Chem. Senses 34: 395–403, 2009
doi:10.1093/chemse/bjp011
Advance Access publication March 13, 2009

Retro-Nasal Aroma Release Depends on Both Subject and Product Differences: A Link to Food Intake Regulation?

Rianne M.A.J. Ruijschop[1], Maurits J.M. Burgering[1], Marc A. Jacobs[1,2] and Alexandra E.M. Boelrijk[1,3]

[1]NIZO Food Research, Kernhemseweg 2, P.O. Box 20, 6710 BA Ede, the Netherlands, [2]Present address: Friesland Foods Corporate Research, Harderwijkerstraat 41006, P.O. Box 87, 7400 AB, Deventer, the Netherlands and [3]Present address: Danone Research Medical Nutrition, Bosrandweg 20, P.O. Box 7005, 6700 CA, Wageningen, the Netherlands

Correspondence to be sent to: Rianne M.A.J. Ruijschop, NIZO Food Research, Kernhemseweg 2, P.O. Box 20, 6710 BA Ede, The Netherlands. e-mail: rianne.ruijschop@nizo.nl

Abstract

It is hypothesized that differences in the extent of retro-nasal aroma release during consumption may be 1 of the reasons that people vary in their satiation characteristics. Using real-time atmospheric pressure chemical ionization mass spectrometry (APcI-MS), *in vivo* retro-nasal aroma release was determined for 30 subjects consuming 9 different food products, varying in physical structure (i.e., [semi]liquid and solid food products). Additionally, for a subset of the subjects *ad libitum* food intake was measured. Retro-nasal aroma release intensity and profile morphology appeared to be subject specific and relatively independent of the type of food product subjects consumed. A subject who was observed as having a relatively high retro-nasal aroma release intensity for a (semi)liquid food product also appeared to have a relatively high retro-nasal aroma release intensity for a solid food product. However, for all subjects, there were absolute differences between food products in the extent of retro-nasal aroma release comparing (semi)liquid and solid food products. This implies that the extent of retro-nasal aroma release is a valid physiological feature that characterizes any individual. Interestingly, a negative trend was observed between extent of retro-nasal aroma release and amount of *ad libitum* food intake ($P = 0.07$). This may have implications for the regulation of food intake.

Key words: APcI-MS, flavor, olfactometry, oral processing, retro-nasal aroma stimulation, satiation

Introduction

Aroma is an important property that contributes to sensory perception of a food product (Meilgaard et al. 1999). It is likely that sensory effects, including aroma stimuli, contribute to a large extent to satiation, resulting in meal termination (Sorensen et al. 2003; Ruijschop et al. 2008). During the consumption of a meal, aroma molecules reach the olfactory epithelium either orthonasally (perceived as originating from the external world) or retro-nasally (perceived as arising from the mouth) (Murphy et al. 1977; Rozin 1982). The brain response, that is, neural brain activation, to a retro-nasally sensed food odor signals the perception of food and is suggested to be related to satiation (Small et al. 2005). Retro-nasal aroma stimulation is mainly related to the event of swallowing, when a small volume of air is exhaled immediately after swallowing, the so-called ''swallow breath.'' It is assumed that this pulse should contain the major part of food volatiles that have been released from the food product prior to swallowing, and should therefore elicit a retro-nasal aroma pulse (Land 1994; Buettner and Schieberle 2000).

From previous work, it is known that the physical structure of a food that is consumed is important for the extent of retro-nasal aroma release during consumption (Linforth et al. 1999; Cook et al. 2003; Lethuaut et al. 2004; van Ruth et al. 2004). Additionally, subject differences are known to be important for the extent of retro-nasal aroma release. These subject differences are factors that are likely to be uncontrolled by a person, for example, saliva production, nasal anatomy, and oral processing habits (Brown et al. 1996; Buettner et al. 2001, 2002; Wright et al. 2003; Pionnier et al. 2004).

It is hypothesized that differences in the extent of retro-nasal aroma release during consumption may be 1 of the reasons that people vary in their satiation characteristics, due to differences in perceived intensity or duration of sensory stimulation.

The aim of the present study was to investigate whether subjects can be segmented based on their extent of retro-nasal aroma release using real-time atmospheric pressure chemical ionization mass spectrometry (APcI-MS) and whether this depends on the type of food product they consume. Ultimately, based on this segmentation, the aim was to determine whether subject differences in the extent of retro-nasal aroma release can be linked to subject differences in sensory satiation and perhaps food intake behavior.

Materials and methods

Subjects

Thirty healthy subjects (13 men and 17 women) aged 18–65 years living in Wageningen (the Netherlands) and surroundings were recruited from an existing database for consumer studies. Subjects did not have any previous experience with APcI-MS measurements. The subjects included normal-weight, overweight, and obese subjects, with a body mass index (BMI) varying between 18 and 32 kg m^{-2}. BMI was calculated as body weight (kg) divided by height (m) squared. Body weight was measured without wearing shoes using a calibrated scale that was accurate to 0.1 kg (Inventum, Veenendaal, the Netherlands). At the same time without wearing shoes, height was measured with a wall-mounted stadiometer (Microtoise mabo 4116, Brevete, France).

Subjects' degrees of dietary restraint were determined using the Dutch translation of the 3-Factor Eating Questionnaire (TFEQ) (Westerterp-Plantenga et al. 1999). Based on the outcome of the TFEQ, these subjects showed no dietary restraint (i.e., scores ≤9), no disinhibition (i.e., emotional eating) (i.e., scores ≤ 8), and no physiological hunger (i.e., scores ≤ 8). Subject characteristics, including TFEQ scores, are summarized in Table 1. Subjects were fully informed about the course of the APcI-MS measurement.

Table 1 Characteristics of the 30 subjects who participated in this study[a]

	Women (n = 17)		Men (n = 13)	
	Normal weight (n = 10)	Overweight (n = 7)	Normal weight (n = 7)	Overweight (n = 6)
Age (years)	43 ± 13	49 ± 14	31 ± 8	59 ± 12
BMI (kg m^{-2})	22 ± 3	28 ± 2	23 ± 2	27 ± 2
TFEQ[b]				
Factor 1 (cognitive restraint)	5 ± 2	7 ± 2	4 ± 3	6 ± 2
Factor 2 (disinhibition)	3 ± 1	3 ± 1	4 ± 1	3 ± 1
Factor 3 (hunger)	3 ± 2	4 ± 2	3 ± 2	2 ± 1

[a]Mean ± SD.
[b]TFEQ: Three-Factor Eating Questionnaire. The value of factors 1, 2, and 3 is in the range of 0–18, 0–13, and 0–14, respectively. A higher value indicates more restraint, disinhibition, or physiological hunger. All values are below medians that are usual.

Food products

Subjects consumed 9 different food products, varying in physical structure (i.e., liquid, semiliquid, and solid food products). All food products were commercially available. In the category liquid food products, the subjects consumed a strawberry-flavored dairy beverage (Fristi, RiedelDrinks, Friesland Foods, Meppel, the Netherlands). In the category semiliquid food products, the subjects consumed banana-flavored custard (private label Albert Heijn Zaandam, the Netherlands) and raspberry pudding (Mona, Campina, Woerden, the Netherlands). In the category solid food products, the subjects consumed cheese-flavored crackers (TUC, LU, General Biscuits Nederland B.V., Danone, Breda, the Netherlands), milk, and dark (extra bitter) chocolate (private label Albert Heijn Zaandam, the Netherlands), young and aged (Gouda type) cheese (private label Albert Heijn Zaandam, the Netherlands), and winegums (candy) (Red Band, LEAF Holland B.V., Oosterhout, the Netherlands).

Measurement of the extent of retro-nasal aroma release with APcI-MS technology

To detect the aroma compounds with the highest response in retro-nasal aroma release, all food products were measured separately. Aroma compounds in the air released from the artificial mouth were monitored by on-line sampling by an Atmospheric Pressure Chemical Ionization Gas-Phase Analyzer attached to a VG Quattro II mass spectrometer (MS-Nose; Micromass UK Ltd., Manchester, United Kingdom) (van Ruth et al. 1994; Taylor and Linforth 1996; Taylor et al. 2000; Weel et al. 2003; Weel et al. 2004). Compounds were ionized by a 3.0 kV discharge (source and probe temperatures were 80 °C) and scanned for m/z 50–250. m/z values (i.e., the ion mass–charge ratio of a specific aroma component) with the highest response were selected (Table 2). *In vivo* retro-nasal aroma release was assessed in exhaled breath of the 30 subjects for 9 food products in triplicate in a fixed volume and size (i.e., mouthful consumption; Table 2). Subjects breathed in and out through the nose. One nostril was placed over a small disposable plastic tube, allowing them to breathe, drink, and eat normally. Aroma compounds in the air released from the breath of subjects were monitored by online sampling part of the exhaled air directly into the APcI-MS via the tube. The air was sampled (75 mL/min) through a capillary tube (0.53 mm internal diameter, heated to 100 °C). The compounds were monitored in selected ion mode (0.08 s dwell on each ion), in 2 independent sets. The cone voltage used was 20 V.

Subjects were free to use their own natural eating habits during the experiments (without any chewing protocol). Between food products, the mouth was rinsed with water. Blank experiments were recorded before the consumption of food products with water following the same protocol. Acetone, present in human breath, was measured at m/z 59 (19 V) as an indicator for the breathing pattern (Weel

Table 2 Food products consumed in a fixed volume and size with their *m/z* value with the highest response in APcI-MS measurement

Product	Category	Serving per measurement	Volume/size per serving	Ion mass (*m/z* value)
Young Gouda type cheese	Solid	1 Piece	2 × 1 × 1 cm; 8 g	73 & 89
Aged Gouda type cheese	Solid	1 Piece	2 × 1 × 1 cm; 8 g	73 & 89
Cheese-flavored cracker	Solid	1 Piece	2 Halves piled up; 4.5 g	73 & 87
Milk chocolate	Solid	1 Piece	6.5 g	87
Dark chocolate	Solid	1 Piece	6.5 g	87
Strawberry-flavored dairy beverage	Liquid	1 Sip	17 mL	117 & 131
Banana-flavored custard	Semiliquid	1 Spoon	20 g	131 & 145
Raspberry pudding	Semiliquid	1 Spoon	20 g	71 & 131
Winegum candy	Solid	1 Piece	5 g	83 & 117

et al. 2002). The area of the resulting breath peaks in the aroma signal was taken as a measure of *in vivo* retro-nasal aroma release. Different parameters could be extracted from each individual retro-nasal aroma release curve, characterizing the extent of retro-nasal aroma release, that is, $T_{-1/2}$, T_{max}, $T_{1/2}$, I_{max}, and Area Under Curve (AUC) (Figure 1). Because we were interested in comparative retro-nasal aroma release between subjects, expression of the extent of retro-nasal aroma release in arbitrary units (AU) was sufficient to analyze differences (Taylor et al. 2000).

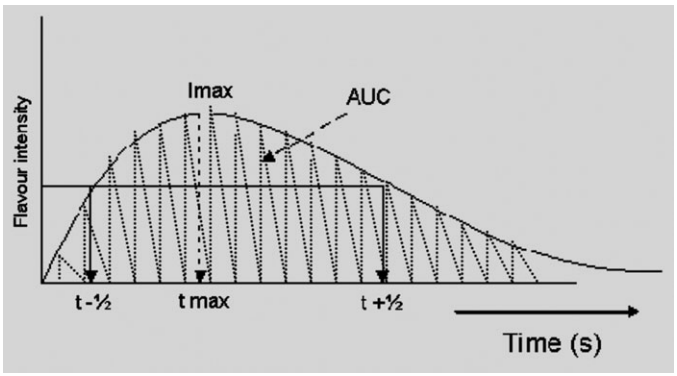

Figure 1 Schematic representation of a retro-nasal aroma release curve and its characteristic parameters (I_{max}: maximum intensity [AU], T_{max}: time at which maximal intensity occurs [min], AUC: total area under the curve [AU], $T_{-1/2}$: time at which half of the maximal intensity occurs, before reaching maximum intensity [min], $T_{1/2}$: time at which half of the maximal intensity occurs, after reaching maximum intensity [min]).

Measurement of ad libitum food intake

In a subsequent study for a subset of 15 subjects (8 men and 7 women; aged 20–30 years; BMI 20–30 kg·m^{-2}), the effect of retro-nasal aroma release intensity on food intake was investigated using a similar approach as described in Ruijschop et al. (2008). The aim was to investigate if people became more or less satiated if they were more or less aroma stimulated than normal. For the 15 subjects their natural retro-nasal aroma release for the given food product had already been assessed using APcI-MS technology. Subsequently, in a double-blind placebo-controlled randomized crossover full factorial design, each subject was, on separate days, administered his or her own natural aroma release profile, a 4 times higher concentrated, and a 4 times lower concentrated aroma release profile using a computer-controlled stimulator based on air dilution olfactometry (OM4, Burghart, Wedel, Germany). The profiles were produced with generic cheese aroma (Givaudan, Naarden, the Netherlands) and administered in a retro-nasal fashion (i.e., approximately 9 cm in length of a silicon tube (suction catheter CH 10, D-Care B.V., Houten, The Netherlands) was placed into the subjects' lower meatus of the right nasal cavity) while the subjects consumed a cold, fusilli tricolore pasta (AH private label Albert Heijn Zaandam, the Netherlands), which was boiled in salty water (100 g pasta boiled in 10 g salt per liter water). After retro-nasal aroma stimulation *ad libitum* food intake of young-matured Gouda type cheese (AH private label Albert Heijn Zaandam) was measured. The total amount of young-matured cheese consumed per subject during his or her 3 test days was calculated, and this amount served as measure for *ad libitum* young-matured cheese intake.

Data analysis

To assess the extent of retro-nasal aroma release, different food products (n = 9) were evaluated in triplicate, that is, on 3 test occasions (n = 3), by all subjects (n = 30) in a cross-over design.

Descriptive (qualitative) data analysis

The triplicate measurements of the characteristic retro-nasal aroma release parameters (T_{max}, I_{max}, and AUC) for each subject per food product were averaged. This was allowed, because test occasion did not explain a significant part of the variance regarding differences in the extent of retro-nasal

aroma release. The mean values of the characteristic parameters of the retro-nasal aroma release curve were standardized for each individual food product, by division of the average value by the standard error. This type of standardization was necessary because all food products had a different magnitude of the characteristic retro-nasal aroma release parameters. The set of data obtained was evaluated by regular statistical means, such as descriptive statistics. In addition, food products and subjects were grouped together in a regular Principal Component Analysis (PCA) projection. Furthermore, Hierarchical Cluster Analysis was applied using Ward's method with the calculation of Euclidean distances among morphology (I_{max} and T_{max}) and intensity (I_{max} and AUC) of the retro-nasal aroma release curve.

Analytical (quantitative) data analysis

Because all food products had a different magnitude of the characteristic retro-nasal aroma release parameters, and it was noticed that the data set was not normally distributed, nonparametric statistical testing was applied to the raw data for the mean value of the triplicate measurements for each subject per food product. To this end, the Spearman rank correlation coefficient was calculated for T_{max}, I_{max}, and AUC across subjects to assess whether subjects could be characterized with respect to their extent of retro-nasal aroma release irrespective of the type of food product consumed.

After ranking the mean value of the triplicate measurements across subjects for each food product for both I_{max} and AUC, the sum of both ranks across subjects for each food product was obtained. When adding the sum of both ranks across subjects for each food product, a final rank for I_{max} and AUC combined was calculated with respect to retro-nasal aroma release intensity across subjects for all food products. In summary:

Final rank = $\sum[(\sum(\text{rank } I_{max} \text{ and rank AUC})_{\text{across subjects for each food product}})$ across subjects for each food product].

For the morphology of the retro-nasal aroma release profile, the sum of ranks for I_{max} and T_{max} across subjects for each food product and subsequently all food products (final rank) was calculated similarly.

Additionally, the extent of the ranked retro-nasal aroma release was linked to subject characteristics, like age and BMI, and food intake behavior.

Statistical analysis was performed with Statistica (1999, StatSoft Inc., Tulsa, OK, United States). P values < 0.05 were considered as being statistically significant.

Results

Validity of response of selected *m/z* signal

Selected aroma compounds detected in the range of *m/z* 20–250 (Table 2), that is, aroma compounds with the highest response showed comparable retro-nasal aroma release profiles for a specific food product. For example, the standardized retro-nasal aroma release parameters (T_{max}, I_{max}, and AUC) for *m/z* 131 and *m/z* 145 for banana-flavored custard appeared to be highly correlated ($R^2 = 0.8$; $P < 0.001$, $R^2 = 0.9$; $P < 0.001$, $R^2 = 0.9$; $P < 0.001$, for T_{max}, I_{max}, and AUC, respectively). Other food products showed comparable results. Therefore, the same retro-nasal aroma release profile was measured, irrespective of the *m/z* value taken (data not shown). Hence, it was appropriate to select *m/z* values with the highest response for a specific food product for the *in vivo* retro-nasal aroma release measurements.

Within-subject reproducibility in the extent of retro-nasal aroma release

Irrespective of the time of measurement, for a specific food product, test occasion did not explain part of the variance regarding differences in the extent of retro-nasal aroma release. The serving number did not affect the magnitude of the characteristic retro-nasal aroma release parameters during consumption of, for example, a spoon of banana-flavored custard or a piece of aged cheese (respectively, for banana-flavored custard Friedman analysis of variance (ANOVA): $P = 0.99$ for T_{max}, $P = 0.98$ for I_{max}, $P = 0.88$ for AUC and for aged cheese Friedman ANOVA: $P = 0.39$ for T_{max}, $P = 0.90$ for I_{max}, $P = 0.65$ for AUC). In summary, the extent of retro-nasal aroma release that was evoked in subjects during consumption of a specific food product appeared to be reproducible with respect to retro-nasal aroma release intensity and profile morphology.

Subject differences affecting the extent of retro-nasal aroma release

Subjects differed in the extent of retro-nasal aroma release regarding intensity (I_{max} and AUC) and morphology (I_{max} and T_{max}) of the retro-nasal aroma release profile. As illustrated by Figure 2, 2 different types of retro-nasal aroma release patterns were identified. Representatives of 2 groups are shown: a group of subjects with a maximum retro-nasal aroma release after swallowing, that is, retro-nasal aroma release likely through the swallow breath (Land, 1994; Buettner and Schieberle 2000) (Figure 2A), and a group of subjects with an earlier maximum retro-nasal aroma release immediately after starting chewing, that is, retro-nasal aroma release likely through the velopharyngeal portal, because the velum is intermittently open during oral processing (Buettner et al. 2001) (Figure 2B1). In addition, within 1 group of subjects with the same retro-nasal aroma release pattern large differences were observed in retro-nasal aroma release intensity among subjects (Figure 2B2). The extent of retro-nasal aroma release that was evoked in subjects during consumption of a specific food product appeared to be reproducible with respect to retro-nasal

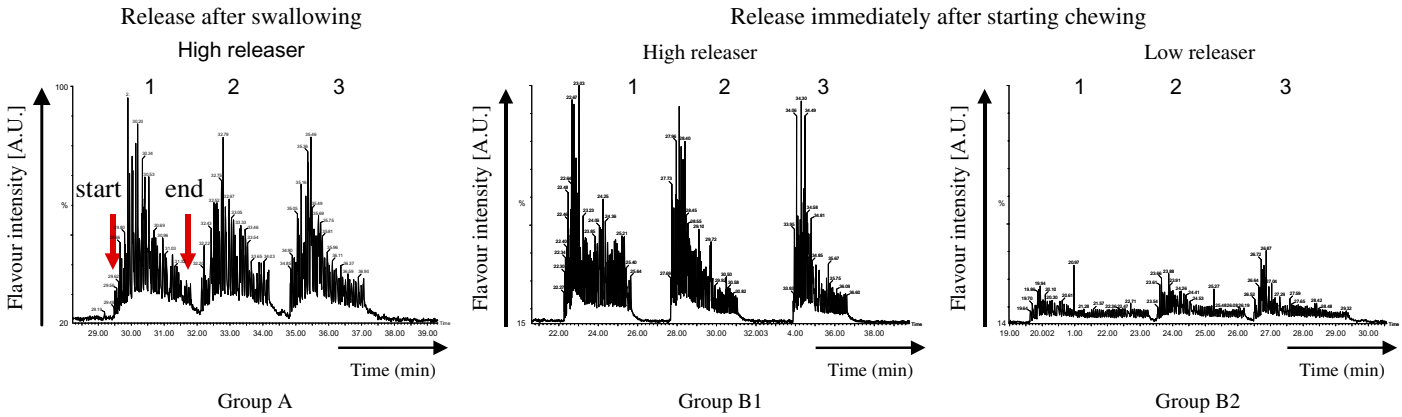

Figure 2 Illustration of the 2 different groups regarding the morphology and intensity of the retro-nasal aroma release profile. Examples given for aged cheese (1, 2, and 3 stand for the triplicate measurements). Start and end of a single retro-nasal aroma release curve are depicted with arrows. For each retro-nasal aroma release curve, the characteristic retro-nasal aroma release parameters (T_{max}, $T_{+1/2}$, I_{max}, and AUC) can be extracted, similarly as represented in Figure 1.

aroma release intensity and profile morphology (Friedman ANOVA: $P > 0.05$).

To investigate whether subjects could be grouped based on their retro-nasal aroma release profiles, subjects were segmented based on the extent of their retro-nasal aroma release intensity (I_{max} and AUC) ("high vs. low–medium releasers") and the morphology of their retro-nasal aroma release curve (I_{max} and T_{max}) ("early vs. late releasers") after consumption of solid and (semi)liquid food products, respectively.

With respect to the extent of retro-nasal aroma release intensity, a number of subjects showed relatively low-to-medium retro-nasal aroma release intensities, whereas other subjects displayed relatively high retro-nasal aroma release intensities after consumption of solid and (semi)liquid food products. As well as the intensity of their retro-nasal aroma release, subjects could also be segmented on the morphology of their retro-nasal aroma release curve. However, segmentation was only possible using the APcI-MS data obtained after consumption of solid food products. This was an expected result, because the morphology of these curves contained more information than the data obtained after consumption of a (semi)liquid food product. (Semi)liquid food products have a relatively short transit time in the oral cavity, and therefore, hardly any oral processing is needed to swallow them. Consumption of (semi)liquid food products thus resulted in relatively short and spiked retro-nasal aroma release patterns, which were difficult to segment based on morphology of the retro-nasal aroma release curve. With respect to solid food products, a number of subjects showed a relatively early start of retro-nasal aroma release, that is, maximal intensity (I_{max}) occurred relatively fast. Conversely, other subjects showed a relatively late start of retro-nasal aroma release. Physiological differences in timing and performance of mastication and swallowing are attributed to be responsible for these differences (Buettner et al. 2001).

Product differences affecting the extent of retro-nasal aroma release

Apart from subject differences in the extent of retro-nasal aroma release, product differences were also factors important for the extent of retro-nasal aroma release. Figure 3 illustrates the differences in the extent of retro-nasal aroma release during consumption of a solid (i.e., aged cheese) compared with a liquid (i.e., strawberry-flavored dairy beverage) food product.

Solid food products required considerable chewing and swallowing, due to their firmer texture. Consequently, most subjects had an immediate and prolonged retro-nasal aroma release. In contrast to consumption of solid food products, during the consumption of (semi)liquid food products, most subjects had a short and spiked retro-nasal aroma release pattern. Intensity levels differed between the subjects. PCA projection supported these observations (data not shown).

Characterization of subjects and food products based on the extent of retro-nasal aroma release

Spearman rank correlation coefficient showed that for the 9 food products, evaluated on 3 test occasions by all subjects, I_{max} and AUC were significantly correlated (data not shown). Therefore, it was justified to calculate a combined rank as the sum of both parameters.

After ranking the mean value of the triplicate measurements across subjects for each food product for both I_{max} and AUC, the sum of both ranks across subjects for each food product was obtained. The sum of the combined rank I_{max} and AUC across subjects for each food product led to a final rank for I_{max} and AUC combined across subjects for all food products.

Spearman rank correlation coefficient across subjects showed that all food products were correlated (i.e., final rank), although solid and (semi)liquid food products were

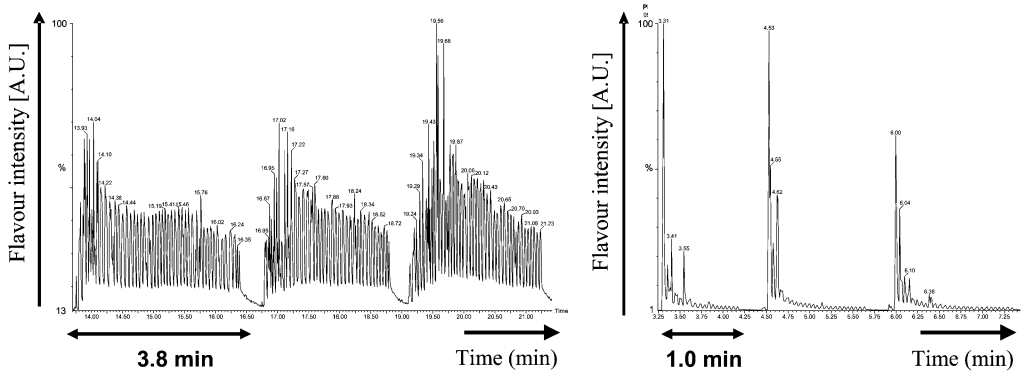

Figure 3 Example of 1 subject illustrating the differences in the extent of retro-nasal aroma stimulation between the consumption of 3 times 1 mouthful (on average 8 g per mouthful) of aged cheese (solid food product; left) and 3 times one sip (on average 17 mL per sip) of strawberry-flavored dairy beverage (liquid food product; right), measured by *in vivo* APcI-MS. For each retro-nasal aroma release curve, the characteristic retro-nasal aroma release parameters (T_{max}, $T_{+1/2}$, I_{max}, and AUC) can be extracted, similarly as represented in Figure 1.

strongly correlated among each other with respect to retro-nasal aroma release intensity (e.g., a correlation between cheese-flavored cracker and milk chocolate and a correlation between banana-flavored custard and raspberry pudding) (Table 3). The only exception was young cheese, which as a solid food product was only correlated to a minority of solid food products, namely, aged cheese and winegum candy.

The morphology of the retro-nasal aroma release profile (in particular explained by T_{max}, i.e., time at which I_{max} occurred) was quite different between (semi)liquid and solid food products (Table 4). Solid food products required a longer duration of oral processing compared with (semi)liquid food products before swallowing. Spearman rank correlation coefficients for combined rank I_{max} and T_{max} across subjects for each food product, and all food products (final rank) were less consistent in characterizing subjects based on the profile morphology of their retro-nasal aroma release (Table 3). All food products were correlated among each other (i.e., final rank), albeit fewer correlations were observed between profile morphology within the group of solid food products (e.g., a correlation between aged cheese and winegum candy), because of different oral processing.

Effects of the extent of retro-nasal aroma release on food intake behavior

Additionally, for half of the subjects, showing no dietary restraint (Table 1), *ad libitum* food intake data of young-matured cheese were available.

When the total amount of cheese consumed *ad libitum* during the three test sessions in the satiation experiment was compared with the extent of the ranked retro-nasal aroma release for these subjects (Table 3), a trend was observed that subjects who had a higher extent of retro-nasal aroma release tended to consume less (Spearman rank correlation coefficient = –0.5; $P = 0.07$) (Figure 4). This result was even significant for the test session in the satiation experiment in which subjects were delivered a 4 times lower concentrated retro-nasal aroma release profile compared with their own natural retro-nasal aroma release profile (Spearman rank correlation coefficient = –0.6; $P = 0.03$).

This may have implications for the regulation of food intake. However, BMI was not directly correlated to the extent of the ranked retro-nasal aroma release (Spearman rank correlation coefficient = 0.1; $P \gg 0.05$). Notably, it appeared that age was positively correlated to T_{max} (time at which I_{max} occurred). Particularly, for solid food products like dark chocolate and winegum candy, T_{max} was later when subjects were older (Spearman rank correlation coefficient = 0.4; $P < 0.05$ for both dark chocolate and winegum candy).

Discussion

In vivo retro-nasal aroma release was assessed for 30 subjects consuming 9 different food products that varied in texture from (semi)liquid to solid using APcI-MS technology.

Selection of specific *m/z* signals (thus a specific aroma compound) (Table 2) did not cause any response bias regarding the obtained retro-nasal aroma release profiles. This implies that aroma release data obtained for a single aroma compound (1 specific *m/z* value) are a good predictor for the relative release of other aroma compounds (other *m/z* values) in order to characterize subjects. Furthermore, the extent of retro-nasal aroma release that was evoked in subjects during consumption of each of the 9 individual food products appeared to be reproducible with respect to retro-nasal aroma release intensity and profile morphology (Figures 2 and 3).

In the present study, the extent of retro-nasal aroma release depended on both subject and product differences. Therefore, it can be concluded that a subject who was observed as having a relatively high retro-nasal aroma intensity for a (semi)liquid food product (e.g., strawberry-flavored dairy beverage), also appeared to have a relatively high intensity for a solid food product (e.g., dark chocolate) (Table 3). Subjects can thus be characterized based on their extent

Table 3 Spearman rank correlation coefficients for combined rank I_{max} and AUC, and combined rank I_{max} and T_{max} (between brackets), across subjects for each food product and all food products (final rank) with regard to retro-nasal aroma release intensity and profile morphology, respectively

	Strawberry-flavored dairy beverage	Banana-flavored custard	Raspberry pudding	Young cheese	Aged cheese	Cheese-flavored cracker	Milk chocolate	Dark chocolate	Winegum candy	Final rank
Strawberry-flavored dairy beverage	1.00 (1.00)[a]	**0.58 (0.52)**[a]	**0.36** (0.17)[a]	0.25 (0.27)	0.34 **(0.38)**	**0.39** (0.00)	0.32 (0.07)	0.35 (0.10)	0.27 (0.18)	**0.58 (0.50)**[c]
Banana-flavored custard	**0.58 (0.52)**[a]	1.00 (1.00)[a]	**0.69 (0.48)**[a]	0.17 (0.15)	0.22 (0.28)	**0.46** (0.26)	**0.56 (0.57)**	**0.46** (0.28)	**0.62** (0.34)	**0.75 (0.68)**[c]
Raspberry pudding	**0.36** (0.17)[a]	**0.69 (0.48)**[a]	1.00 (1.00)[a]	**0.38** (0.18)	0.35 (0.20)	**0.53** (0.27)	**0.75 (0.47)**	**0.59** (0.19)	**0.68 (0.43)**	**0.84 (0.56)**[c]
Young cheese	0.25 (0.27)	0.17 (0.15)	**0.38** (0.18)	1.00 (1.00)	**0.67 (0.63)**	0.20 (0.05)	0.32 (0.05)	0.15 (−0.02)	**0.41** (0.24)	**0.54 (0.46)**[c]
Aged cheese	0.34 **(0.38)**	0.22 (0.28)	0.35 (0.20)	**0.67 (0.63)**	1.00 (1.00)[b]	**0.44** (0.09)[b]	**0.42** (0.24)[b]	**0.40** (0.32)[b]	0.34 **(0.51)**[b]	**0.64 (0.70)**[c]
Cheese-flavored cracker	**0.39** (0.00)	**0.46** (0.26)	**0.53** (0.27)	0.20 (0.05)	**0.44** (0.09)[b]	1.00 (1.00)[b]	**0.60 (0.58)**[b]	**0.66 (0.45)**[b]	**0.60 (0.40)**[b]	**0.75 (0.50)**[c]
Milk chocolate	0.32 (0.07)	**0.56 (0.57)**	**0.75** (0.47)	0.32 (0.05)	**0.42** (0.24)[b]	**0.60 (0.58)**[b]	1.00 (1.00)[b]	**0.76 (0.71)**[b]	**0.61 (0.48)**[b]	**0.83 (0.73)**[c]
Dark chocolate	0.35 (0.10)	**0.46** (0.28)	**0.59** (0.19)	0.15 (−0.02)	**0.40** (0.32)[b]	**0.66 (0.45)**[b]	**0.76 (0.71)**[b]	1.00 (1.00)[b]	**0.56 (0.47)**[b]	**0.73 (0.63)**[c]
Winegum candy	0.27 (0.18)	**0.62 (0.34)**	**0.68 (0.43)**	**0.41** (0.24)	0.34 **(0.51)**[b]	**0.60 (0.40)**[b]	**0.61 (0.48)**[b]	**0.56 (0.47)**[b]	1.00 (1.00)[b]	**0.78 (0.74)**[c]
Final rank	**0.58 (0.50)**[c]	**0.75 (0.68)**[c]	**0.84 (0.56)**[c]	**0.54 (0.46)**[c]	**0.64 (0.70)**[c]	**0.75 (0.50)**[c]	**0.83 (0.73)**[c]	**0.73 (0.63)**[c]	**0.78 (0.74)**[c]	1.00 (1.00)[c]

A, B, and C represent the correlations (significant; i.e., in bold) for the extent of retro-nasal aroma release intensity and profile morphology across subjects for (semi)liquids, solids, and all food products, respectively. Solids (B) and (semi)liquids (A) were strongly correlated among each other with respect to retro-nasal aroma release intensity. Fewer correlations were observed between profile morphology within the group of solids, because of different oral processing.

of retro-nasal aroma release, independent of the type of food product they consumed. However, there were absolute differences between food products in the extent of retro-nasal aroma release comparing (semi)liquid and solid food products (Figure 3). Differences in structure and composition of the food product and the oral processing it evoked are thought to be responsible for these differences (Linforth et al. 1999; Cook et al. 2003; Lethuaut et al. 2004; van Ruth et al. 2004). Among (semi)liquid food products (strawberry-flavored dairy beverage, banana-flavored custard, and raspberry pudding), *in vivo* retro-nasal aroma release profiles were comparable and correlated. These types of food matrices apparently did not evoke significant differences in oral processing and subsequently in retro-nasal aroma release. The observed similarity in retro-nasal aroma release profiles for the selection of (semi)liquid food products enables future measurements to characterize a person's extent of retro-nasal aroma release based on 1(semi)liquid food product. With respect to solid food products, this was more complicated. Among solid food products, there are larger differences in matrix structures compared with (semi)liquid food products, evoking significant differences in oral processing and subsequently in retro-nasal aroma release.

The demonstrated subject and product differences with respect to the extent of retro-nasal aroma release may be 1 of the reasons that people vary in their satiation characteristics and may have implications for the regulation of food intake. It is known that the extent of sensory stimulation may be related to meal termination (Hetherington et al. 1989; Hetherington and Boyland 2007). Liquid foods appear to be less satiating than (soft) solid foods (Haber et al. 1977; Mattes and Rothacker 2001; Mattes 2005; Tsuchiya et al. 2006). Differences in the extent of retro-nasal aroma release due to differences in structure and composition and the oral processing it evokes may be responsible for this effect (Linforth et al. 1999; Cook et al. 2003; Lethuaut et al. 2004; van Ruth et al. 2004). Additionally, subject differences in oral processing parameters, like salivary flow rate, nasal anatomy, bite size, and eating speed may have an effect on the extent of retro-nasal aroma release (Brown et al. 1996; Buettner et al. 2001, 2002; Wright et al. 2003; Pionnier et al. 2004). The development of mathematical models for aroma release during consumption of liquid, semiliquid, and solid food products is ongoing, including both physicochemical and physiological parameters (e.g., Normand et al. 2004; Trelea et al. 2008).

In the present study, a negative trend was observed between the extent of retro-nasal aroma release and total amount of *ad libitum* food intake. The brain response, that is, neural brain activation, to a food odor sensed retronasally signals the perception of food, which is hypothesized to be related to satiation (Small et al. 2005). Limited extent of retro-nasal aroma release may result in less sensory stimulation, which in turn may lead to decreased feelings of satiation and increased food intake. The current findings tend

Table 4 Morphology (mean values) of retro-nasal aroma release profiles for the 30 subjects consuming 9 different food products in triplicate.

Food product	Mean T_{max}[a] (min)	Minimum T_{max}[a](min)	Maximum T_{max}[a] (min)	Standard error (min)	Food matrix
Strawberry-flavored dairy beverage	0.083711	0.002000	0.381000	0.064102	(Semi) liquid
Banana-flavored custard	0.134111	0.010000	0.353000	0.081199	(Semi) liquid
Raspberry pudding	0.191045	0.001000	0.547000	0.113245	(Semi) liquid
Cheese-flavored cracker	0.405844	0.008000	1.213998	0.194112	Solid
Milk chocolate	0.620766	0.076000	1.652000	0.321189	Solid
Dark chocolate	0.672543	0.238998	1.720001	0.321654	Solid
Young cheese	0.705489	0.021000	2.457000	0.493131	Solid
Aged cheese	0.762844	0.043000	1.989000	0.381746	Solid
Winegum candy	0.853833	0.259998	4.243999	0.437076	Solid

There is a distinct difference in (semi)liquid and solid food products for T_{max} (i.e., time at which maximal intensity occurs [min]).
[a] T_{max}: time at which maximal intensity occurs (min).

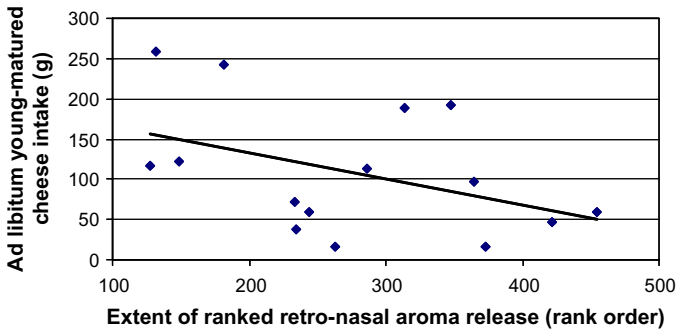

Figure 4 The total amount of young-matured cheese consumed *ad libitum* by 15 subjects after aroma stimulation using olfactometry in relation to their extent of ranked retro-nasal aroma release (i.e., final rank = $\sum[(\sum(\text{rank } I_{max} \text{ and rank AUC})_{\text{across subjects for each food product}})$ across subjects for each food product]).

to support the hypothesis that subject differences in the extent of retro-nasal aroma release are linked to subject differences in sensory satiation and food intake behavior. This assumption is even strengthened by the result of the session in the satiation experiment, in which subjects were aroma stimulated with a 4 times lower concentrated retro-nasal aroma release profile. Here, a subject's original extent of *in vivo* retro-nasal aroma release, while eating normally, was negatively related to the amount of *ad libitum* food consumed in the aroma-stimulated satiation experiment. Due to the preload *ad libitum* setting of the satiation experiment (Ruijschop et al. 2008), it was expected that the aroma stimulation (preload) would affect the amount of *ad libitum* food intake, as a result of the development of sensory satiation. This may act upon a possible relationship between a subject's extent of *in vivo* retro-nasal aroma release and amount of *ad libitum* food intake. From the 3 different aroma intensities, the least concentrated aroma stimulation was envisaged to hardly affect *ad libitum* young-matured cheese consumption in terms of sensory satiation. Indeed, we found that under this condition, a significant negative correlation appeared between a subject's original extent of *in vivo* retro-nasal aroma release and amount of *ad libitum* food intake. However, a follow-up study with a larger subject population is needed to ultimately demonstrate an immediate, significant effect of the extent of retro-nasal aroma release on food intake behavior.

The results of the present study are promising and will serve as input for upcoming studies that investigate the role of retro-nasal aroma release in satiation, both from food products and subject point of view. Ultimately, the aim is to develop food products containing triggers that are able to regulate food intake behavior. Examples of applications could be the development of food products with an increase of aftertaste, an increase or lingering of aroma release via flavor delivery systems or encapsulation technology, or the development of long chewable food structures in beverages that evoke substantial oral processing and an increase in transit time in the oral cavity. These applications may lead to a more efficient retro-nasal aroma release and sensory stimulation, which in turn may affect satiation and food intake behavior.

Funding

DiOGenes (Diet, Obesity and Genes' supported by the European Community; Contract no. FOOD-CT-2005-513946). The Parties of the project are listed on the website of the project (http://www.diogenes-eu.org/). Special acknowledgment is made to the Dutch Dairy Organization for cofinancing this project.

Acknowledgements

We acknowledge Wageningen Centre for Food Sciences (now Top Institute Food and Nutrition) for putting their consumer panel at

our disposal for subject recruitment. We also thank Jan van Riel and Annereinou Dijkstra for their skillful assistance with the measurements.

References

Brown WE, Dauchel C, Wakeling I. 1996. Influence of chewing efficiency on texture and flavour perceptions of food. J Texture Stud. 27:433–450.

Buettner A, Beer A, Hanning C, Settles M. 2001. Observation of the swallowing process by application of videofluoroscopy and real-time magnetic resonance imaging-consequences for retro-nasal aroma stimulation. Chem Senses. 26:1211–1219.

Buettner A, Beer A, Hanning C, Settles M, Schieberle P. 2002. Physiological and analytical studies on flavor perception dynamics as induced by eating and swallowing process. Food Qual Pref. 13:497–504.

Buettner A, Schieberle P. 2000. Exhaled odorant measurement (EXOM) – a new approach to quantify the degree of in-mouth release of food aroma compounds. Lebensm——Wiss Technol. 33:553–559.

Cook DJ, Linforth RST, Taylor A. 2003. Effects on hydrocolloid thickeners on the perception of savory flavors. J Agric Food Chem. 51:3067–3072.

Haber GB, Heaton KW, Murphy D, Burroughs LF. 1977. Depletion and disruption of dietary fibre. Effects on satiety, plasma-glucose, and serum-insulin. Lancet. 2:679–682.

Hetherington M, Rolls BJ, Burley VJ. 1989. The time course of sensory-specific satiety. Appetite. 12:57–68.

Hetherington MM, Boyland E. 2007. Short-term effects of chewing gum on snack intake and appetite. Appetite. 48:397–401.

Land DG. 1994. Perspectives on the effects of interactions on flavor perception: an overview. In: McGorrin RJ, Leland J, editors. Flavor–food interactions. Washington: ACS.: ACS Symposium Series 633. p. 2–11.

Lethuaut L, Weel KG, Boelrijk AE, Brossard CD. 2004. Flavor perception and aroma release from model dairy desserts. J Agric Food Chem. 52:3478–3485.

Linforth RST, Baek I, Taylor AJ. 1999. Simultaneous instrumental and sensory analysis of volatile release from gelatine and pectin/gelatine gels. Food Chem. 65:77–83.

Mattes R. 2005. Soup and satiety. Physiol Behav. 83:739–747.

Mattes RD, Rothacker D. 2001. Beverage viscosity is inversely related to postprandial hunger in humans. Physiol Behav. 74:551–557.

Meilgaard M, Civille GV, Carr BT. 1999. Sensory evaluation techniques. 3rd ed. Boca Raton, FL: CRC Press. p. 7–8.

Murphy C, Cain WS, Bartoshuk LM. 1977. Mutual action of taste and olfaction. Sens Processes. 1:204–211.

Normand V, Avison S, Parker A. 2004. Modeling the kinetics of flavour release during drinking. Chem Senses. 29:235–245.

Pionnier E, Chabanet C, Mioche L, Le Quere JL, Salles C. 2004. 1. In vivo aroma release during eating of a model cheese: relationships with oral parameters. J Agric Food Chem. 52:557–564.

Rozin P. 1982. Taste–smell confusions and the duality of the olfactory sense. Percept Psychophys. 31:397–401.

Ruijschop RMAJ, Boelrijk AEM, de Ru JA, de Graaf C, Westerterp-Plantenga MS. 2008. Effects of retro-nasal aroma release on satiation. Br J Nutr. 99:1140–1148.

Small DM, Gerber JC, Mak YE, Hummel T. 2005. Differential neural responses evoked by orthonasal versus retro-nasal odorant perception in humans. Neuron. 47:593–605.

Sorensen LB, Moller P, Flint A, Martens M, Raben A. 2003. Effect on sensory perception of foods on appetite and food intake: a review of studies on humans. Int J Obes Relat Metab Disord. 27:1152–1166.

Taylor AJ, Linforth RST. 1996. Flavour release in the mouth. Trends Food Sci Tech. 7:444–448.

Taylor AJ, Linforth RST, Harvey BA, Blake A. 2000. Atmospheric pressure chemical ionisation mass spectrometry for *in vivo* analysis of volatile flavour release. Food Chem. 71:327–338.

Trelea IC, Atlan S, Déléris I, Saint-Eve A, Marin M, Souchon I. 2008. Mechanistic mathematical model for in vivo aroma release during eating of semiliquid foods. Chem Senses. 33:181–192.

Tsuchiya A, Almiron-Roig E, Lluch A, Guyonnet D, Drewnowski A. 2006. Higher satiety ratings following yoghurt consumption relative to fruit drink or dairy fruit drink. J Am Diet Assoc. 106:550–557.

van Ruth SM, de Witte L, Uriarte AR. 2004. Volatile flavor analysis and sensory evaluation of custard desserts varying in type and concentration of carboxymethyl cellulose. J Agric Food Chem. 52:8105–8110.

van Ruth SM, Roozen JP, Cozijnsen JL. 1994. Comparison of dynamic headspace in mouth model systems for flavour release from rehydrated bell pepper cuttings. In: Maarse H, van der Heij DG, editors. Trends in flavour research. Amsterdam: Elsevier Science. p. 59–64.

Weel KGC, Boelrijk AEM, Alting AC, van Mil PJJM, Burger JJ, Gruppen H, Voragen AGJ, Smit G. 2002. Flavor release and perception of flavoured whey protein gels: perception is determined by texture rather than by release. J Agric Food Chem. 50:5149–5155.

Weel KGC, Boelrijk AEM, Burger JJ, Gruppen H, Voragen AGJ, Smit G. 2003. A protocol for measurement of *in vivo* aroma release from beverages. J Food Sci. 68:1123–1128.

Weel KGC, Boelrijk AEM, Burger JJ, Verschueren M, Gruppen H, Voragen AGJ, Smit G. 2004. New device to stimulate swallowing and in vivo aroma release in the throat from liquid and semi-liquid food systems. J Agric Food Chem. 52:6564–6571.

Westerterp-Plantenga MS, Rolland V, Wilson SAJ, Westerterp KR. 1999. Satiety related to 24h diet-induced thermogenesis during high protein/carbohydrate versus high fat diets, measured in a respiration chamber. Eur J Clin Nutr. 53:1–8.

Wright KM, Sprunt J, Smith AC, Hills BP. 2003. Modeling flavor release from a chewed bolus in the mouth. Part 1. Mastication. Int J Food Sci Technol. 38:351–360.

Accepted February 16, 2009