), KCl (0.24 g/L), $CaCl_2 \cdot 2H_2O$ (0.44 g/L) and 2.16 g/L porcine stomach mucin (Sigma, Steinheim, Germany). No α-amylase was added. The pH was set to 7.0 with HCl.

Preparation of solutions. Aroma solutions were prepared in two mixtures, one with a high concentration for *in vivo* measurements, and another with low concentration for artificial throat, calibration and static headspace measurements (Table 4.1). The use of a high and a low aroma concentration assured that the MS-Nose measurements had a high signal-to-noise level and linearity of response in all systems for each aroma compound. Ethanol was used to dissolve the compounds and was present at 0.01% (w/w) in all solutions used throughout the study. These aroma concentrations were used in aqueous solutions with added whey protein and/or buffer, or carrageenan, or without any further additive, dependent on the experiment.

Table 4.1. Applied aroma concentrations and analysis parameters of the compounds analysed

	Concentration *In vivo* (ppm)	Concentration in artificial throat (ppm)	Ion mass (m/z)	Cone Voltage (V)
Mixture 1				
Butanal	2	0.1	73.0	21
Hexanal	2	0.1	101.0	21
Octanal	2	0.1	129.0	21
Geranyl acetate	1	0.1	137.0	20
Mixture 2				
Diacetyl	25	1	86.6	19
Ethyl butanoate	0.1	0.01	116.7	20
Nonanal	2	0.1	143.0	21
Citral	5	1	153.0	20

Whey protein solutions consisted of 50 mM $NaH_2PO_4 \cdot HCl$ buffer (pH 7) with 3% (w/v) whey protein isolate. The aroma solutions without protein, to which the protein solutions were directly compared, also contained the same buffer. In order to dissolve the whey protein, the solutions were stirred for at least 3 h before addition of aroma.

Carrageenan solutions of 0.25; 0.50; and 0.75% (w/v) were heated at 70ºC for 30 min and first cooled down to 50ºC before aroma was added. All solutions were stored in sealed containers at 4ºC until use.

Measurement of aroma release with the MS-Nose. Aroma concentrations in air coming from the artificial throat, from the breath of panelists, from static headspace flasks, or from the calibration were monitored by on-line sampling by an Atmospheric Pressure Chemical Ionisation Gas Phase Analyser (APCI-GPA) attached to a VG Quattro II mass spectrometer (MS-Nose; Micromass UK Ltd., Manchester, UK). The air was sampled (75 mL/min) through a capillary tube (0.53 mm internal diameter, heated to 100°C). Source and probe temperature were 80°C. The compounds were ionised by a 3.0 kV discharge and monitored in selected ion mode (0.08 s dwell on each ion), in two independent sets. See Table 4.1 for m/z values and cone voltages used. For *in vivo* experiments acetone release was measured in both sets at 58.8 m/z (19 V) as an indicator of the panelists' breathing pattern. The chosen m/z values were unique for each compound. There was no difference in response between an aroma compound dissolved in a mixture and dissolved apart. None of the other ingredients used gave any signal at these masses.

Artificial throat. *In vivo* aroma release was simulated by an artificial throat (Figure 4.1). This device consists of vertical glass tubing (internal diameter 12 mm). The MS-Nose sampling capillary samples air from the top end of the tube. An essential part of the system is a 3 mm thick tubing of Viton rubber (11 mm internal diameter, DuPont Dow Elastomers L.L.C., Wilmington, DE) in the middle of the glass tubing that can be closed and opened by a clamp. Above this rubber section several liquids can be added simultaneously or subsequently from syringes through capillaries, ending in the glass tubing. A water mantle surrounds the glass tubing and is coupled to a waterbath, equipped with a thermostat (set to 37°C). An air inlet, which is pointing upwards, is located below the water mantle. The liquid can leave the system from the down end of the glass tubing.

At the start of the experiment, the clamp is closed and 4 mL of liquid is loaded above the clamp. When using human or artificial saliva, 2 mL saliva or water is poured in first, followed by 2 mL aroma solution. After a contact time of 10 s the clamp is opened. The liquid pours down along the glass tubing. The wetted part is 30 cm (5 cm rubber and 25 cm glass). A thin liquid layer remains on the surface. Ten seconds after opening of the clamp, a stream of air (1.0 L/min) enters the tube and flows upwards, where it can freely flow out of the system, while a small part of the air is sampled by the MS-Nose.

The inner glass surface was hydrophilised, by rinsing the surface with sulphuric acid (95-98% w/w) and subsequent rinsing with tap water. The glass kept its good wetting properties for over 50 measurements. The area below the release curve was integrated and used for calculation of the total amount of aroma released. Measurements were done in 5 replicates.

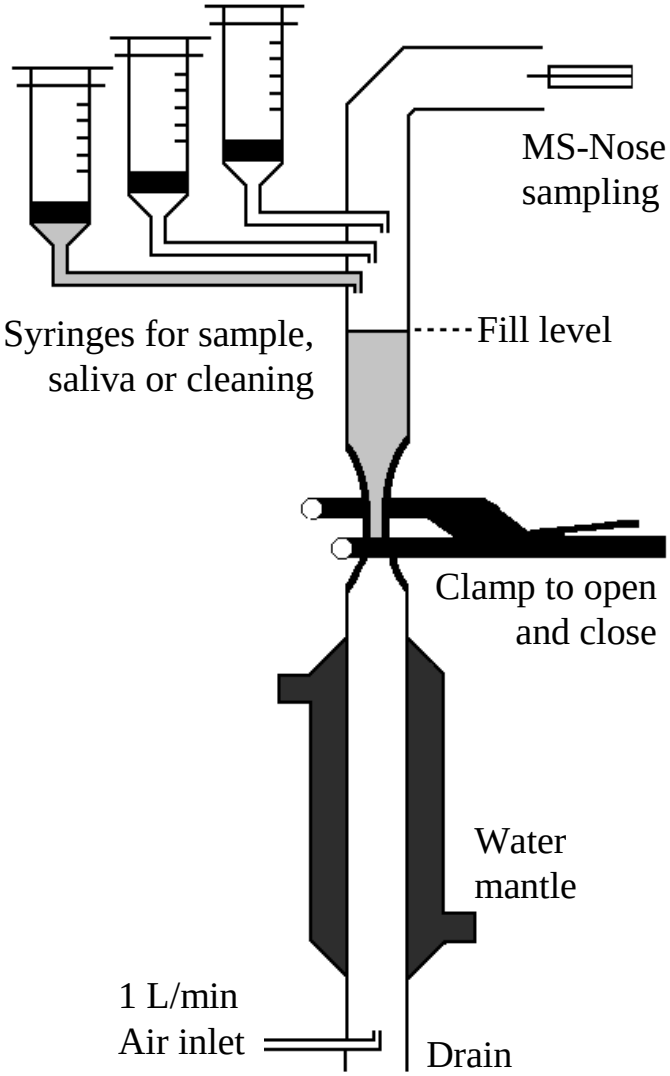

Figure 4.1. Schematic overview of the artificial throat configuration.

Measurement of *in vivo* aroma release in exhaled air. Aroma release measurements in exhaled air were conducted using the MS-Nose according to a strict protocol, developed for liquid samples (Weel *ea*, '03b) to control chewing, breathing, and swallowing, in order to reduce experimental error. The area under the first exhalation peak after swallowing was integrated and used for calculation of the amount of aroma released in this breath. The panelists were trained and considered to be sufficiently trained when their averaged relative standard deviation of the area for all samples of a training session did not exceed 15%. Two panelists assessed all samples in 5 replicates.

Static headspace measurements. Static headspace measurements were performed by inserting the sampling tip of the MS-Nose through a hole drilled in the cap of a 100 mL flask containing 20 mL solution. Aliquots (10 mL) of human saliva (or water) and aroma solution were added, equilibrated for 10 minutes at ambient temperature before analysis in duplicate. This resulted in a plateau value from which an equilibrium aroma concentration in the sampled air could be calculated.

Calibration of aroma release measurements. MS-Nose measurements were calibrated in order to quantify the results *in vivo*, in the artificial throat, and static headspace. An aliquot of 1 mL of aroma solution with a known concentration (Table 4.1) was put in a glass vessel, which was connected to the MS-Nose sampling capillary. A magnetic stirrer mixed the liquid continuously. The headspace above this solution was continuously sampled into the MS-Nose source at a constant flow speed of 60 mL/min. The removed air was replaced by fresh air through a secondary opening. The release signal increased and subsequently returned to the baseline, indicating that the entire amount of aroma initially present in the solution, had released. The area under this curve corresponds to the total amount of aroma present in the solution. Compounds were measured in two replicates and at two concentrations. The concentration of the aroma compound in the air Cg (μg/L) can be calculated from the release signal in au (arbitrary units), according to eq. 4.1.

$$C_g = \frac{M}{A \times F} \times signal \qquad (4.1)$$

in which M is the amount of aroma compound present in the solution (μg), A is the area under the calibration curve (au × min), and F is the flow of sampled air (L/min).

Breath flow measurement. Breath flow of panelists was measured using a ALNOR compuflow anemometer (Shoreview, Minnesota), giving air speed in m/s, which was recalculated to a flow rate (L/min) using the diameter of the tube in the nose (5 mm).

Viscosity measurements. Viscosity measurements were carried out with a Haake Rotovisco RV20 rotational viscometer (ThermoInstruments, Breda, The Netherlands). The concentric cylinder measuring system M5, equipped with the MV1 bob, was used. The radii of the bob and cup were 20.0 mm and 21.5 mm, respectively. The sample was subjected to a linearly increasing shear rate from 0 to 400 s^{-1} in 4 min and returning to 0 in 4 min, while the shear stress was measured. The dynamic viscosities quoted in this study were taken at a shear rate of 300 s^{-1}.

Measurement of layer thickness in artificial throat. The amount of liquid remaining in the artificial throat was determined by weighing the glass and rubber parts before (dry) and 10 s after pouring a sample through. The layer thickness could then be calculated in combination with the internal diameter and length of the tube.

Results

Effect of flow rate. In Figure 4.2 typical examples are given of release curves obtained by the artificial throat and by *in vivo* release measurements. In the artificial throat a solution containing 0.1 ppm octanal was sampled at different air flow rates. Panelists were subjected to solutions of 2 ppm octanal. The panelists breathed shallowly, normally and deeply, to obtain different flow rates. The flow rates differed considerably between panelists.

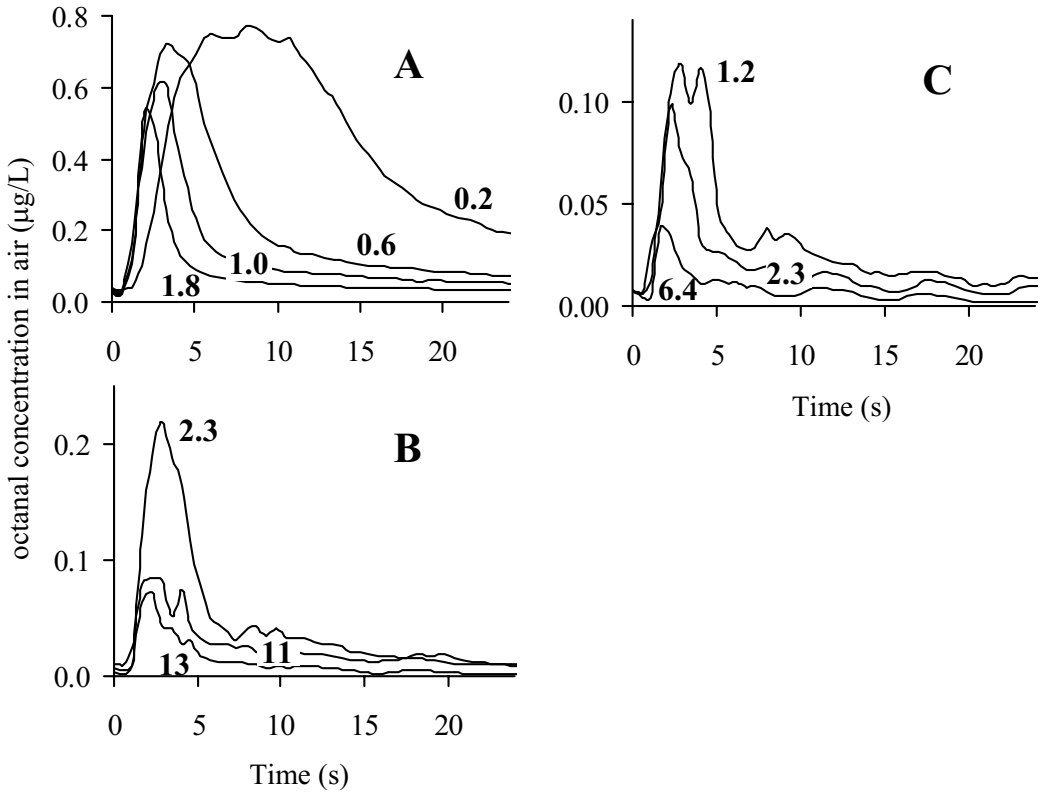

Figure 4.2. Release of octanal (0,1 ppm in the artificial throat, 2 ppm *in vivo*) from an aqueous solution in either the artificial throat (A) or *in vivo* for panelists 1 and 2 (B and C, respectively), at different air flow rates (indicated in the graph in L/min). Each curve is the average of five replicates.

An increase in flow rate resu1ts in a decrease in the released peak area, both *in vivo* and in the artificial throat. This effect is observed for all aroma compounds studied. At a flow rate of 1.0 to 1.2 L/min the peak width at 50% height in the artificial throat is typically around 5 seconds. This period is comparable to *in vivo* aroma release during shallow breathing (3 seconds in our *in vivo* protocol) and indicates the potential of the artificial throat to simulate the dynamics of *in vivo* aroma release of liquid foods. All further artificial throat measurements were performed at 1.0 L/min. This value fits into the flow rates measured in the present study for shallow breathing *in vivo* (1-2 L/min).

Effect of sample volume. The sample volume used in the artificial throat was varied between 2 and 4 mL. A different volume did not induce a significant difference in released peak area. The same result was found for *in vivo* measurements. A four-fold increase of sample volume (from 5 to 20 mL), yielded only a less than 2-fold increase in *in vivo* released amount of aroma (data not shown). The amounts used differ between both systems for practical reasons, but the trends are the same.

Effect of aroma concentration. The aroma concentrations of the solutions show a high correlation with the released amount both *in vivo* and in the artificial throat. Linear relationships with high correlation coefficients were found for all compounds, over an aroma concentration

range of at least 2 orders of magnitude. An example of this is given for ethyl butanoate in Figure 4.3.

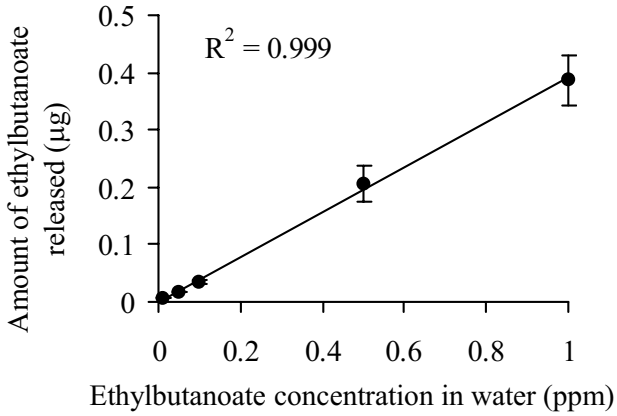

Figure 4.3. Amounts released of ethyl butanoate (µg) from the artificial throat at ethyl butanoate concentrations in the solution ranging from 0.01 to 1 ppm. Error bars represent standard deviations.

Effect of temperature. The temperature of the sample was varied from 5 to 66 °C, but this did not significantly change the release of any of the compounds studied neither *in vivo* nor in the artificial throat (data not shown).

Effect of presence of whey protein. Previously, we have demonstrated that aldehyde retention in solutions containing whey protein isolate is less strong under *in vivo* conditions, compared to static headspace conditions (Weel *ea*, '03a). Here, the effect of presence of whey protein on release of aldehydes has been investigated in the artificial throat as well. The released amount of butanal, hexanal, octanal, and nonanal decreased due to the presence of 3% whey protein with 11%, 14%, 15%, and 20%, respectively.

Effect of contact time. The effect of differences in surface behaviour between the human and the artificial throat can be visualised by changing the time between swallowing and exhalation in both systems. For the artificial throat it is the time between opening of the clamp and application of the air flow. Table 4.2 lists the relative change in amounts released of aroma for *in vivo* and artificial throat measurements due to longer delay time between swallowing and exhalation. Generally, the amount of aroma released drops, as the contact time increases. This is observed *in vivo* with 2 panelists and in the artificial throat. However, the decrease is much stronger *in vivo*, than with the artificial throat.

Table 4.2. Relative change in released amounts of aroma (%) from the artificial throat or *in vivo* with 10 and 30 s between swallowing and exhalation, compared to no time in-between.

	Art. throat		Panelist 1		Panelist 2	
	10 s	30 s	10 s	30 s	10 s	30 s
Diacetyl	-17%	-25%	-26%	-45%	-35%	-64%
Ethyl butanoate	-17%	-26%	-38%	-56%	-20%	-58%
Geranyl acetate	+3%	-8%	-46%	-65%	-41%	-67%
Citral	-14%	-24%	-45%	-70%	-43%	-73%
Butanal	-19%	-15%	-68%	-78%	-57%	-77%
Hexanal	-25%	-26%	-66%	-73%	-54%	-76%
Octanal	-15%	-10%	-70%	-79%	-66%	-81%
Nonanal	-36%	-43%	-63%	-78%	-56%	-88%

Persistence in the breath. The *in vivo* persistence of the aroma compounds in the human breath can play a role in aftertaste. Persistence can be calculated according to the method of Linforth and Taylor (Linforth *ea*, '00). For the persistence in the artificial throat, the peak width at 50% of the peak height was taken. The results are displayed in Table 4.3. A high correlation between panelists was found for their persistence data ($R^2 = 0.91$) for the eight aroma compounds studied. The correlation between the artificial throat and the panelists was reasonable as well (R^2 was 0.70 and 0.82 for panelists 1 and 2, respectively).

Table 4.3. Persistence of aroma compounds in the artificial throat and 2o panelists[1]

Compound	artificial throat	panelist 1	panelist 2
Diacetyl	0.51	0.74	0.83
Ethylbutyrate	0.07	0.27	0.45
Geranyl ac	0.37	0.54	0.56
Citral	0.6	0.52	0.57
Butanal	0.07	0.21	0.21
Hexanal	0.065	0.23	0.18
Octanal	0.068	0.17	0.16
Nonanal	0.072	0.23	0.31

[1] Persistence values in the artificial throat are calculated as the peak width at half height in minutes. The *in vivo* persistence is calculated as the ratio between the peak height of the second and the first peak after swallowing.

Effect of viscosity. The effect of viscosity on aroma release *in vivo* and in the artificial throat was studied by means of a range of aroma solutions with increasing carrageenan concentrations. The carrageenan solutions showed a lower dynamic viscosity at higher shear rates (shear thinning behaviour), but the curves of the increasing and decreasing shear rate closely overlapped, indicating that the solutions were not thixotropic. The results are shown in Figure 4.4 for ethyl

butanoate, diacetyl, and hexanal. An increase in viscosity results in an increase in released amount of aroma in the artificial throat. The other aroma compounds showed similar effects (results not shown).

The artificial throat measurements presented in Figure 4.4 were conducted at a flow rate of 1.0 L/min. Measurements were also done using flow rates of 0.2, 0.6, 1.2, and 1.8 L/min (data not shown). Although the area under the release curve decreased with increasing flow rate (as shown in Figure 4.2), the qualitative effect of differences in viscosity was similar for each flow rate.

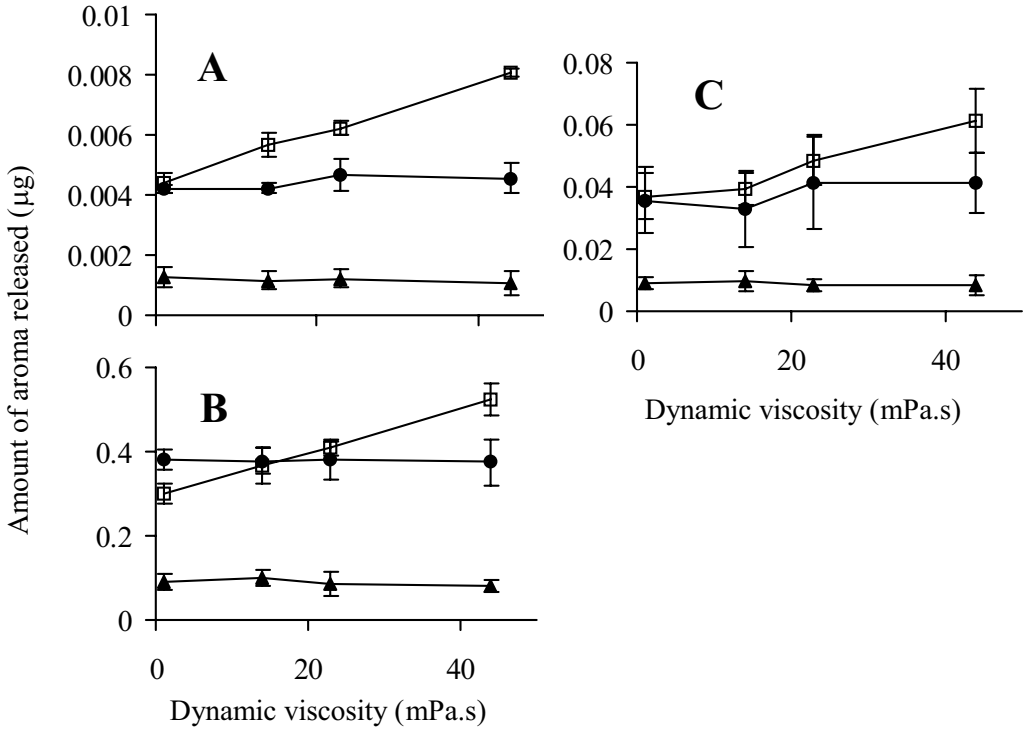

Figure 4.4. Amounts released (µg) of ethyl butanoate (A), diacetyl (B), and hexanal (C) with increasing dynamic viscosity (mPa·s) for the artificial throat (□), panelist 1 (●) and panelist 2 (▲). Error bars represent standard deviations.

The measurements were repeated to investigate the influence of dilution with saliva on aroma release in the artificial throat. Now, 2 mL non-aromatised water or artificial saliva was added first, followed by 2 mL of an aroma solution with the same range of viscosities. Both solutions were poured into the artificial throat, without further external mixing. The results for ethyl butanoate, diacetyl and hexanal with addition of artificial saliva are shown in Figure 4.5. The amount of aroma released increases only at low viscosities. This was also observed for the other aroma compounds analysed (results not shown). These results contrast to the effect of viscosity without addition of water or saliva, which displayed a steady increase within the viscosity range studied. No differences were found between the results obtained with addition of artificial saliva and those with water for all aroma compounds examined (no further results shown, ANOVA, $p = 0.18$).

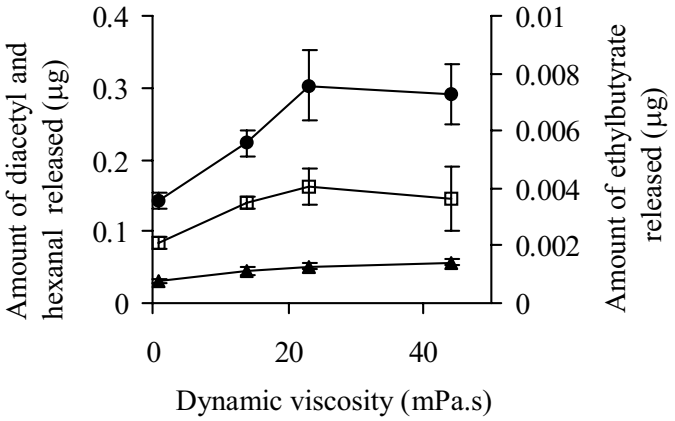

Figure 4.5. Amounts released (µg) of diacetyl (●), hexanal (▲), and ethyl butanoate (□) from 2 mL aroma solutions with increasing viscosity (mPa·s) in the artificial throat, with 2 mL artificial saliva added first. Error bars represent standard deviations. The viscosity on the x-axis reflects the viscosity in the aroma solution before mixing with water or saliva.

Discussion

Aroma release from thin liquid layer. The design of the artificial throat was inspired on the hypothesis that the majority of aroma release from liquids *in vivo* originates from the layer that remains at the inner surface of the human throat after swallowing (Weel *ea*, '03a). The results obtained here are well in line with this hypothesis. The effects of sample volume, aroma concentration, sample temperature, air flow rate, and presence of whey protein, show similar trends in the artificial throat compared to *in vivo*, as will be discussed below.

With respect to the sample volume, the amount of liquid that remains on the surface is only slightly dependent on the amount of liquid passing through, as long as the total amount is much larger than the amount constituting the film at the inner surface. When the aroma concentration is higher, there is proportionally more aroma compound available for release. Linear increase in *in vivo* aroma release with liquids with increasing aroma concentrations was found previously as well (Weel *ea*, '03b).

The sample temperature does not influence the total aroma release, neither *in vivo* nor in the artificial throat, because all aroma present in the thin film will rapidly release, once the exhalation or air flow starts. At equilibrium conditions however, a higher temperature leads to a stronger partitioning of volatiles into the air phase. This has been demonstrated previously for a range of aroma compounds (Roberts *ea*, '95, Deibler *ea*, '99).

A decrease in measured release with increasing flow rate is also expected, because only a part of the air exhaled either by the artificial throat or the panelists, is sampled. The aroma concentration is determined in the part sampled. When the flow rate is higher, the aroma compounds are more diluted, resulting in a lower concentration. The total amount of aroma released does not change within the range of flow rates studied, because all aroma compounds present will release. The decrease in peak width at higher flow rate indicates that the reservoir of aroma molecules present

in the thin liquid film (coating the inner surface of both the human throat and the artificial throat) is exhausted faster.

Aldehyde-protein interactions and their effect on aldehyde release have been investigated previously for static headspace, mouth model, and *in vivo* measurements (Friel *ea*, '01, Van Ruth *ea*, '01, Weel *ea*, '03a). Considerable reversible interactions were found during static headspace measurements (binding up to 90%). These interactions appeared to be much less pronounced under *in vivo* conditions. Given a reversible interaction, it was hypothesised earlier (Weel *ea*, '03a) that after swallowing all aldehydes present in the layer coating the inner throat would release; the free aldehydes as well as those reversibly bound to the protein. This explanation is similar to the "thin layer" hypothesis formulated above. Figure 4.6 gives an indication of the interaction between a homologous range of aldehydes and whey protein in the artificial throat, the mouth model, under *in vivo* and static headspace conditions. From Figure 4.6 it is clear that the *in vivo* results deviate from the results obtained with static headspace and the mouth model. The artificial throat results are much more similar to the *in vivo* results.

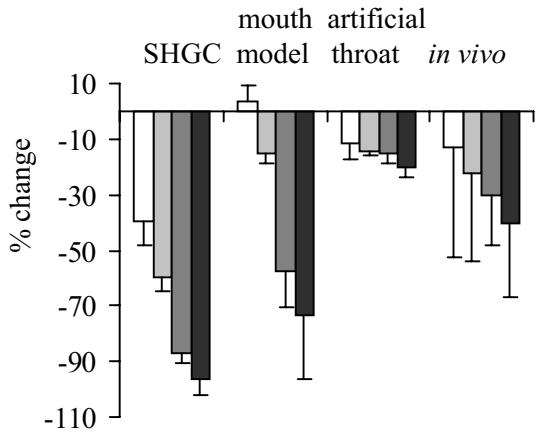

Figure 4.6. Relative change (%) in release of butanal (white bars), hexanal (light gray bars), octanal (dark gray bars), and nonanal (black bars) due to presence of 3% whey protein for different systems (graph contains data from Weel et al (Weel *ea*, '03a)). Error bars represent standard deviations.

The assumption that all aroma present in the thin layer remaining in the human throat after swallowing will release, was verified using the artificial throat. For this purpose, the total amount of aroma released during artificial throat measurements was calculated in two independent ways. The total amount of aroma released (and thus the amount of aroma initially present in the liquid film) could be calculated from the area integrated below the release curve. The other approach was to weigh the amount of liquid in the film at the glass wall and the rubber, and to combine it with the known aroma concentration in the solution. Results of both calculations are shown in Table 4.4.

Table 4.4. Total released amount of aroma during one artificial throat run (µg). The amount is calculated based on either the integrated release peak area, or on the weight of the film remaining on the glass wall.

Compound	Based on area integrated	Based on film weight
Diacetyl	0.36	0.33
Ethylbutyrate	0.0054	0.0033
Geranyl acetate	0.041	0.033
Citral	0.32	0.33
Butanal	0.039	0.033
Hexanal	0.047	0.033
Octanal	0.047	0.033
Nonanal	0.041	0.033

The amounts calculated based on the two methods are both in the same order of magnitude and highly correlated ($R^2 = 0.99$). Conform our hypothesis, the bulk of the aroma compounds present in the liquid film in the artificial throat has indeed released within 10 seconds into the air flowing along the liquid film. Similarly to these results, the aroma present in the liquid layer in the human throat after swallowing will probably release rapidly as well. Based on the weight of the liquid film and the dimensions of the artificial throat, the layer thickness of the film was calculated to be 0.1 mm for the rubber part and 0.015 mm for the glass part.

Contact with throat surface. Gravity driven clearance of the liquid layer on the glass surface is the major factor responsible for the decrease in release in the artificial throat at longer time intervals between opening of the clamp and application of the air flow (Table 4.2). *In vivo*, there are additional processes involved, explaining the stronger loss of aroma compounds there. These are fluid excretion of the mucosa and partitioning of the aroma compounds into the mucus (Buettner *ea*, '02, Weel *ea*, '03a). It was shown previously that the extent of the interaction between aroma compounds and mucosa depends heavily on the contact time. When aldehyde solutions were rinsed in the mouth for 3 s and subsequently expectorated, 1% had released into the air and 7% of the aldehydes could not be retrieved, and were probably lost to the inside coating of the mouth (Weel *ea*, '03a). Buettner and co-workers (Buettner *ea*, '02) reported a decrease of 30-40% upon 10 min rinsing of pyrazine solutions in the mouth. The measurements presented do not reveal the absolute amount lost due to interaction with the inner surface of the human throat during the normal residence time of the film in the throat. Nevertheless, these results clearly show that the inner surface of the human throat is not as inert as the glass surface of the artificial throat.

Persistence data of artificial throat and *in vivo* measurements correlated well, despite the different calculation methods. Especially diacetyl showed a high persistence in both systems. Due to its high hydrophilicity this compound remains in the liquid layer forming a reservoir for continued release. Linforth and Taylor (Linforth *ea*, '00) have shown that hydrophobicity and vapour

pressure are the main predictors for *in vivo* persistence. In our present study, a high persistence is also observed in the artificial throat for diacetyl, indicating that presence of mucus is no prerequisite for high persistence, and thus stressing the importance of the liquid layer for persistence.

Sample viscosity and dilution with saliva. The effect of viscosity was studied with and without addition of saliva. When the viscosity of the samples increases, a thicker layer remains at the inner surface of the tube. This thicker layer will contain proportionally more aroma compounds, and consequently the total amount released will be higher. Determining the increase in weight of the artificial throat after pouring the samples through revealed that the amount of liquid remaining at the inner surface of the artificial throat 10 s after opening the clamp had increased 5-fold when comparing solutions with a viscosity of 1 and 44 mPa·s (0.25 g and 1.21 g, respectively). The released amount of aroma however, increased less than 5-fold (Figure 4.4). This can be explained as follows. The time scale of diffusion (τ), is given by eq. 4.2,

$$\tau = \frac{L_D^{\,2}}{D} \qquad (4.2)$$

where L_D is the layer thickness (m), and D is the diffusion coefficient (m^2/s). The value of D ranges from 10^{-10} to 10^{-9} m^2/s for small molecules in water, as derived from the Hayduk-Minhas correlation (Reid *ea*, '87). Considering aqueous solutions, the time scale of diffusion is 0.5 s for the liquid layer on the glass tube (0.015 mm) and 20 s for the liquid on the rubber part (0.1 mm). When the amount of liquid remaining at the inner surface of the artificial throat is five times more viscous, the layer is approximately five times thicker as well (the layer is very thin compared to the diameter of the tube). The time scale of diffusion is 2.5 s and 500 s, for the liquid layers on the glass tube and on the rubber part, respectively. Consequently, a prolonged tailing release is obtained. However, this is not incorporated in the interpretation, leading to the differences observed.

In contrast to the artificial throat results, *in vivo* measurements showed no effect of viscosity on the amount of aroma released. This can be caused by differences in swallowing mechanism and by dilution with saliva. Swallowing in the artificial throat is driven by gravity, while *in vivo* the liquid is forced downwards by pharyngeal peristalsis. It seems reasonable to assume that the film formed *in vivo* will therefore be different. Future versions of the artificial throat should take peristalsis effects into account.

Dilution with saliva could play a role as well. When saliva or water is added to the artificial throat, the increase in release with higher viscosity levels off. In the measurements corresponding to the results in Figure 4.5, the viscous solution is injected above an equal amount of water or saliva before the clamp is opened. This situation lasts for 10 s but no stirring or mixing is imposed. Therefore, in case of the more viscous solution, the mixing is limited, resulting in an inhomogeneous distribution of aroma throughout the sample. Improper mixing results in less aroma on the wall, after the sample was poured down. Dilution always occurs under *in vivo* conditions, and this could also explain why there is no effect of viscosity *in vivo*. *In vivo* aroma

release measurements have demonstrated before that viscosity does not influence aroma release (Cook *ea*, '03a).

No difference was found between addition of artificial saliva and water. Based on previous work, reversible interactions between aroma compounds and saliva could be expected in static headspace measurements (Friel *ea*, '01, Van Ruth *ea*, '01, Weel *ea*, '03a), but as was shown for the effect of presence of whey protein (Figure 4.6), reversible interactions are of minor importance in the artificial throat. This suggests that the role of saliva is dilution, rather than aroma binding, under these dynamic conditions.

Conclusions

The newly developed artificial throat successfully simulates aroma release from liquids under *in vivo* conditions. Artificial throat and *in vivo* results correlate well. The device can be used to increase understanding of *in vivo* aroma release. Aroma release of beverages is determined by the principle of total exhaustion of aroma compounds present in a thin liquid layer, both *in vivo* and in the artificial throat.

Chapter 5

The effect of emulsion properties on release of ester compounds under static headspace, *in vivo* and artificial throat conditions in relation to sensory intensity

This chapter is submitted for publication by the authors K.G.C. Weel, A.E.M. Boelrijk, J.J. Burger, M.A. Jacobs, H. Gruppen, A.G.J. Voragen, and G. Smit.

Abstract

The effects of oil content and droplet size distributions of dilute oil-in-water emulsions on release of 4 esters with different hydrophobicities were studied under *in vivo*, static headspace, and artificial throat conditions. The effect of oil content on orthonasal and retronasal perceived intensity of ethyl hexanoate was studied using a 7-person panel. With increasing oil content and with a higher hydrophobicity of the aroma compound, a stronger decrease in aroma release was found. This effect was stronger under static headspace conditions than under *in vivo* and artificial throat conditions, and the sensory intensity of ethyl hexanoate was perceived stronger orthonasally than retronasally. The lowest effective oil content was determined for all systems. Of the compounds tested, droplet size distribution only influenced the *in vivo* release of geranyl acetate. The artificial throat results correlated well with *in vivo* release, giving support to the assumption that a thin layer of liquid remaining in the throat after swallowing determines aroma release.

Introduction

Oils are generally recognised as the non-volatile food ingredient with the largest impact on aroma release when compared to proteins and polysaccharides. Understanding the impact of oil on aroma release is valuable for the optimisation of increasingly popular low-fat products (Overbosch *ea*, '91, De Roos, '97). Changes in oil content affect aroma release profiles, but also change appearance and texture of the product. Reduction of the oil content results in a higher aroma release and perception and a lower persistence of the aroma compound, depending on its hydrophobicity (Brauss *ea*, '99). The flavour balance of an aroma mixture will be disturbed when the oil content is changed, given the variance in hydrophobicity of aroma compounds. The oil phase is a potential sink for hydrophobic molecules. Knowledge of aroma release from foods containing oils, could therefore be applied to mask off-flavours (McGorrin *ea*, '94).

Predicting release and perception of aroma compounds from food products that contain an oil phase has been the goal of several mathematical (McNulty, '87, De Roos *ea*, '94, Harrison *ea*, '97a, Harrison *ea*, '97c) and empirical (Carey *ea*, '02) models. These studies demonstrated that the oil content of an emulsion and the hydrophobicity of the aroma compounds are key factors for predicting the release. Several other studies have also indicated this, using either analytical (Haahr *ea*, '00, Doyen *ea*, '01, Carey *ea*, '02, Van Ruth *ea*, '02a, Malone *ea*, '03) or sensorial methods (Miettinen *ea*, '03), or using both (Roberts *ea*, '03a). Some authors (De Roos *ea*, '94, Doyen *ea*, '01, Malone *ea*, '03, Roberts *ea*, '03a) have compared the effect of oil content in liquid emulsions on *in vivo* aroma release or on sensory aroma perception with static headspace measurements. These studies have shown that the effect of oil content on *in vivo* aroma release or perception is smaller than expected from equilibrium headspace studies.

Recently, an artificial throat system has been developed that simulates *in vivo* aroma release (Weel *ea*, submitted). A thin liquid layer of product is formed in the human throat upon swallowing a liquid sample. Subsequently, a relatively large flow of exhaled air extracts aroma molecules from this thin layer into the breath. The results obtained with oil-free products correlated well with *in vivo* measurements of liquid samples (Weel *ea*, submitted). The current study aims at explaining why the effect of oil content on *in vivo* aroma release or perception is smaller than expected from equilibrium headspace studies, using this artificial throat.

To our knowledge, no detailed information about the minimal effective oil content under *in vivo* conditions is available, despite the general acceptance of the importance of oil for aroma release. Carey and co-workers (Carey *ea*, '02) have shown a significant effect of 0.025% (w/w) C8 triglyceride oil on release in static headspace experiments, but no systematic variation in oil content has been performed for *in vivo* aroma release measurements.

Oil droplet size distribution is another well-studied emulsion property possibly influencing aroma release. However, contradictory results have been reported. For static headspace, Carey and co-workers (Carey *ea*, '02) found no effect, but an increased aroma retention with larger droplet

diameters was reported by Van Ruth and co-workers (Van Ruth *ea*, '02a) . In another study, Van Ruth and co-workers reported an increase in aroma release with an increase in droplet diameter under the dynamic conditions of the artificial mouth (Van Ruth *ea*, '02b). Rabe and co-workers found no effect of droplet diameter on aroma release, under the dynamic conditions of their model system (Rabe *ea*, '03). In order to clarify this matter, we investigated the effect of three different droplet size distributions, under *in vivo* conditions, in the artificial throat and in static headspace experiments.

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

Materials and methods

Materials. MCT oil (medium chain triglycerides, mainly consisting of octanoic and decanoic acid), gum arabic, and geranyl acetate (ester of acetic acid and a mixture of 3,7-dimethyl-2-trans, 6-octadien-1-ol [geranyl acetate] and 3,7-dimethyl-2-cis, 6-octadien-1-ol [neryl acetate]) were provided by Quest International (Naarden, The Netherlands). Ethyl acetate and ethyl butanoate were obtained from Fluka Chemie (Buchs, Switzerland). Sigma (Zwijndrecht, The Netherlands) supplied ethyl hexanoate. Ethanol (100%) was purchased from J.T. Baker (Deventer, The Netherlands). Citric acid was obtained from Analar (Poole, U.K.). Table 5.1 presents air-water and oil-water partition coefficients (P_{aw} and P_{ow}, respectively) and boiling points (Bp) of the ester compounds used. P_{ow} values of the esters used were calculated from their molecular structure by the logP software module of ACD/Labs (Toronto, Canada).

All percentages given in this chapter are based on weight/weight ratio.

Table 5.1. Physicochemical properties of the ester compounds

	P_{aw} (-)[1]	P_{ow} (-)	Bp (ºC)
Ethyl acetate	0.00628	5.4	77.2
Ethyl butanoate	0.0146	59	121
Ethyl hexanoate	0.018	676	168
Geranyl acetate	0.0037	12882	138

[1] obtained from (Sander, '99)

Sample preparation. MCT oil was slowly added to a final concentration of 5% to an aqueous solution of 5% gum arabic while stirring in a Cyclotron mixer (Kinematica AG, Luzern, Zwitzerland). The mixing continued for 5 min, yielding a crude emulsion. A Rannie 250 homogeniser (APV, Hendrik Ido Ambacht, The Netherlands) processed this crude emulsion at

two regimes: single-valve at 30 bar and double-valve at 230 and 30 bar. The latter emulsion was processed three times at these conditions. This resulted in 3 emulsions with clearly different droplet size distributions: the crude emulsion (A), the low energy homogenised emulsion (B), and high energy homogenised emulsion (C) with mean volume-based ($D_{(4,3)}$) oil droplet diameters of 10, 2.3 and 0.4 µm, and mean surface-based ($D_{(3,2)}$) oil droplet diameters of 4.3, 1.5, and 0.4 µm respectively.

These 3 emulsions were mixed by overnight shaking in a bottle shaker at 4ºC and 50 rpm, with aqueous solutions of ester compounds, water, and gum arabic solution, to produce a range of emulsions with oil contents of 0.01%, 0.02%, 0.05%, 0.10%, 0.20%, 0.50%, 1.0%, and 2.0%. The emulsion with an oil content of 5% was aromatised by adding a small amount of a concentrated solution of aroma compounds in ethanol (10-20 mg). The final concentrations of esters were 0.05 ppm ethyl acetate, 0.01 ppm ethyl butanoate, 0.01 ppm ethyl hexanoate, and 0.1 ppm geranyl acetate, for artificial throat and calibration measurements, and 1, 0.2, 0.2, and 1 ppm, respectively for *in vivo* aroma release and static headspace measurements. All final emulsions contained 0.05% ethanol, 0.125% citric acid, and 1% gum arabic, irrespective of the oil content. The emulsions with 2 and 5% oil however, contained 2 and 5% gum arabic, respectively. All solutions were prepared using demineralised water. The solutions were stored at 4ºC and used within 1 week.

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

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

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

The inner glass surface was hydrophilised, by rinsing the surface with sulphuric acid (95-98%), followed by rinsing abundantly with tap water. The glass kept its good wetting properties for over fifty measurements. These measurements were done in 2 replicates. The areas under the aroma release curves obtained, were determined for the first minute of measurement.

Layer thickness was determined by weighing the separate pieces of the artificial throat (glass and rubber) before and 10 s after pouring a 4-mL aliquot of sample through. The syringe with sample and a collection bin below the artificial throat were also weighed before and after, to obtain a complete mass balance. These measurements were done in 6 replicates.

***In vivo* aroma release measurements.** Aroma release measurements in exhaled air were conducted using the MS-Nose according to a strict consumption protocol. This protocol has been developed for liquid samples (Weel *ea*, '03b) to control mouth movements, breathing, and swallowing, in order to reduce experimental error. The area under the first exhalation peak after swallowing was integrated and used for calculation of the amount of aroma released in this breath. The panelists were trained and considered to be sufficiently trained when their averaged relative standard deviation of the area for all samples of a training session did not exceed 15%. Two panelists assessed all samples in 5 replicates.

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

Droplet size distribution. A Mastersizer X laser diffractometer was used to determine the droplet size distribution (Malvern Instruments LTD, Malvern, U.K.).

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

Sensory rating. The intensity of ethyl hexanoate was tested for six emulsions containing 0%, 0.05%, 0.1%, 0.2%, 0.5% and 1% MCT-oil by a panel consisting of 7 judges, who were all experienced with the procedure of sensory rating. The panel was familiarised to the aroma of ethyl hexanoate. An aliquot of 15 mL emulsion was presented in 20 mL glass bottles. The bottles were covered with aluminium foil to avoid any visual clues. All samples were assessed in duplicate and presented in a different random order for each panelist. All intensities were scored on a 0-10 scale. Fizz software (Biosystemes, Couternon, France) was used to acquire the data. In the orthonasal session the panel evaluated the orthonasal intensity of 2 ppm ethyl hexanoate by sniffing the headspace of the solutions. The orthonasal intensities of aqueous solutions with 0.4 ppm and 1.6 ppm were defined as anchor points in advance as 20% and 80% of the scale, respectively. In the retronasal session the panelists took the sample (containing 10 ppm ethyl hexanoate) in the mouth, while avoiding sniffing. First, they evaluated the fattiness mouthfeel. The fattiness mouthfeel of emulsions with 0.2% and 1% oil were defined as anchor points in advance as 20% and 80% of the scale, respectively. Next, they swallowed the entire sample and judged the retronasal ethyl hexanoate intensity during exhalation. The retronasal intensities of aqueous solutions with 2 ppm and 8 ppm were defined as anchor points in advance as 20% and 80% of the scale, respectively.

Results and discussion

Effect of oil content on aroma release under static headspace and *in vivo* conditions. The partitioning of the esters over air and emulsion phases is shown in Figure 5.1A. The data shown are measured by SHGC and calculated based on the physicochemical properties of the ester compounds (Table 5.1) and the dimensions of the SHGC configuration. The calculated and measured data correlate well, except for geranyl acetate. The software used for estimation of the P_{ow}'s apparently overestimated the value of geranyl acetate. Ester compounds with a higher hydrophobicity are more retained in the emulsion phase. The relatively hydrophilic ethyl acetate is not affected by the presence of oil from 0 to 5%, while the hydrophobic geranyl acetate remains almost completely in the emulsion phase at 5% oil. Ethyl butanoate and ethyl hexanoate show intermediate behaviour.

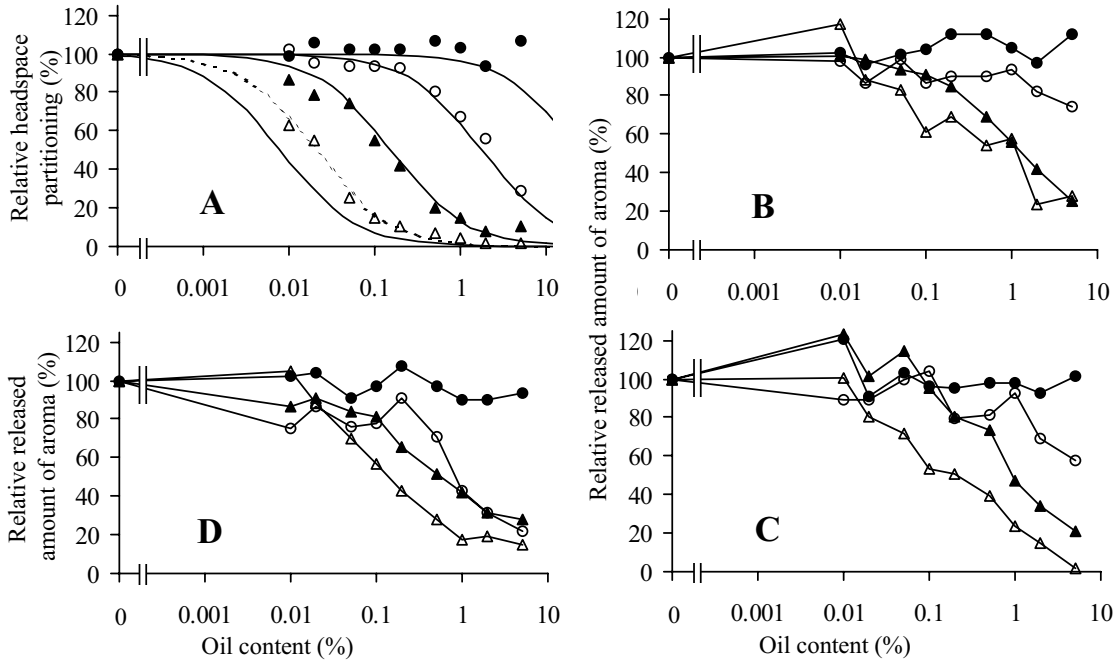

Figure 5.1. Relative SHGC peak area's (%) (A), and relative released amounts (%) *in vivo* for panelists 1 and 2 (B, C, respectively) and in the artificial throat (D) of ethyl acetate (•), ethyl butanoate (○), ethyl hexanoate (▲), and geranyl acetate (Δ) at different oil contents (%, logaritmic scale), compared to partitioning (A) or released amount (B, C, D) at 0% oil. A: Solid lines represent the theoretical expected partitioning. The dotted line represents a fit for the geranyl acetate partitioning, with a modified P_{ow}-value (5000).

Figures 5.1B and 5.1C show the effect of oil content on the amount of 4 esters released in the first breath after swallowing the emulsion for 2 panelists. The data of both panelists correlate well linearly ($R^2 = 0.86$). Similar to the SHGC results, the oil content does not influence the release of ethyl acetate under *in vivo* conditions. Geranyl acetate and ethyl hexanoate show a strong decrease in aroma release at higher oil contents, while ethyl butanoate shows intermediate behaviour. However, the decrease in amount released as a function of oil content under *in vivo* conditions is less strong than the decrease in partitioning under static headspace conditions, especially at low and high oil contents. This can be seen in Figure 5.2A, where the results obtained under *in vivo* conditions have been plotted against the SHGC results. A polynomial trend line was fitted to the data ($R^2 = 0.84$). No theoretical background could be given to support the choice of a polynomial trend line or to specify exactly the expected physical relationship between the results of these static and dynamic processes.

Several other authors observed before that the retention effect of oil on aroma release is smaller under *in vivo* conditions than under static headspace conditions (De Roos *ea*, '94, Doyen *ea*, '01, Malone *ea*, '03, Roberts *ea*, '03a). For example, the results obtained for release of 3 esters under static headspace and *in vivo* conditions by Doyen and co-workers (Doyen *ea*, '01) using C8 triglyceride oil, matches very closely with the results reported in the present work.

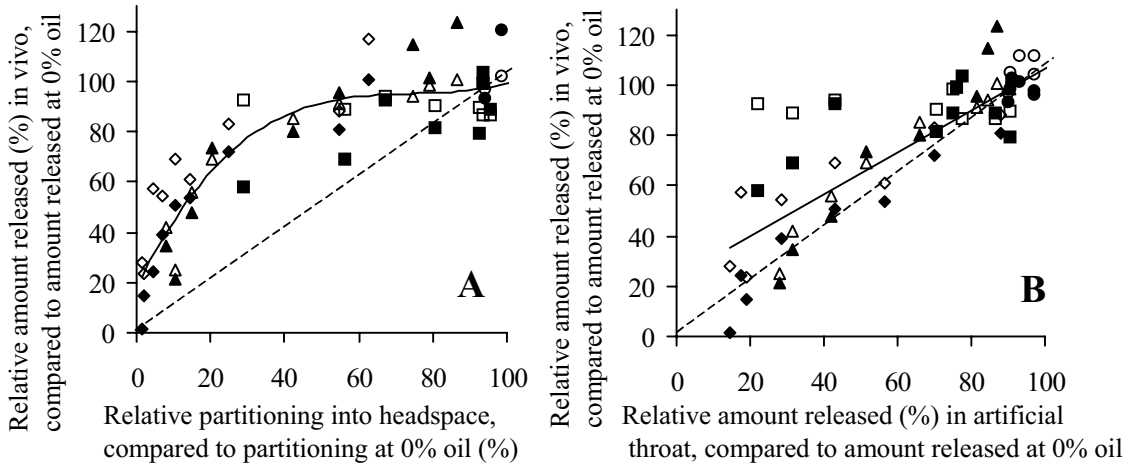

Figure 5.2. Correlation between relative amount released of ethyl acetate (•), ethyl butanoate (■), ethyl hexanoate (▲), and geranyl acetate (♦) under *in vivo* conditions (%) for 2 panelists (open and closed symbols represent panelist 1 and 2, respectively) and (A) the relative partitioning (%) into headspace (SHGC) and (B) the relative amount released (%) in the artificial throat. The solid lines represent a polynomial (A) or a linear (B) fit. The dashed line indicates perfect linear correlation.

Effect of oil content on perception of ethyl hexanoate. A panel scored orthonasal and retronasal intensities of ethyl hexanoate, and fattiness mouthfeel of emulsions containing a range of oil contents (Table 5.2). The aroma perception decreases with increasing oil content, both during orthonasal and retronasal evaluation (ANOVA: $p < 10^{-8}$, and $p = 0.013$, respectively). The effect is less strong at retronasal evaluation compared to orthonasal ($p = 0.11$, homogeneity-of-slopes model). A significant increase in perceived fattiness was found at higher oil concentrations (ANOVA, $p = 0.0005$). No significant panelist effect was found in any of the ANOVA's. The Levene's test for homogeneity of variances was not significant for any factor, indicating homogeneity of variances throughout the datasets.

Table 5.2. Averaged perceived orthonasal and retronasal intensities of ethyl hexanoate, and fattiness of emulsions with different oil contents, scored on a 0-10 scale.

Emulsion oil content	0%	0.05%	0.1%	0.2%	0.5%	1.0%
Orthonasal aroma intensity[1]	7.1^{A}	6.1^{AB}	5.8^{AB}	5.0^{BC}	3.5^{CD}	2.9^{D}
Retronasal aroma intensity[1]	5.8^{A}	6.0^{A}	5.2^{AB}	4.4^{AB}	4.6^{AB}	3.8^{B}
Fattiness mouthfeel[1]	2.7^{A}	4.3^{BC}	3.2^{AB}	3.8^{AB}	4.7^{AB}	5.8^{C}

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

Orthonasal aroma intensity was assessed by sniffing from freshly opened bottles containing emulsions. This process is similar to SHGC analysis and the aroma release is governed by the partition coefficient. The aroma partitioning of ethyl hexanoate into the headspace decreases at

higher oil contents (Figure 5.1A), and consequently, the orthonasal aroma perception decreases as well.

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

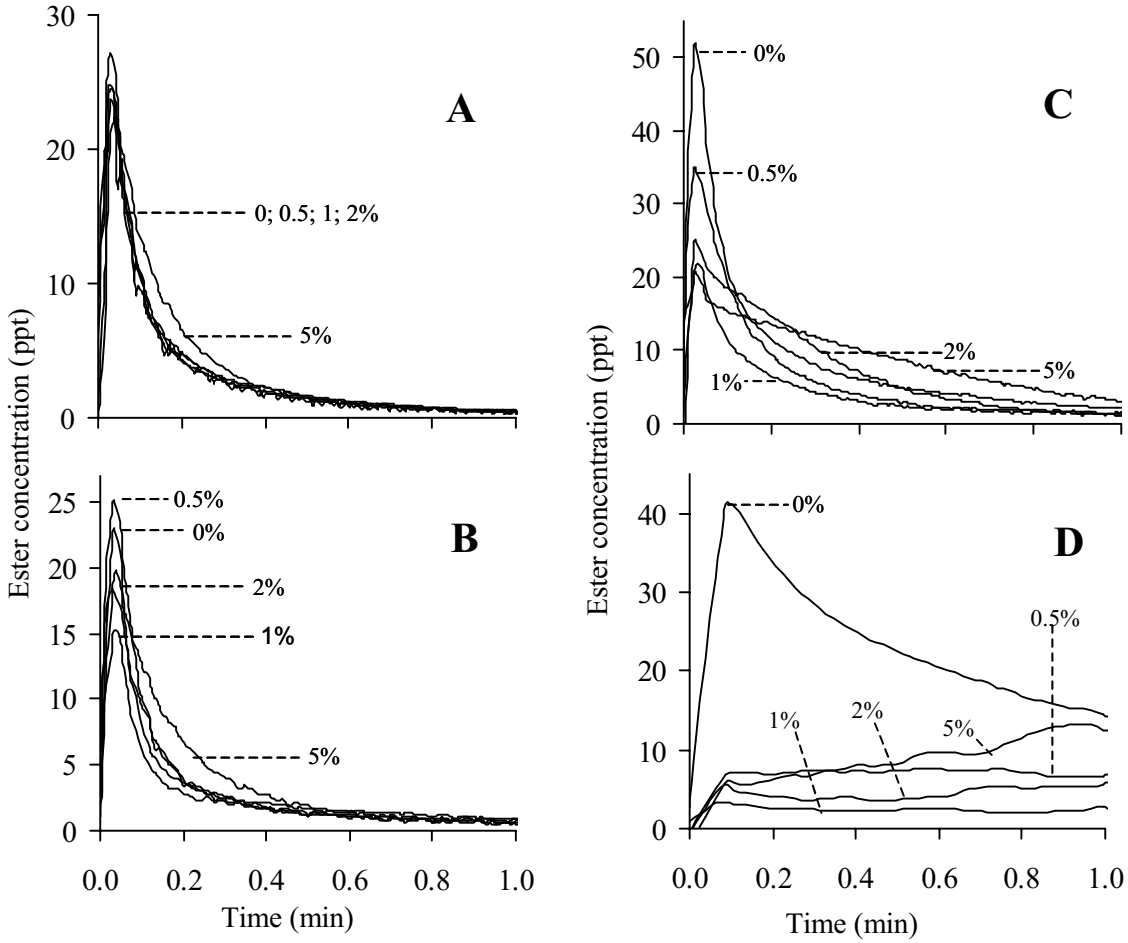

Figure 5.3. Release curves for ethyl acetate (A), ethyl butanoate (B), ethyl hexanoate (C), and geranyl acetate (D) of artificial throat measurements of emulsions with different oil contents.

Effect of oil content on aroma release in the artificial throat. The artificial throat simulates aroma release in the situation that a thin liquid layer is remaining in the throat after swallowing (Weel *ea*, submitted).

Figure 5.3 shows release curves of the ester compounds from emulsions with different oil contents obtained from artificial throat measurements. An increase in oil content results in a lower maximum intensity (I_{max}) and more peak tailing at higher oil contents. The decrease in I_{max} and increase in peak tailing at higher oil contents are stronger for compounds with a higher hydrophobicity. The I_{max} of the ethyl butanoate curve at 0.5% oil is higher than the one at 0% oil. This is no systematic effect, but an example of experimental error in the data, which is generally less than 10%.

The P_{ow} values of the hydrophobic compounds used, show that the concentration of an aroma compound in the oil phase of an emulsion is much higher than the concentration in the liquid phase (Table 5.1). When the oil content is increased, the aroma concentration in the oil phase will decrease as a result of dilution, and consequently the concentration in the water phase will decrease as a result of partitioning. The latter results in a less steep concentration gradient between the emulsion and air, and consequently in a smaller driving force for release. Therefore, the release rate reduces significantly, which leads to low gas phase concentrations and long release times. At a certain point, this release becomes too small to be analysed accurately by the MS-Nose. Because of this, peak areas cannot be compared directly amongst curves acquired at different oil contents.

An increase in release of geranyl acetate is observed for emulsions with higher oil contents (1-5%). This might be attributed to the increase observed in layer thickness of the emulsion remaining on the inside of the rubber tube of the artificial throat, after pouring the sample through. The increase in layer thickness is caused by an increase in emulsion viscosity (from 1.3 to 3.4 mPa·s for emulsions with 1 and 5% oil, respectively), possibly combined with an interaction between the apolar rubber surface and the emulsion surface, which has increased in hydrophobicity. No increase in layer thickness is observed for the hydrophilic glass tube. The layer thickness of the rubber tube increased from 0.045 to 0.11 mm, for emulsions with 1 and 5% oil, respectively. Consequently, a larger amount of aroma compounds will be available for release. A thicker layer also gives a higher diffusion time scale, which results in a slower release. However, these considerations do not explain why an increase in release for emulsions with higher oil contents (1-5%) is only observed for geranyl acetate, and not for the other compounds.

The effects of oil content on release of several esters in the artificial throat are shown in Figure 5.1D, and are comparable to the effect observed under *in vivo* conditions (shown in Figure 5.1B and C). The effect of oil content on ester release in the artificial throat is smaller than under static headspace conditions (Figure 5.1A). Figure 5.2B shows the correlation between the relative amount of ester released under *in vivo* conditions and the relative amount released in the artificial throat within 1 min. Although quite some scattering is present in the data, a linear correlation was observed between the results obtained under artificial throat and *in vivo* conditions ($R^2 = 0.75$). *In vivo* release measurements and static headspace results are less correlated at high and low oil contents. Therefore, artificial throat measurements have a higher predictive power for *in vivo* aroma release than static headspace measurements (Figure 5.2).

Smallest effective oil content. The dataset acquired in this study allowed the determination of the smallest oil content that influences release or perception of aroma significantly ($\alpha = 0.05$), when compared to oil free solutions, for the various systems used (Table 5.3). Lower effective oil contents are found for esters with a higher hydrophobicity. When the analysis is based on partition (SHGC and orthonasal perception), instead of release dynamics from a thin liquid layer (artificial throat, *in vivo* release, and retronasal perception), lower effective oil contents are found as well. In case of ethyl hexanoate, the effective oil content of the artificial throat (0.2%), of the *in vivo* aroma release measurements (0.5-1%), and of the retronasal perception (1%) are all within the same range in this study, indicating the relevance of artificial throat and *in vivo* aroma release measurements for aroma perception.

Table 5.3. Lowest oil contents from emulsions that differ significantly from 0% oil emulsions, for various analytical techniques. All tested ($\alpha = 0.05$) by single sided t-tests, except panel results (single sided Fisher LSD test, to take the panelist effect into account).

	Ethyl acetate	Ethyl butanoate	Ethyl Hexanoate	Geranyl Acetate
Panelist 1	(>5%)[1]	(>5%)[1]	0.5 %	0.1 %
Panelist 2	(>5%)[1]	(>5%)[1]	1 %	0.05 %
SHGC	(>5%)[1]	0.5 %	0.01 %	0.01 %
Artificial throat	(>5%)[1]	0.5 %	0.2 %	0.05 %
Panel orthonasal	N.m.[2]	N.m.	0.2 %	N.m.
Panel retronasal	N.m.	N.m.	1 %	N.m.
Panel fattiness	N.m.	N.m.	0.5 %	N.m.

[1] A possible significant effect at higher oil content

[2] N.m. = not measured

Effect of droplet size distribution on aroma release. The effect of droplet size distribution on the release of ethyl butanoate, ethyl hexanoate, and geranyl acetate, was tested at 2 oil contents (0.1% and 1%), under static headspace, *in vivo*, and artificial throat conditions. The release of ethyl acetate was not measured because an oil content of 1% did not influence its release at all. The SHGC and artificial throat measurements did not yield a significant effect of droplet size distribution (Fisher LSD-test, $\alpha = 0.05$, results not shown). No effect of droplet size on release in static headspace was observed by Carey and co-workers (Carey *ea*, '02). The results of *in vivo* release of esters are shown in Table 5.4. A significant effect of droplet size distribution was found for geranyl acetate at an oil content of 0.1% under *in vivo* conditions. The release from the crude emulsion (A) was lower than the release from the emulsions homogenised at low and high energy (B and C, respectively). This was found similarly for both panelists.

Table 5.4. Amounts of ester released (ng) for both panelists from emulsions with different droplet size distributions (A, B and C).

	%oil	Panelist 1			Panelist 2		
Emulsion[1]		A	B	C	A	B	C
$D_{(4,3)}$ (µm)		0.9	0.9	0.8	0.6	0.6	0.7
Ethyl butanoate	0.1	0.5	0.5	0.5	0.7	0.6	0.6
	1.0	2.6	3.0	2.9	2.6	3.1	3.6
Ethyl hexanoate	0.1	1.1	1.4	1.4	1.6	2.1	1.9
	1.0	1.1	2.5	2.9	1.0	2.3	2.9
Geranyl acetate	0.1[2]	1.1^{A}	2.5^{B}	2.9^{B}	1.0^{A}	2.3^{B}	2.9^{B}
	1.0	0.3	0.5	0.6	0.7	0.8	0.8

[1] A: crude emulsion, B and C: low and high energy homogenised emulsions, respectively.

[2] Different characters indicate a significant difference (Fisher single sided LSD-test, $\alpha = 0.01$).

The difference between the *in vivo* measurements and artificial throat measurements for the effect of droplet size distribution on release of geranyl acetate may be related to a difference in time scales. The time scale of diffusion (τ) within an oil droplet is given by eq. 5.1, in which d is the droplet diameter (m), and D is the diffusion coefficient (ranging from 10^{-10} to 10^{-9} m^2/s for small molecules in water, derived from the Hayduk-Minhas correlation (Reid *ea*, '87). Using the $D_{(4,3)}$ measured this results in a τ in the order of roughly 1 s for the crude emulsion. For the low-energy, homogenised emulsion τ is approximately 0.05 s.

$$\tau = \frac{d^2}{D} \qquad (5.1)$$

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

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

In literature, droplet size has been reported to increase, to decrease, as well as to have no effect on aroma release for different systems (Carey *ea*, '02, Van Ruth *ea*, '02a, Van Ruth *ea*, '02b, Rabe *ea*, '03). The present results indicate that the effect of droplet size distribution on aroma release strongly depends on the hydrophobicity of the aroma compound, the emulsion characteristics and the dynamics of the measurement. The effect of droplet size distribution on *in vivo* release of

geranyl acetate shows the potential of the emulsion structure to influence the *in vivo* release of very hydrophobic compounds. Therefore, a relatively large change in release under *in vivo* conditions might be accomplished modulating the oil droplet size distribution, without changing the oil content.

Conclusions

The effect of oil on aroma release is smaller under *in vivo* conditions than under static headspace conditions, and smaller for retronasal perception of aroma than for orthonasal perception. The thin layer of emulsion remaining in the human throat after swallowing plays a crucial role in explaining this difference. The effect of oil on aroma release in the artificial throat correlated well with the effect under *in vivo* conditions, providing further support for the 'thin layer' hypothesis, and thus showing that the artificial throat is a suitable device to simulate *in vivo* aroma release accurately.

Chapter 6

Aroma release and perception of aromatised whey protein gels: perception is determined by texture rather than by release

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Abstract

Five whey protein gels, with different gel hardnesses and waterholding capacities, were aromatised with ethyl butanoate or diacetyl and evaluated by a 10-person panel to study the relation between the gel structure and the sensory perception, as well as the aroma release during eating. The sensory perception of the aroma compounds was measured by the time-intensity method, while simultaneously the aroma release was monitored by the MS-Nose. The aroma release was found to be independent of the gel hardness or waterholding capacity. However, significant changes in aroma intensity between the gels were perceived by the majority of the panelists, despite the fact that the panelists were instructed to focus only on aroma perception and not to take texture into account. From these observations it is concluded that texture of gels determines perception of aroma intensity rather than the in-nose aroma concentration.

Introduction

The influence of product structure and composition on the temporal aspects of aroma release has been the subject of many studies. The reason for this interest is that understanding of the principles governing aroma release and perception during eating might help to formulate food products with improved aroma characteristics. This is especially important for low-calorie products with, for instance, reduced levels of fat, sugar, or alcohol. The structure of a product will influence the transport of volatiles into the oral and nasal cavities, while the composition of a product will influence the interactions between aroma and non-aroma ingredients. Various authors have reviewed these aroma-matrix interactions (Solms, '86, Overbosch *ea*, '91, Land, '96, Taylor, '99).

Studies with liquid systems containing hydrocolloids (e.g., xanthan, hydroxypropylcellulose, sodium alginate, carboxymethylcellulose, and guar gum) have revealed that an increased viscosity due to an increase in hydrocolloid concentration results in a reduction of the aroma perception (Pangborn *ea*, '74, Pangborn *ea*, '78, Baines *ea*, '87).

Protein and carbohydrate gels have been used to study the effect of matrix properties, such as gel hardness, waterholding capacity, and microstructure, on the perception of aroma. In general, the same trend was found for the gel systems as for liquid systems, i.e., an increase in gelling agent concentration causes a decrease in the sensory rating of the aroma perception (Clark, '92, Jaime *ea*, '93, Carr *ea*, '96).

In addition to these studies, which used sensory rating, the aroma perception of aromatised protein and carbohydrate gels has been monitored by time-intensity (TI) methodology (Guinard *ea*, '95, Bakker *ea*, '96, Wilson *ea*, '97). It was shown that the maximum perceived aroma intensity (I_{max}) decreased with increasing gel hardness. An increase in gel hardness was achieved by an increase in gelling agent concentration. A similar TI study has been done with gels with equal gelling agent concentration (Gwartney, '99). In this study gels were prepared with equal protein concentrations but with variation in salt type and ionic strength during preparation, which resulted in different rheological, microstructural, and waterholding properties. However, ionic strength and salt type might influence the interactions between aroma compounds and proteins. No relationship between gel hardness (fracture stress) and perceived intensity of aroma was found. However, the gel structure (i.e., stranded or particulate structure) seemed to have an effect. Gels with a particulate structure and low waterholding capacity had a lower maximum perceived intensity than that of the gels with a stranded structure (Gwartney, '99). Release of aroma compounds was not measured in these studies.

Several methods have been developed to measure aroma concentrations in real time in the breath of test-persons during eating (Soeting *ea*, '88, Lindinger *ea*, '98b, Taylor *ea*, '00). In the present study, we used the MS-Nose, developed by Taylor and co-workers (Taylor *ea*, '00). The system consists of a mass spectrometer equipped with an APCI (atmospheric pressure chemical

ionisation) source, which is modified to allow analysis of the human breath. A small part of the test-person's breath is continuously sampled into the mass spectrometer. The system allows sensitive and fast monitoring of the *in vivo* aroma release. The release of various aroma compounds from gelatin gels has been measured with this instrument (Baek *ea*, '99, Linforth *ea*, '99). In both studies aroma release measurements and time-intensity recordings were performed simultaneously. Baek and co-workers, who worked with furfuryl acetate, found a significant decrease in I_{max} and an increase in T_{max} (time to maximum intensity) for TI as the gelatin concentration increased. No correlation between I_{max} of aroma release and gelatin concentration was found. However, the rate of volatile release seemed to correlate well with the sensory data (Baek *ea*, '99). Linforth and co-workers compared T_{max} values of aroma release and sensory TI for menthol and dimethylpyrazine. When the maximum was reached fast, the sensory perception was found to lag behind the aroma release. When the maximum was reached after a longer period of time, the T_{max} of perception preceded the T_{max} of aroma release, due to a sensory adaptation effect (Linforth *ea*, '99).

Recently, we have developed a gel system based on whey protein, which allowed different rheological properties at equal protein concentrations (Alting *ea*, '00). The rheological variation between the gels was obtained by variation in protein concentration during heating. In contrast to the gels of Gwartney no salts or sugars which could influence the aroma perception (taste-aroma interaction) were necessary for this purpose. Another advantage of the gelation system used in this study is that the used aroma compounds are not exposed to heat, as gelation was performed at ambient temperature by mild acidification. Furthermore, the level of protein-related off-flavours is minimised by mild heating conditions.

The aim of the present study was to determine whether aroma release and perception would be affected by gel hardness and waterholding capacity and whether changes in aroma release correlate with changes in aroma perception. Diacetyl and ethyl butanoate were chosen as aroma compounds, because they represent a typical hydrophilic and hydrophobic compound, respectively. Release and perception of these aroma compounds from five different whey protein gels were studied by sensory TI and measurement of aroma release using the MS-Nose.

Materials and methods

Materials. Whey protein isolate (Bipro, JE 153-9-420) was obtained from Davisco Foods International Inc., Le Sueur, Minnesota. Specifications were as follows: pH 7.23; non- protein nitrogen 0.17%; ash 1.8%; lactose 0.34%; calcium 0.13%; total protein 93.39% (N × 6.38); α-lactalbumin 12.6%; bovine serum albumine 3.2%; immunoglobuline G 5.2%; β-lactoglobulin A 33.2% and β -lactoglobulin B 37.1%. Quest International (Naarden, The Netherlands) provided diacetyl and ethyl butanoate. Glucono-δ-lactone (GDL) was supplied by Sigma Chemical Co. (St. Louis, MO) and ethanol (100%) was from J.T. Baker (Deventer, The Netherlands).

Preparation of the gels. Whey protein isolate was solubilised in demineralised water at initial concentrations of 4%, 7.5%, and 11% (w/w) by gentle stirring for at least 2 h at ambient temperature. To obtain gels with different physical parameters (i.e., gel hardness and waterholding capacity) at equal protein concentrations, a two-step gelation procedure was followed (Table 6.1). First, solutions were heated at 68.5°C in a waterbath for 3 h. During this heating step, the protein molecules formed aggregates of different sizes, depending on the initial protein concentration. After cooling to ambient temperature, parts of the protein solutions of 7.5% and 11% were diluted to final concentrations of 4% by addition of demineralised water. At this point, ethyl butanoate or diacetyl was added to all solutions to a final concentration of 150 ppm. Ethyl butanoate was diluted in ethanol before addition to the protein solution. This introduced 0.14% (w/w) ethanol into the ethyl butanoate gels. Diacetyl was diluted in water before addition to the protein solution. GDL was added to the solutions with final protein concentrations of 4, 7.5, and 11% (w/w), to a final concentration of 0.32, 0.52, and 0.86% (w/w), respectively. After addition of GDL the pH decreased slowly to a final value of 5.0, toward the pI of the protein (pH 5.1). During a period of 15 h at ambient temperature, the gels were formed. Subsequently, the gels were stored at 4°C for a maximum period of 2 days.

Table 6.1. Physical properties of the whey protein gels. The initial and final protein concentrations during the preparation of the gels are also indicated.

Gel Number	Initial protein conc. (%)	Final protein conc. (%)	Hardness (kPa)	WHC (%)
1	4.0	4.0	13.4	71
2	7.5	4.0	41.6	74
3	11	4.0	35.0	76
4	7.5	7.5	114.8	84
5	11	11	211.5	86

To determine the sensitivity of the aroma release measurements, gels with increasing concentrations of diacetyl and ethyl butanoate were prepared, according to the procedure described above. The protein concentrations of the solutions used for these gels during heating and gelation were 11 % and 4 %, respectively. Diacetyl and ethyl butanoate were both present in each gel at concentrations of 100, 125, 150, 175, and 200 ppm.

Gel hardness. Gel hardness was determined by a texture analyser (type TA-XT2, Stable Micro Systems Ltd., Godalming, U.K.). Approximately 24 h after the addition of GDL, a grid was pressed into the gels. The device consisted of four blades (45 × 1.5 × 2 mm) of stainless steel arranged in a double cross. A force-time curve was obtained at a constant rate of 0.3 mm/s for a 10-mm displacement. Gel hardness was expressed as the stress (Pa) at the maximum peak of the force-time curve (Bourne, '78, Luyten *ea*, '91).

Waterholding capacity (WHC). A 40-g portion of gel was minced with a plunger in 60 crushing movements and put into a centrifuge tube. The gel was centrifuged for 30 min at 160 g. After

centrifugation the serum was removed and weighed. The WHC (%) of the gel was defined as the mass fraction of the retained water from the total amount of water present in the gel (Harwalkar *ea*, '83, Kocher *ea*, '93).

General setup of aroma release measurements and Time-Intensity recordings. Ten panelists were familiarised to the aroma of diacetyl and ethyl butanoate and trained to produce TI curves, while the aroma release in their noses was measured simultaneously by the MS Nose, during 5 training sessions in which 3 samples were judged by each panelist. The panelists were instructed to chew regularly (independent of the gel hardness) for 30 s without swallowing, then to swallow the entire bolus, and, after that, to continue chewing for 60 s.

The different gels aromatised with either diacetyl or ethyl butanoate were presented in triplicate to each panelist as cylinder-shaped samples of 2 mL (3 samples of each type of gel). The order of the samples was randomised and to prevent sensory fatigue the samples were presented in 5 sessions of 3 gels. Before every session a non-aromatised gel with medium hardness (gel no.3) was presented as a blank, followed by an aromatised gel with medium hardness (gel no.3) as reference. TI recordings and aroma release measurements were conducted simultaneously.

No information about the purpose of the experiment or the kind of presented sample was given to the panelists.

Aroma release measurements. While panelists were eating the gels, the aroma release of diacetyl or ethyl butanoate was monitored by sampling the air flow from one nostril over a 1.5-min period. By resting one nostril at a plastic tip, attached to a pipe, the tidal flow of air from the nostril was allowed to pass back and forth through this pipe. In this way, the normal breathing pattern was not disturbed. A small part of the breath in the pipe was sampled through a capillary tube (0.53 mm internal diameter, heated to 100°C) positioned at a right angle with the pipe, within the flow of breath. Because only a small part of the breath was sampled, the measurement was independent of the nostril used.

The sampled part of the breath was introduced (75 mL/min) into the source of a MS Nose Atmospheric Pressure Chemical Ionisation Gas Phase Analyser (APCI-GPA) attached to a VG Quattro II mass spectrometer (Micromass UK Ltd., Manchester, U.K.).

The compounds were ionised by a 3.0 kV discharge. Source and probe temperature were 80°C.

Diacetyl and ethyl butanoate were analysed in selected ion mode (0.2 s dwell on each ion), at cone voltages of 19 and 20 V, respectively. Acetone, always present in the human breath as a result of the fatty acid metabolism, was measured as an indicator of the panelists breathing pattern. Breath volatile concentrations were expressed as peak height in arbitrary units and divided by a factor 5×10^6.

Time-Intensity recordings. TI curves were recorded by FIZZ software (Biosystemes, Couternon, France) over a 1.5-min period. Panelists were instructed to rate the perceived diacetyl or ethyl butanoate aroma on a scale from 0 to 10. The maximum intensity of the reference gel was agreed by the panelists to have an aroma intensity of 5 on the 0-10 scale. The panelists were instructed not to let textural effects influence their perception of the aroma intensity.

Gel preparation for *in vitro* measurement of aroma release. Each gel was directly prepared in a 10-mL headspace vial (3 mL of gel per vial) and aromatised with 5, 10 or 25 ppm of diacetyl and ethyl butanoate, according to the procedure described above.

Another series of gels was prepared according to the procedure, except for the protein concentration during gelation, which was 10 times diluted for each sample to avoid gel formation. The solutions were centrifuged (30 min, 9600 g) and the resulting pellet was freeze-dried (72 h). The dry protein was suspended in demineralised water to the original concentrations and amounts of 3 mL were put in 10-mL headspace vials. Diacetyl and ethyl butanoate were added to final concentrations of 5, 10 and 25 ppm.

Static headspace gas chromatography (SHGC). The headspace aroma concentrations were allowed to reach equilibrium during overnight storage at ambient temperature. The headspace aroma concentration was analysed by gas chromatography. To this end 1000 µL of headspace was injected splitless on the column after 20 min incubation at 35°C. A GC-8000^{top} gas chromatograph (CE Instruments, Milan, Italy) was equipped with a CP-SIL 5 CB Low bleed column (30 m × 0.25 mm; film thickness: 1.0 µm, Chrompack, Middelburg, The Netherlands) and a flame ionisation detector (FID). The oven temperature was initially 40°C for 5 min, then rised with 15°C/min to 150°C and was kept at 150°C for 5 min. Inlet and detector temperatures were 250 and 225°C, respectively. Gas flow-rates were as follows: hydrogen, 35 mL/min; air 350 mL/min; makeup nitrogen, 30 mL/min. The headspace concentrations were expressed as peak areas in arbitrary units.

Confocal scanning laser microscopy (CSLM). Imaging was performed using a Leica confocal scanning laser microscope, type TCS-SP, configured with an inverted microscope, and an ArKr laser for single-photon excitation. The protein gels were stained by applying 2 mL of an aqueous solution of 0.05% (w/w) Rhodamine B to 200 mL gel. The 568 nm laser line was used for excitation inducing a fluorescent emission of Rhodamine B, detected between 600 and 700 nm.

Results and discussion

Preparation and physical properties of the gels. The hardness and WHC of the five different gels produced are shown in Table 6.1. Gels 1, 2 and 3 have the same final protein concentration (4%) and they can be compared without taking into account compositional effects. To obtain a set of gels with a wider range of physical parameters, two gels with a higher final protein concentration (gels 4 and 5; 7.5 and 11% protein, respectively) were included.

In addition to measurement of the WHC and gel hardness, the microstructure of the five different gels was determined by CSLM. Figure 6.1 shows the microstructure (length scale in µm) of gel 1 and 5. It is clearly seen that gel 1 has a coarser structure than gel 5, which is in good agreement with the results of the hardness and WHC measurements (Table 6.1). A gel with a more open structure will, in general, be softer than a gel with a more compact structure (Van Marle, '98), and a relatively weak gel with an open structure will have a lower WHC (Verheul *ea*, '98).

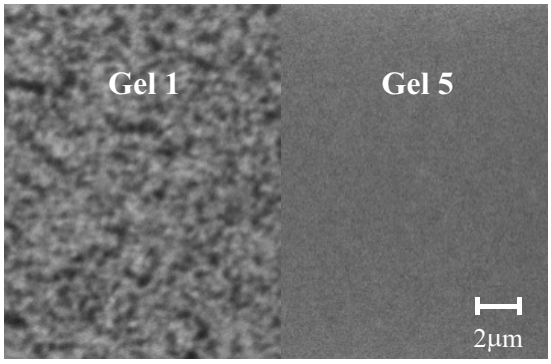

Figure 6.1. CSLM images of the structures of gels nr 1 and 5 (Table 6.1).

The molecular interactions of diacetyl and ethyl butanoate with whey protein gels were studied by SHGC measurements. The equilibrium headspace concentrations of diacetyl and ethyl butanoate above the gels were on average 15% and 40%, respectively, lower than above water (Figure 6.2). However, there were no differences observed in equilibrium headspace aroma concentrations between the gels. If the molecular binding of diacetyl or ethyl butanoate with whey protein was important, one would expect a lower static headspace concentration for gels 4 and 5 compared to gels 1,2, and 3. This is not observed in Figure 6.2. In addition to this, aroma binding was studied with nongelled samples with protein concentrations and applied heating conditions similar to those of the gelled samples. On rehydration the protein formed an insoluble dispersion. SHGC analysis of the headspace of these samples did not reveal any differences in aroma binding for the different solutions (data not shown). Therefore, the differences in aroma release and perception of the studied gels can be directly explained by physical gel parameters, without the need to take final protein concentration and aroma-protein interactions into account.

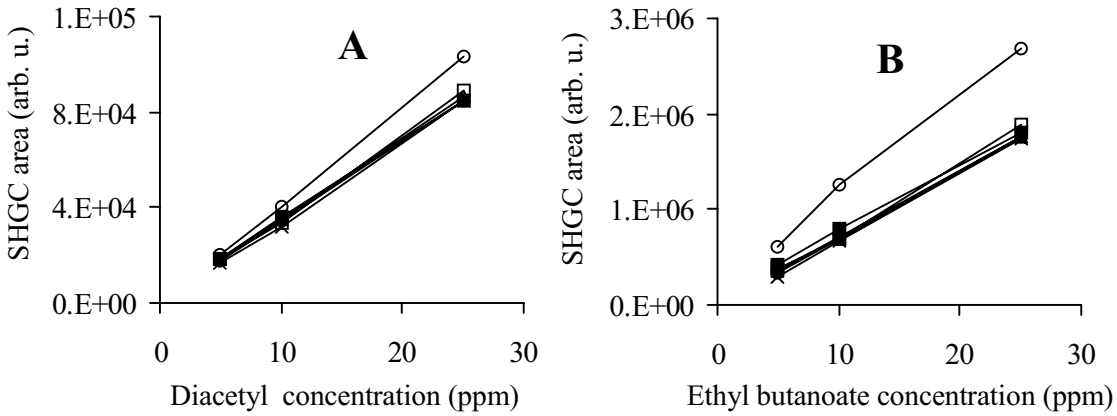

Figure 6.2. Equilibrium headspace concentration of diacetyl (A) and ethyl butanoate (B) above whey protein gels 1 (●), 2 (□), 3 (Δ), 4 (×) and 5 (■) (Table 6.1). The open circles (o) represent water samples.

Effect of gel properties on aroma release and perception. Figure 6.3 shows the averaged TI profiles and the corresponding averaged aroma release curves of the 5 gels, eaten by 10 panelists

in triplicate for diacetyl (A) and ethyl butanoate (B). Each curve in both figures represents the average of 30 single curves.

It can be seen in Figure 6.3 that the perceived intensity of diacetyl and ethyl butanoate generally decreases with an increase in gel hardness (going from gel 1 to 5), averaged over the 10-person panel. However, no clear differences between the gels were found in the averaged aroma release curves.

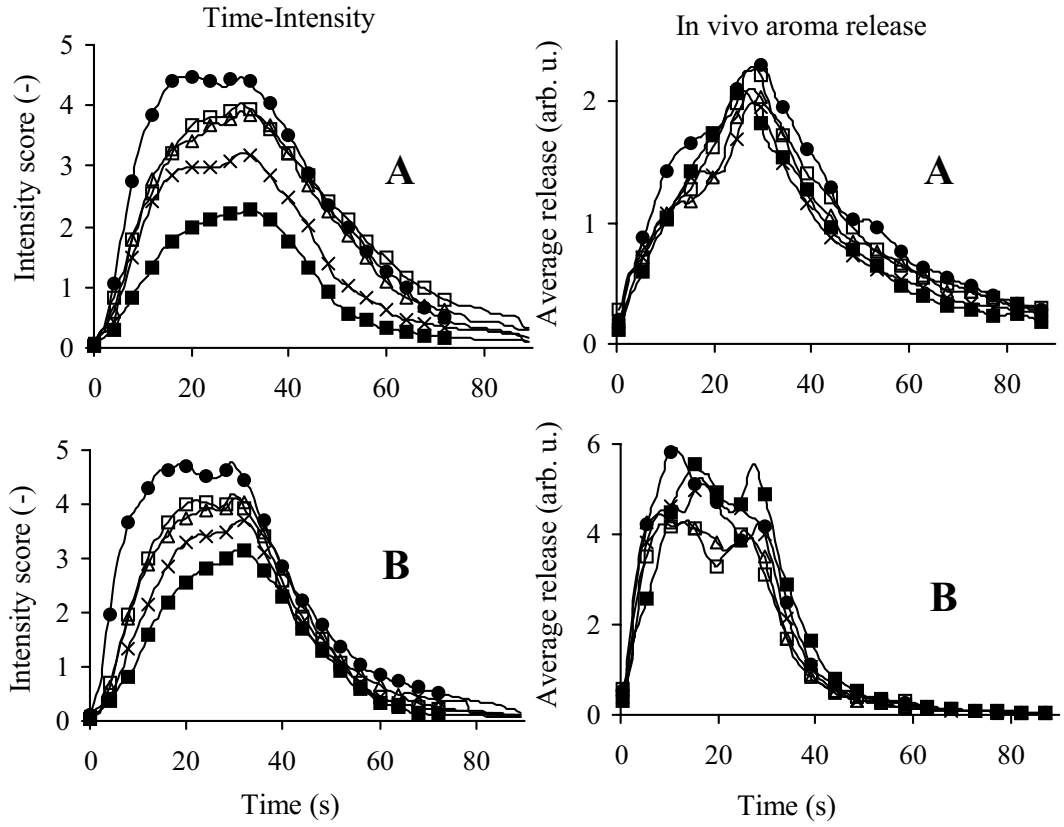

Figure 6.3. Averaged time-intensity recordings and relative averaged release profiles for gels 1 (●), 2 (□), 3 (Δ), 4 (×) and 5 (■) (Table 6.1), aromatised with diacetyl (A) and ethyl butanoate (B).

The results for both aroma compounds are summarised in Figure 6.4. Averaging across the whole panel of I_{max} values of the TI curves and the aroma release profiles shows a decrease of TI-I_{max} (maximum intensity of time intensity), while release-I_{max} (maximum intensity of aroma release) remains constant.

An explanation for the difference in release and perception might be that the current setup of the aroma release measurements, using 10 panelists who assessed each gel in 3 replicates, was not sensitive enough to show subtle differences, which can be made visible by TI. Such an effect in aroma release might be hidden because of a large person-to-person variation. To determine the sensitivity of the aroma release method, physically identical gels (gel no. 3) with increasing concentrations of diacetyl and ethyl butanoate were eaten by the panel. A higher concentration of the aroma compounds resulted in a higher average aroma release curve (results not shown). The

regression coefficients of the linear regression between aroma concentration and I_{max} of all individual curves were 0.99 and 0.97 for diacetyl and ethyl butanoate, respectively. This experiment clearly shows that a linear relationship exists between aroma concentration in the gel and release-I_{max} and that the method used to measure aroma release is sufficiently sensitive to detect differences in aroma release. Therefore, the fact that no differences in aroma release are found between the 5 structurally different gels cannot be explained by a lack of sensitivity. The person-to-person variation does not hide the aroma release effect.

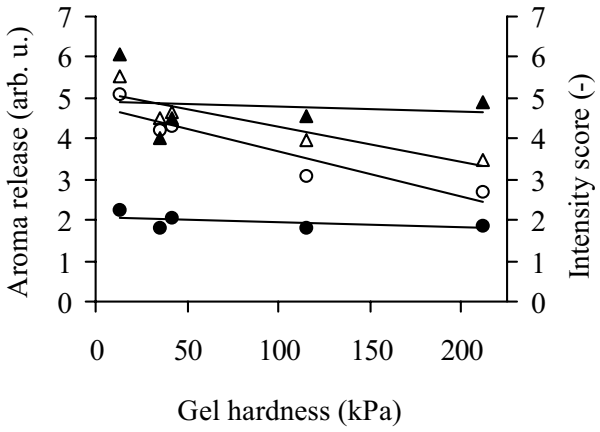

Figure 6.4. Relation between gel hardness and overall averaged values of I_{max} of NSC (closed symbols) and Time Intensity (open symbols) for ethyl butanoate (▲ and Δ) and diacetyl (● and ○). The lines reflect a linear regression estimate of the effect.

Clear differences in aroma release were found, but interestingly, the panelists indicated afterward that differences in aroma perception between the gels with different concentrations of diacetyl and ethyl butanoate were small. So, in this experiment an increase in gel aroma concentration caused an increase in the aroma release, but it did not seem to increase the aroma perception. The opposite was the case in the study with gels with increasing gel hardness: the aroma release did not change, while the aroma perception decreased (Figure 6.4).

Several other authors who studied various gel systems have reported a decrease in aroma perception with increasing gel hardness (Jaime *ea*, '93, Guinard *ea*, '95, Bakker *ea*, '96, Carr *ea*, '96, Wilson *ea*, '97). However, in these studies the actual aroma release was not measured by *in vivo* measurements. On basis of the results of our study we would suggest that the release of aroma compounds from gels might not decrease with firmer gels, but that the aroma perception by panelists could be influenced by the textural properties of the gels in a psychophysical way. Psychophysics is the study of the relationship of what exists in the real world (stimulus or stimuli) and the human experience of these events (Meiselman, '72). The influence of texture on aroma perception could be explained by the integration of the signals from various senses that reach the brain simultaneously, during eating of a gel.

Statistical analysis of curve parameters. In addition to graphical analysis of the data by averaging the aroma release and TI curves for all panelists, statistical analysis has been applied.

WHC and gel hardness are correlated (Table 6.1). Because of this, WHC and gel hardness have not been used in multivariate statistical analysis of the data, but strictly as univariate predictors. To facilitate the statistical analysis, each individual TI and aroma release curve was summarised by a T_{max} and an I_{max} value. ANOVA with gel hardness and panelist as predictors, and T_{max} and I_{max} of both TI and aroma release curves as dependent variables, showed that the factor 'panelist' was highly significant ($p < 10^{-8}$). This means that the average values of the TI and aroma release are different for each panelist. The Levene's test for heterogeneity in variance was significant ($p < 0.001$), indicating that variance also differs between panelists. Hence, the effect of gel hardness and WHC on the aroma release and TI data was not analysed across the whole panel. These data have been separately analysed for each panelist. Linear regression has been applied to the dependent variables TI-I_{max}, TI-T_{max}, release-I_{max} and release-T_{max}, against gel hardness and WHC as predictors for each panelist separately, for both ethyl butanoate and diacetyl. Table 6.2 summarises for each combination of predictor and dependent variable the number of panelists showing a significant slope at a significance level of 0.05.

Table 6.2. Number of panelists with a significant ($\alpha = 0.05$) slope for the linear regression of WHC and gel hardness against I_{max} and T_{max} values of the aroma release and TI signals for gels with diacetyl and ethyl butanoate.

Dependent / Predictor		release-I_{max}	Release-T_{max}	TI-I_{max}	TI-T_{max}
Diacetyl	WHC	2	0	6	1
	Gel hardness	0	0	6	2
Ethyl but.	WHC	2	2	6	4
	Gel hardness	1	3	6	2

TI-I_{max} is the only dependent variable for which a considerable number (6 out of 10) of significant slopes was found, for both ethyl butanoate and diacetyl. For release-I_{max} and T_{max}, and TI-T_{max}, only a few significant slopes were observed. This confirms the conclusions drawn from the graphical presentation in Figure 6.4, namely that only the TI-I_{max} is significantly correlated with the gel hardness. The actual p-values of the TI-I_{max} data have been summarised in Table 6.3. For each aroma compound the panelists can be divided into two groups: group I, consisting of six panelists who show a significant effect of gel hardness on TI-I_{max} and group II, with the 4 other panelists who do not exhibit this effect. Except for panelists 2 and 6, all panelists fall in the same group for each aroma compound. Panelist 2 belongs to group I for diacetyl and to group II for ethyl butanoate. For panelist 6 the opposite situation is observed.

A hypothesis to explain the fact that the correlation between gel hardness and TI-I_{max} is not significant for group II, is that the panelists in this group produce nonconsistent results, and, therefore, do not show a significant correlation with gel properties. In that case, group II will have a higher standard error of their estimated slope. This has been tested for diacetyl and ethyl butanoate, and gel hardness and WHC, using ANOVA. There was no significant difference

between groups I and II in their standard error values. This shows that the nonsignificance of group II is not caused by nonconsistent panelist performance. The panelists in group II produce results different from those of group I, but still in a consistent and reproducible way. The groups could not be characterised by any differences in age, experience, or gender.

Table 6.3: P-values of the linear regression per panelist of gel hardness and WHC against I_{max} values of the TI signals for gels with diacetyl and ethyl butanoate.

	Diacetyl		Ethyl butanoate	
Panelist	Gel hardness	WHC	Gel hardness	WHC
1	0.02[a]	0.03[a]	0.00[a]	0.00[a]
2	0.01[a]	0.01[a]	0.15	0.15
3	0.00[a]	0.00[a]	0.00[a]	0.00[a]
4	0.02[a]	0.02[a]	0.01[a]	0.02[a]
5	0.18	0.18	0.89	0.83
6	0.19	0.19	0.01[a]	0.00[a]
7	0.59	0.59	0.72	0.88
8	0.66	0.66	0.23	0.45
9	0.00[a]	0.00[a]	0.00[a]	0.03[a]
10	0.00[a]	0.00[a]	0.00[a]	0.00[a]

[a] significant ($\alpha = 0.05$)

A second hypothesis is that the panelists in group I allow that their aroma perception is influenced by gel texture, whereas the panelists in group II are able to separate aroma perception from texture perception. Interestingly, the panelists were explicitly instructed not to let textural effects influence their perception of the aroma intensity. Despite this, an effect of texture on aroma perception was found for a considerable number of panelists. Moreover, for the general consumer, perception occurs spontaneously, and psychophysical interactions between the senses are likely to occur, which emphasises the potential importance of texture for aroma perception.

Influence of the eating-protocol for *in vivo* measurement on the T_{max}-value. The studies of Baek and co-workers (Baek *ea*, '99) and Linforth and co-workers (Linforth *ea*, '99) are comparable to our work, because they have also measured TI and aroma release simultaneously, using gelatin gels. In contrast to our study, in which the panelists followed a strict protocol, the panelists in these two studies were allowed to chew and swallow whenever they wished. Similar to our results, Baek and co-workers found no significant difference in the release-I_{max} values, but a significant difference in TI-I_{max} and TI-T_{max} values ($p < 0.001$, ANOVA).

In addition to this, a statistically significant ($p < 0.001$) decrease in the rate of release with increasing gelatin concentration was found. The rate of release was defined as the release-I_{max} divided by the time span between the time points of the increasing phase corresponding with 25% and 75% of the T_{max}-value. For our dataset a significant correlation ($p = 0.037$) was found between gel hardness and rate of release for ethyl butanoate. For diacetyl a nonsignificant p-value

of 0.24 was obtained. The fact that we find a much lower significance for ethyl butanoate than Baek and co-workers and no significance at all for diacetyl, can be explained by comparing the different eating protocols of both studies. Because the T_{max} is usually determined by the moment of swallowing (Buettner *ea*, '00a), the effects in T_{max} measured by Baek and co-workers are primarily caused by the fact that people chew longer on harder gels. In our eating protocol, the panelists had to swallow the entire bolus after 30 s of chewing, irrespective of the gel hardness. In this way, the chewing and swallowing time becomes independent of the gel hardness, which should result in a more objective monitoring of the effect of gel structure on the rate of release. To prove this, our set of gels with increasing gel hardness were eaten in triplicate by 3 panelists without the use of the eating-protocol, while their aroma release was measured simultaneously. In contrast to the experiments in which a protocol with a fixed swallowing time was used, a much higher significant positive correlation between gel hardness and release-T_{max} was found for both aroma compounds. P-values of 6.8×10^{-7} and 0.01 were obtained for ethyl butanoate and diacetyl, respectively (no further results shown).
Linforth and co-workers have found differences in the ratio between sensory and instrumental T_{max} values. These effects are not reproduced in our study, due to the fixation of T_{max} at 30 s by use of a protocol, as discussed above.

Conclusion

The main conclusion is that for the used whey protein gel system a change in texture determines the perception of aroma intensity in a psychophysical way, through a change in texture, and not through a change in the aroma release. Apparently, aroma release does not always determine aroma perception, suggesting that for some food applications changing product structure might be an effective tool to adjust aroma perception.

Chapter 7

General discussion

The release and perception of aroma compounds in food systems has been studied for decades. When this project started in 1999, the MS-Nose had recently been introduced as a new tool to measure the *in vivo* release of aroma compounds. This new tool made it possible to actually measure real-time aroma release during the consumption of food. However, only limited validation was performed. Therefore, prior to the application of the technique, validation studies were performed and intra and interpersonal variation were studied. Subsequently, a more accurate *in vitro* testing device (the artificial throat) was developed for liquids and the MS-Nose was used to investigate the interaction between perception and release of aroma during consumption, thereby unravelling cross-modal interactions.

Requirements for valid *in vivo* aroma release measurements

To perform valid *in vivo* release measurements with the MS-Nose, the possibility to measure compounds simultaneously, the lower and upper detection limits, the time resolution, and the calibration have to be considered.

APCI (atmospheric pressure chemical ionisation) can be used to measure the release of compounds simultaneously, because it is a soft ionisation technique. During ionisation mainly molecular ions are formed. This results in spectra that are easy interpretable and provides the possibility to measure the release of more than one aroma compound simultaneously. In addition to the molecular ion, fragment ions can be generated (by increasing the cone voltage), and this gives another option for independent analysis of compounds in a mixture. Spectra of the daughter ions of the molecular ions can be recorded by a second in-line MS to prove that the fragments originate from specific molecular ions.

The latter approach was chosen for instance in Chapter 2, where citral, nonanal, and geranyl acetate, representing a lemon-lime aroma, were to be analysed simultaneously in a mixture. Analysis of this specific combination of aroma compounds showed that most of the molecular and fragment ions overlapped. However, when analysing nonanal at its molecular ion (m/z 143), and citral and geranyl acetate at their fragment ions 95 and 137 m/z, respectively, it was possible to determine the release of the three compounds simultaneously and independently. Spectra of the daughter ions of the molecular ions of citral and geranyl acetate were recorded by a second in-line MS, and proved that the fragments chosen originated from the correct aroma compounds.

The detection threshold of the MS-Nose is low enough to measure aroma compounds, at concentrations normally present in aromatised products. For the compounds analysed in this thesis the lower *in vivo* detection limits were determined to be 0.1 - 1 ppm (concentration in aqueous solutions, assessed using the protocol for liquid samples). The concentrations of most aroma compounds naturally occurring in food products (meat, cheese, fruit) are in the range of 0.02 - 0.05 ppm. This is only a rough indication, and examples exist of higher (alcohols) and lower (sulphur compounds) concentrations occurring (Nijssen ea, '96). These are compositional

data, and the amount of aroma readily available for release could well be lower, which might challenge the lower detection threshold.
The upper limit of detection is determined by the ionising capacity of the APCI source, in which a certain amount of protons is constantly generated from water molecules present in the air analysed. In case of overfeeding the source with volatiles to be ionised, the surplus of volatiles will not be detected. When this value is approached, volatiles will compete for the protons, governed by their proton affinities. Every volatile compound in the food product that is released during eating, and reaches the source, takes its share of the ionising capacity available. In this way the upper detection limits of the compounds of interest can be reduced. Linearity of response was proven for all concentrations measured in this thesis. Most compounds have a linear response of approximately 3 orders of magnitude, depending on the aroma compound and measurement system (i.e. ethyl butanoate: 0.001 - 10 ppm for artificial throat and mouth model measurements, 0.01 - 10 ppm for *in vivo* measurement of liquids, 10 - 200 ppm for *in vivo* measurement of solid products).
The time resolution of the MS-Nose is sufficiently high (approximately 9 data points/s) to record an accurate breath-by-breath signal of a single m/z value in SIR (selected ion recording) mode. Simultaneous recording of 5 or 6 m/z values is possible, but the time-resolution should not become too low to measure individual breathes (which usually take about 2 s).
According to the literature, the MS-Nose signal can be calibrated by injecting solutions of aroma compounds in organic solvents into the nitrogen entering the APCI-source (Taylor *ea*, '00). In our laboratory however, this method never yielded consistent results. Therefore, an alternative method was developed and has been described in Chapter 4. A known volume (1 mL) of a solution with known aroma concentration is continuously stirred in a glass tube. The headspace is constantly removed with a known flow rate. The release is analysed and followed until all aroma has released. The area under this curve can be used to calculate a calibration line. In this way the release could be accurately quantified.

Intra and interpersonal variation in aroma release and perception

When *in vivo* aroma release measurements are performed, intra and interpersonal variation have to be properly dealt with. The sources of intra and interpersonal variation in the absolute amount of aroma released between people are many: differences in mouth, nose and throat geometry, and chewing, swallowing, and breathing behaviour and saliva production (Brown *ea*, '96, Wright *ea*, '03b, Pionnier *ea*, '04). MS-Nose can be used to study these effects accurately.
An example of how interpersonal variation in aroma release can influence aroma release and perception is given by the following experiment conducted in our laboratory. Three groups could be distinguished in a panel assessing dairy desserts by simultaneous Time-Intensity (TI) and *in vivo* aroma release measurements. They were instructed to swallow after 20 seconds. One group had their highest release during the initial chewing. A second group had initial release, but they

peaked at swallowing. Finally, 1 panelist had no release at all before swallowing, and the aroma released only upon swallowing. The TI profiles of these 3 groups closely matched their release curves. This illustrates how opening of the tongue-velum border during chewing can vary between persons and can result in differences in aroma release and perception. It also illustrates the importance of considering individual release data, instead of simply averaging across people (Lethuaut *ea*, In press).

When *in vivo* aroma release of liquids is measured, reduction of experimental variation is of critical importance to obtain meaningful results. The release is determined in a single exhalation after swallowing, while for solid products relatively long release profiles are recorded. One irregular breath does not change this release profile for solids dramatically, but it will have a large effect on the amount of aroma compound released in the first exhalation after swallowing a liquid sample. Reproducible release measurements of liquids are not possible without control of swallowing, breathing and mouth movements. For this purpose, a protocol was developed accounting for these factors (Chapter 2). The relative standard deviation of trained panelists was reduced to 10-15%. Similar variation is generally found for *in vivo* release measurements of solid products.

MS-Nose as a new tool to study *in vivo* aroma release

Static headspace gas chromatography (SHGC) measurements provide information about the partitioning of aroma compounds over product and air phase at equilibrium. To obtain more information about the release of aroma compounds, a range of mouth models has been developed to mimic *in vivo* aroma release during the last decades (Lee, '86, Van Ruth *ea*, '94, Naßl *ea*, '95, Roberts *ea*, '95, Elmore *ea*, '96, Bakker *ea*, '98, Springett *ea*, '99, Rabe *ea*, '02). They generally consist of temperature-controlled chambers in which a relatively large amount of product is stirred or blended. A relative small amount of air is removed from the headspace, and analysed for its volatile content. This approach is thought to be representative for the human mouth.

However, *in vivo* aroma release measurements show the amount of aroma compound that actually releases during consumption. Especially in the case of liquid products, aroma release occurs when exhaling air just after swallowing. A thin liquid layer remains in the human throat after swallowing, and a subsequent exhalation transports the aroma compounds present in that layer to the olfactory epithelium in the nose for detection. A steep aroma concentration gradient exists between this thin layer and the large stream of air flowing along the surface. Under these dynamic conditions, factors that determine partitioning under static headspace conditions and in mouth models are less relevant. These factors are for instance reversible binding to macromolecules or partitioning into hydrophobic phases.

This assumption is supported by most of the parameters that have been investigated in this thesis for their effect on aroma release under *in vivo*, static headspace, and mouth model conditions. Saccharose at concentrations commonly used in beverages (10%) and intense sweeteners, did not

influence the *in vivo* release of 3 lemon-lime aroma compounds (Chapter 2). Whey protein did not exhibit the strong retention of 4 aldehydes *in vivo* (Chapter 3). In contrast, the effects of saccharose and whey protein on aroma release are well established in static headspace and mouth model studies. An increase in oil content gives a decrease in release of hydrophobic aroma compounds under both static headspace and *in vivo* conditions. Once again, this effect is stronger under static headspace conditions than under *in vivo* conditions (Chapter 5). SHGC studies reported large effects of saliva on aroma release, but *in vivo* aroma release studies of liquids with MS-Nose showed that saliva plays only a minor role in aroma release and perception. Table 7.1 provides an overview of the effects of product parameters and saliva on aroma release for different analysis methods.

All these results together clearly show that SHGC and mouth model measurements are much less relevant for *in vivo* aroma release. Thus, conclusions about *in vivo* aroma release, solely based upon these 2 measurements, should be considered carefully. Only by using the MS-Nose for *in vivo* aroma release, it was possible to reveal the effects of compositional product parameters and physiological parameters under actual *in vivo* conditions.

Table 7.1 Effect of product parameters and saliva on aroma release for different analysis methods

Parameter	Chapter	SHGC	Mouth models	*In vivo* release	Artificial throat
Saccharose	2	Effect on release	Not analysed	No effect on release	Not analysed
Whey protein	3 and 4	Strong aroma retention	Strong aroma retention	Weak aroma retention	Weak aroma retention
MCT-oil	5	Strong aroma retention	Not analysed	Weak aroma retention	Weak aroma retention
Saliva	3 and 4	Dilution, aroma binding and enzymatic conversions	Dilution	Minimal dilution	Minimal dilution

Development and use of an artificial throat

Model systems focusing on the simulation of *in vivo* aroma release from liquid products, should focus on the events in the throat rather than those in the mouth, as discussed above. Chapter 4 describes such a device. The artificial throat consists of a vertical tube in which a thin liquid layer is formed when a liquid sample flows down. Subsequently, a large upward stream of air is applied, and aroma compounds release from the thin liquid film and are measured by the MS-

Nose. Similar release profiles were observed when comparing the artificial throat with *in vivo* measurements. Many parameters showed good correlations, such as breath flow, sample volume, aroma concentration, reversible protein interactions (Chapter 4), and oil content (Chapter 5). Therefore, the artificial throat simulates *in vivo* aroma release better than conventional model systems. Moreover, the artificial throat has lower experimental variation than *in vivo* aroma release measurements.

Cross-modal interactions

The development of methods to measure the actual release of aroma compounds during eating made it possible to study the relation between release and perception of aroma. Panelists can score the perceived aroma intensity with Time-Intensity, while the released amount of aroma in their noses is simultaneously measured. Such experiments have been performed for gels with different hardnesses (Chapter 6). The aroma release was not influenced, but the aroma perception decreased, as the gel hardness increased. This is an example of a cross-modal interaction. The texture perception of the gel structure influences the perceived aroma intensity. This was published in 2002. Since then, similar results for textural effects have been reported in literature (Hollowood *ea*, '02, Cook *ea*, '03a, Cook *ea*, '03b). Textural attributes and taste were shown to influence aroma perception without an effect on aroma release. These findings are supported by an increasing amount of neurological studies that show the existence of single neurons that are responsive to different modalities (Cerf-Ducastel *ea*, '01). These findings have increased the understanding of aroma perception and will open the way to new approaches to modulate perceived aroma intensity.

Summary

Until the mid 1990's analytical aroma research predominantly focused on identification and quantification of aroma compounds in food products. However, the aroma composition of a product is not necessarily the same as the aroma compounds that release from the food during eating, which is the way the aroma compounds are perceived by consumers. In the late 1990's, new techniques were developed that combined a high sensitivity and a high time resolution to allow the measurement of the release of aroma compounds in the breath of persons during consumption. Each individual breath is registered. This created the possibility to investigate the effect of food properties on *in vivo* aroma release and to correlate these results to the sensory perception of the aroma and the product as a whole. One of these new techniques is the MS-Nose, developed by Taylor and Linforth of Nottingham University. The aim of this thesis was (1) to develop and validate methods to measure aroma release, using the MS-Nose, and (2) to study the effects of product-related and physiological parameters on aroma release and perception. Chapter 1 gives an introduction to this research.

In vivo aroma release measurements of solid food products give release profiles over a typical time period of 10 to 20 exhalations. These profiles are characterised by an initial increase to a certain maximum, usually associated with swallowing, followed by a decrease. It is not possible to record such a profile for liquid products. When a liquid is placed in the mouth, it is swallowed directly, leaving only a few seconds to measure the *in vivo* aroma release. Full control of breathing, swallowing, and mouth movements are necessary for a reproducible *in vivo* aroma release measurement. Chapter 2 describes the development of such a protocol. The relative standard deviation of *in vivo* aroma release measurements of liquids could be reduced to 10-15%. The smallest detectable aroma concentration difference was 17%. Subsequently, the effect of sweeteners on *in vivo* aroma release was tested, using this protocol. The sweetener concentrations used were relevant for soft drink applications. No differences in *in vivo* aroma release were found for solutions sweetened with either 10% (w/v) saccharose or a mixture of 0.09% sodium cyclamate and 0.015% (w/v) sodium saccharinate (equisweet to 10% saccharose (w/v), or solutions without any sweetener. Differences in aroma perception were reported in literature for aromatised solutions containing different sweeteners (at equisweet concentrations). The results presented here, show that these differences cannot be explained by differences in *in vivo* aroma release.

Chapter 3 deals with the effect of reversible interactions between whey protein and aldehydes on the *in vivo* release of aldehydes from liquids. This effect is well-established in static headspace studies. Under static headspace conditions, the aroma compounds partition between the air and water phase based on their air-water partition coefficient. In the water phase, they are bound to protein or they are free in the solution based on the binding constant between the protein and the

aroma compound. The equilibruim concentration in the air phase is measured. Static headspace studies demonstrated a large decrease in headspace aroma concentrations as a result of strong aroma compound-protein interactions. This was also observed for aldehydes and whey protein in Chapter 3. However, *in vivo* aroma release measurements showed a much smaller decrease. The difference between static headspace and *in vivo* aroma release could not be attributed to the effect of saliva, which was investigated using a mouth model. Other experiments showed that the mucous membranes of mouth, nose, and throat could not explain the reduced effect of whey protein on *in vivo* aldehyde release either. The most likely explanation of the difference is that the results of a static and a dynamic system are compared. The majority of a liquid sample disappears into the esophagus upon swallowing and is not available for aroma release. However, a thin liquid layer remains at the inner surface of the throat. During subsequent exhalation, a relatively large stream of air flows along this thin liquid layer. An air stream of a few seconds is already long enough to remove all the aroma compound present in this layer and to transport it to the nasal cavity. Not only aroma compounds that are free in the solution will release, but also those reversibly bound to whey protein. This is the reason why the effect of reversible whey protein is much smaller under *in vivo* conditions, compared to static headspace conditions.

The crucial role of this thin layer in the throat for *in vivo* aroma release of liquids was the basis for a newly developed model system to simulate *in vivo* aroma release of liquids. This so-called artificial throat has been described in Chapter 4 and consists of a vertical glass tube with a rubber section that can be closed with a clamp. Aromatised solution is added above this closed part. When the clamp opens, the liquid pours down, leaving a thin liquid layer on the rubber and the glass surface. Subsequently, an upward stream of air is applied in the tube. Finally, this air is analysed by the MS-Nose. The release profiles obtained with the artificial throat were highly comparable to the *in vivo* release profiles. Many parameters showed good correlation between artificial throat and *in vivo* aroma release, such as air flow rate, aroma concentration, sample volume, and reversible interaction with protein. An advantage of a model system with high correlation to the *in vivo* situation is the fact that parameters influencing the release can be easily changed and studied to increase understanding of *in vivo* aroma release. Moreover, the experimental variation of the artificial throat is smaller than *in vivo* measurements. In addition, the artificial throat can be used to measure the aroma release of samples that are too dangerous or too repulsive to be analysed *in vivo*.

The effects of oil content and oil droplet size distribution on release of a set of ester compounds have been studied under *in vivo*, artificial throat and static headspace conditions, using dilute oil-in-water emulsions (Chapter 5). The effect of oil content on perception of ethyl hexanoate has been studied using a 7-person panel. The aroma intensity was assessed both orthonasally (sniffing of a freshly opened bottle), and retronasally (swallowing the sample, followed by exhalation). The effect of oil content was stronger under static headspace conditions than under *in vivo* and artificial throat conditions, and stronger for orthonasal perceived intensity than for retronasal perceived intensity. The relatively large breath flow along the thin emulsion layer in the throat after swallowing does not only transport ester compounds from the water phase, but also those

from the oil phase (through the water phase). This reduces the effect of oil content on *in vivo* aroma release. The results obtained under *in vivo* and artificial throat conditions correlated linearly. The droplet size distribution only affected the release of the hydrophobic geranyl acetate and only under *in vivo* conditions.

Chapter 6 describes a study in which the aroma release and perception were determined for a range of gels with increasing gel hardnesses. The *in vivo* aroma release was monitored for 10 panelists during 1.5 min (swallowing after 0.5 min). Simultaneously to the aroma release measurement, the panelists scored the perceived aroma intensity using the Time-Intensity method. The aroma release was independent of the gel hardness. However, the aroma perception decreased when the gel hardness increased (for 6 out of 10 panelists). This was attributed to a psychophysical effect. The texture perception influences the aroma perception, while the aroma release does not change.

Chapter 7 contains the main conclusions and the general discussion. The MS-Nose can be used for quantitative analysis of aroma release, provided that the signal measured is unique for the aroma compound studied, and the response is linear. In addition, the experimental variation should be reduced, by using the protocol for liquids. The results of *in vivo* aroma release with sweeteners, protein, and oil, have shown that *in vivo* aroma release measurements are needed to determine the effect of product parameters on *in vivo* aroma release during consumption. This is not possible based on static headspace and mouth model measurements alone. In case of liquid samples, the thin layer in the throat has to be taken into account. This concept allowed development of the artificial throat. The release profiles of this model system correlated well with *in vivo* results. Finally, the availability of a technique to measure *in vivo* aroma release made it possible to demonstrate the existence of cross-modal interactions.

Samenvatting

Analytisch aroma onderzoek van levensmiddelen heeft zich tot midden jaren ’90 voornamelijk geconcentreerd op het identificeren en kwantificeren van aromastoffen in levensmiddelen. De aroma samenstelling van een product is echter niet noodzakelijkerwijs representatief voor de aromastoffen die vrijkomen tijdens het eten of drinken van een product, zoals ze door de consument waargenomen worden. Eind jaren ’90 kwamen er technieken beschikbaar die door een hoge gevoeligheid èn tijdresolutie geschikt waren om het vrijkomen van aromastoffen in de adem van etende proefpersonen te kunnen meten (ook wel: *in vivo* aroma release). Elke afzonderlijke ademhaling wordt geregistreerd. De mogelijkheid was ontstaan om het effect van producteigenschappen op het vrijkomen van aromastoffen tijdens consumptie te bepalen en te relateren aan de gewaarwording van het aroma en het product als geheel (aroma en product perceptie). Een van deze technieken is de MS-Nose, ontwikkeld door Taylor en Linforth van de Universiteit van Nottingham. Het doel van het onderzoek beschreven in dit proefschrift was (1) het ontwikkelen en valideren van methoden om *in vivo* aroma release te meten, gebruikmakend van de MS-Nose, en (2) de effecten van productgerelateerde en fysiologische factoren op aroma release en perceptie te bepalen. Hoofdstuk 1 geeft een inleiding tot dit onderzoek.

In vivo aroma release metingen van vaste producten waarop enige tijd gekauwd kan worden, geven release profielen over een tijdspanne van 10 tot 20 ademhalingen. Deze worden gekenmerkt door een initiële toename tot een maximum, meestal geassocieerd met het doorslikken, gevolgd door een afname. Bij vloeistoffen is het niet mogelijk een dergelijke release curve op te nemen. Na het in de mond nemen volgt direct doorslikken, waardoor er slechts een paar seconden zijn waarin de *in vivo* aroma release gemeten kan worden. Volledige controle van ademhaling, slikken en mondbewegingen is noodzakelijk voor een reproduceerbare *in vivo* aroma release meting. Hoofdstuk 2 beschrijft de ontwikkeling van een dergelijk protocol. De relatieve standaardfout van de *in vivo* aroma release metingen van vloeistoffen kon gereduceerd worden tot 10-15%. Het kleinste significant detecteerbare verschil in aroma concentratie was 17%. Verder is gebruikmakend van het vloeistofprotocol, ook het effect van verschillende zoetstoffen op de *in vivo* aroma release onderzocht. De gebruikte zoetstofconcentraties waren relevant voor toepassing in frisdranken. Er bleek geen verschil in aroma release te zijn tussen 10% (w/v) saccharose, een mengsel van 0,09% (w/v) natriumcyclamaat en 0,015% (w/v) natrium saccharinaat (dat even zoet smaakt als 10% (w/v) saccharose), en een oplossing zonder zoetstoffen. In de literatuur zijn verschillen in aromaperceptie bij aroma oplossingen met verschillende zoetstoffen (met gelijke zoetheid) beschreven, maar uit de hier gepresenteerde resultaten blijkt dat die sensorische verschillen niet aan *in vivo* aroma release toegeschreven kunnen worden.

Hoofdstuk 3 behandelt het effect van reversibele interacties tussen wei-eiwit en aldehyden op de *in vivo* aroma release van aldehyden uit vloeistoffen. Dit effect is uitvoerig beschreven in de

literatuur voor statische headspace omstandigheden. De aroma componenten hebben zich in die situatie verdeeld over de lucht-, en waterfase op basis van hun lucht-water verdelingscoëfficiënt, en zijn in de waterfase vrij of gebonden aan eiwit op basis van hun bindingsconstante met het eiwit. De evenwichtsconcentratie in de headspace wordt gemeten. Dergelijke studies lieten zeer grote afnamen van de headspace aroma concentratie zien, als gevolg van sterke eiwit-aroma interacties. Dit werd ook gevonden voor aldehyden en wei-eiwit in hoofdstuk 3. De *in vivo* aroma release metingen lieten echter een veel kleinere afname in aldehyde release zien. Dit verschil tussen statische headspace en *in vivo* release kon niet toegeschreven worden aan het effect van speeksel, zo bleek uit experimenten met een kunstmond. Andere experimenten toonden aan dat de slijmvliezen van mond-, neus-, en keelholte ook niet het verminderde effect van wei-eiwit op aldehyde retentie *in vivo* konden verklaren. De meest waarschijnlijke verklaring voor dit verschil ligt in het feit dat hier een statisch systeem met een zeer dynamisch systeem wordt vergeleken. Tijdens slikken verdwijnt het grootste deel van een slok te onderzoeken (vloeibaar) product in de slokdarm en is niet meer beschikbaar voor aroma release. Er blijft echter wel een dun laagje materiaal zitten aan de binnenwand van de keel. Bij de eerstvolgende uitademing stroomt een relatief grote luchtstroom langs dit uiterst dunne vloeistoflaagje. Een ademstroom van enkele seconden is al voldoende om al het aroma dat zich in het laagje bevindt, mee te nemen. Hierbij komt niet alleen het vrij in water opgeloste aroma vrij, maar ook het aroma dat op dat moment reversibel met eiwit gebonden is. Door de dynamiek van dit systeem is het effect van reversibele eiwitbinding op aroma retentie in vloeistoffen veel kleiner *in vivo* dan onder statische evenwichtsinstelling.

De cruciale rol die het dunne laagje in de keel speelt bij *in vivo* aroma release van vloeistoffen, vormde de basis voor een nieuw model systeem om de *in vivo* aroma release van vloeistoffen te simuleren. Deze zogenaamde kunstkeel is beschreven in hoofdstuk 4 en bestaat uit een verticale glazen buis met een rubber sectie die met een klem dichtgeknepen kan worden. Hier bovenop wordt een gearomatiseerde vloeistof aangebracht. Als de klem opent, stroomt de vloeistof door de buis naar beneden en laat een dun laagje aan de wand achter. Vervolgens voert een luchtstroom door de buis omhoog. Bovenin wordt de uitstromende lucht geanalyseerd door de MS-Nose. Release profielen van de kunstkeel bleken zeer vergelijkbaar met *in vivo* aroma release profielen. Voor veel parameters werd een goede correlatie gevonden tussen kunstkeel en *in vivo* aroma release, zoals de ademsnelheid, aroma concentratie, monstervolume en reversibele interactie met eiwitten. De voordelen van een goed correlerend model systeem voor *in vivo* aroma release zijn dat veel parameters makkelijk gevarieerd kunnen worden, wat leidt tot een groter inzicht in aroma release. Verder is de meetfout van de kunstkeel kleiner dan bij *in vivo* metingen. Ook kan de aroma release gemeten worden van monsters die te vies of te gevaarlijk zijn voor menselijke consumptie.

De effecten van het oliegehalte en de oliedruppelgrootteverdeling op aroma release zijn voor een verdunde olie-in-water emulsie onderzocht voor een reeks esterverbindingen onder *in vivo*, kunstkeel en statische headspace omstandigheden (hoofdstuk 5). Verder is het effect van het oliegehalte op de perceptie van ethyl hexanoaat met een 7-koppig panel onderzocht. Hierbij werd

de aroma intensiteit zowel orthonasaal (ruiken aan een net geopend potje) als retronasaal (monster doorslikken, gevolgd door uitademen) beoordeeld. Het effect van het oliegehalte was groter onder statische headspace omstandigheden dan onder *in vivo* en kunstkeel omstandigheden en groter bij orthonasale perceptie dan bij retronasale perceptie. Door de sterke ademstroom langs het dunne emulsielaagje in de keel komt niet alleen het aroma uit de waterfase vrij, maar ook vanuit de oliefase (via de waterfase). Dit vermindert het effect van het oliegehalte op de *in vivo* aroma release. De resultaten onder *in vivo* en kunstkeel omstandigheden vertoonden opnieuw een lineaire correlatie. De oliedruppelgrootteverdeling speelde alleen een rol bij het zeer hydrofobe geranyl acetaat onder *in vivo* omstandigheden.

In hoofdstuk 6 wordt een studie beschreven waarbij de release en de perceptie van aroma uit een reeks gelen met sterk oplopende hardheid is bepaald. De *in vivo* aroma release werd gedurende anderhalve minuut (doorslikken na 30 s) gemeten bij 10 proefpersonen. Tegelijkertijd gaven zij continu de waargenomen intensiteit aan op een computerscherm (de zogenaamde Time-Intensity methode). De aroma release bleek onafhankelijk te zijn van de gelsterkte. De aromaperceptie ging echter wel omlaag, bij toenemende gelsterkte (bij 6 van de 10 proefpersonen). Dit is toegeschreven aan een psychofysisch effect. De perceptie van de textuur beïnvloedt de perceptie van het aroma, ondanks dat de release gelijk blijft.

De belangrijkste conclusies en algemene discussie staan in hoofdstuk 7. De MS-Nose kan gebruikt worden voor kwantitatieve analyse van aroma release tijdens consumptie, zolang er gecontroleerd wordt of het gemeten signaal uniek is voor de te bestuderen aromastof en of de respons lineair is. Verder moeten er maatregelen genomen worden om een reproduceerbare meting te garanderen, zoals het gebruiken van een protocol. De resultaten met suiker, eiwit en olie, hebben aangetoond dat *in vivo* aroma release metingen nodig zijn om de werkelijke effecten van product parameters op aroma release tijdens consumptie te bepalen. Op basis van gegevens van de statische headspace en kunstmond metingen alleen, ontstaat een onvolledig beeld. Bij vloeistoffen moet er rekening gehouden worden met een dun vloeistoflaagje dat zich tijdens slikken vormt in de keel. Door dit inzicht was het mogelijk de kunstkeel te ontwikkelen, waarvan de aroma release goed correleert met *in vivo* aroma release. Ten slotte kon dankzij de beschikbaarheid van een techniek om *in vivo* aroma release te meten, interzintuiglijke beïnvloeding tussen aroma en textuur aangetoond worden.

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Dankwoord

24 cm, 250 g, kerngezond…en we noemen hem: ‘Release and perception of aroma compounds during consumption’. Na vier jaar blijde verwachting is ie er. Het was een questie van een lange adem, want vanaf begin af aan kwamen de metingen bij mij al de neus uit…en bij alle proefpersonen… Maar, la situation est sous control en we kunnen nu concluderen dat Mercedes rijden alleen niet voldoende is om te slagen als Aio. De hulp van anderen is ook essentieel, daarom: iedereen bedankt!

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KWeel

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Curriculum vitae

Koen Weel werd geboren op 19 juni 1976, te Roermond. Hij behaalde het diploma Gymnasium in 1994 aan het Christelijk Lyceum te Arnhem. Aansluitend studeerde hij Levensmiddelen-technologie aan de toenmalige Landbouwuniversiteit te Wageningen. In het kader van deze studie deed hij afstudeervakken bij de vakgroepen Levensmiddelenchemie en Biochemie, en liep hij stage bij de Kauno Technologijos Universitetas te Kaunas in Litouwen en bij Quest International te Naarden. Het ingenieursdiploma werd behaald in september 1999. Van oktober 1999 tot januari 2004 deed hij promotie-onderzoek bij NIZO food research, te Ede, in opdracht van Quest/ICI en in samenwerking met de leerstoelgroep Levensmiddelenchemie van de Wageningen Universiteit. Dit onderzoek staat beschreven in dit proefschrift. Sinds maart 2004 is hij werkzaam als scientist product and process design bij Numico Research te Wageningen.

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