British Journal of Nutrition (2014), **111**, 554–562
doi:10.1017/S0007114513002729

Aroma exposure time and aroma concentration in relation to satiation

Mariëlle G. Ramaekers[1]*, Pieternel A. Luning[1], Rianne M. A. J. Ruijschop[2], Catriona M. M. Lakemond[1], Johannes H. F. Bult[2,3], Gerrit Gort[4] and Martinus A. J. S. van Boekel[1]

[1]*Food Quality and Design, Wageningen University, Bornse Weilanden 9, PO Box 17, 6700 AA Wageningen, The Netherlands*
[2]*NIZO Food Research, Kernhemseweg 2, PO Box 20, 6710 BA Ede, The Netherlands*
[3]*Top Institute Food and Nutrition, Nieuwe Kanaal 9a, PO Box 557, 6700 AN Wageningen, The Netherlands*
[4]*Biometris, Wageningen University and Research Centre, Droevendaalsesteeg 1, PO Box 100, 6700 AC Wageningen, The Netherlands*

(Submitted 4 January 2013 – Final revision received 12 June 2013 – Accepted 14 July 2013 – First published online 28 August 2013)

Abstract

The present study investigated the effect of aroma exposure time and aroma concentration on *ad libitum* intake and subjective satiation. In a within-subject study, thirty-eight unrestrained, healthy female participants (age: 18–39 years; BMI: 18·5–26·0 kg/m^2) were asked to consume tomato soup during lunchtime, until they felt comfortably full. Every 30 s, the participants consumed 10 g of a bland soup base while tomato soup aroma was delivered separately through the nose via a retronasal tube that was attached to an olfactometer. This gave the impression of consuming real tomato soup. For each sip, the aroma varied in exposure time (3 and 18 s) and concentration (5×), resulting in four different test conditions. *Ad libitum* food intake and appetite profile parameters were measured. A 9 % lower food intake was observed when the participants were exposed to the condition with 18 s exposure time and a high concentration than when exposed to the other three conditions. These results indicate that changing the retronasal aroma release by aroma concentration and aroma exposure time affects food intake.

Key words: **Food intake: Aroma concentration: Aroma exposure time: Sensory-specific satiation**

Understanding the factors that influence meal size can be helpful for finding strategies to limit overconsumption. It has been widely accepted that sensory processes play an important role in the development of satiation[1–5]. Satiation is the process that brings a meal to an end. Although Brunstrom and colleagues[6,7] suggested that sensory properties might be more important for meal onset and meal planning than for meal termination, there are strong indications that sensory processes influence meal termination and determine meal size; for example, sensory variety in a meal increases food intake[8–11].

Another example is a lower *ad libitum* food intake after a longer oral exposure time per volume of consumed food[12–17]. The decreases in food intake found in these studies varied between 9 and 30 %. These studies focused on the effect of total flavour exposure time, which is a combination of aroma, taste and mouthfeel. Ruijschop *et al.*[18], however, focused on the unimodal effect of aroma exposure time on satiation. The participants first received a fixed preload of ten sips from a sweetened milk drink during a short or long aroma delivery. Ruijschop *et al.*[18] found that an increase in exposure time to strawberry aroma increased subjective satiation, measured on visual analogue scales (VAS). The effects of sensory exposure time on food intake and subjective satiation were attributed to sensory-specific satiation (SSS). SSS is the decrease in the pleasantness of a food eaten to satiation, relative to uneaten foods[9,10]. The subsequent *ad libitum* intake of a normal strawberry drink, in the study carried out by Ruijschop *et al.*[18], showed no differences between the conditions. The previously mentioned increase in subjective satiation with an increase in aroma exposure time[18] might reduce *ad libitum* food intake when measured directly during aroma delivery in a different experimental set-up.

Besides the effect of sensory exposure time on satiation, researchers have investigated the relationship between flavour intensity and satiation/SSS. Flavour intensity is the perceptual consequence of a certain stimulant's concentration. The effects of flavour intensity on satiation are not consistent though. Vickers *et al.*[19] and Lucas & Bellisle[20] showed, for example, that people consumed less when given the better-liked high-sweetened yogurt than when given the low-sweetened yogurt. This tendency of people satiating more from products with high taste or flavour intensities has been observed in a number of other studies[21–24], whereas others have reported

Abbreviations: SSS, sensory-specific satiation; VAS, visual analogue scales.

* **Corresponding author:** M. G. Ramaekers, fax +31 317 483669, email marielle.ramaekers@wur.nl

no effects of flavour intensity[25–27] or have even found an opposite effect[28]. Chung & Vickers[28] found a lower SSS after drinking an optimal-sweet tea than after drinking a low-sweet tea. The inconsistency in the outcomes could be partially explained by the different test foods and different methods used to assess satiation, such as *ad libitum* food intake, subjective appetite ratings on VAS and decrease in pleasantness to assess SSS. For example, participants drank 25 % less from the equally liked lemon-flavoured ice tea with the strongest flavour intensity (including sweet taste), but did not show differences in appetite ratings[23]. Moreover, in some studies, only taste intensity has been reported to vary[19,20,22,28–30], while others have focused on total flavour intensity, which includes both taste and aroma[21,23–25,27]. So far, the unimodal effect of aroma intensity on satiation has never been investigated. It is unknown whether an increase in aroma concentration would lead to a lower food intake.

Aroma volatiles are released from foods in the mouth while eating. After swallowing, these volatiles pass through the pharynx to the nasal cavity where they reach the olfactory epithelium. We refer to this pathway as retronasal aroma stimulation, as opposed to orthonasal aroma stimulation, which occurs when odorants enter by the inhalation of volatiles through the nose. The concentration of aroma volatiles that is released over time during consumption of a single bite is referred to as the aroma release profile. During food consumption, aroma release profiles depend on food properties such as texture, temperature and composition[31–35] and also on human characteristics such as chewing behaviour, salivation and morphology of the nose[36–38]. Since the use of aromas does not contribute to the energy density of foods, any suppressive effects of aroma on food intake could, therefore, reduce energy intake.

The objective of the present study was to investigate whether retronasal aroma concentration and/or aroma exposure time affect satiation, measured as *ad libitum* food intake. We examined the effect of well-defined aroma release profiles, presented retronasally by an olfactometer, on the development of satiation. Aromas that are presented retronasally are processed differently than aromas presented orthonasally[39–42]. Especially, the pathway-specific contribution to the perception of taste[39] and mouthfeel[43] may add to the satiating properties of the aroma. In order to verify a possible relationship between aroma concentration and food intake, we maximised the differences in concentrations within the limits of acceptability. Besides food intake, we measured appetite profile parameters on VAS. We hypothesised that an increase in both aroma concentration and aroma exposure time increases SSS, which in turn increases subjective satiation and decreases *ad libitum* food intake.

Materials and methods

Participants

For the present study, healthy women aged 18–45 years and with a BMI of 18·5–26 kg/m^2 were recruited from the surrounding areas of Ede and Wageningen. Unrestrained eaters on the basis of the Dutch Eating Behaviour Questionnaire (score $<2{\cdot}91$)[44] and women who liked tomato soup (score >5 on a nine-point scale, reported in the online inclusion questionnaire) were included. Women who had followed an energy-restricted diet during the last 2 months, had change in body weight >5 kg during the last 2 months, were pregnant or breast-feeding during the last 6 months or had a lack of appetite for any reason were excluded. The olfactory function of the participants was tested using Sniffin' Sticks (Burghart Medical Technics) as described by Hummel *et al.*[45]. The test consisted of an examination of odour threshold (*n*-butanol), odour discrimination and odour identification. Women with a total score <27 on threshold, discrimination and identification were also excluded. In total, forty-three women aged 24 (SD 5) years and with a BMI of 22·5 (SD 1·6) kg/m^2 were enrolled for the study. Due to reports of discomfort due to the retronasal tube, newly discovered pregnancy or dislike of the test products, five participants were excluded from statistical analysis. To reduce the number of missing data due to sickness, hay fever or misinterpretation of the instructions, eight participants came for an additional test session. The participants were unaware of the change in aroma concentration and aroma exposure time and were informed that the influence of taste and smell on tomato soup consumption was being investigated. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Medical Ethical Committee of Wageningen University. Written informed consent was obtained from all the participants.

Test products

Tomato soup was used as the model product, because it meets the criteria of being homogeneous, liquid, commonly consumed during lunch and familiar to the participants. A bland soup base (with little tomato aroma) was given orally, with the well-defined retronasal aromas being presented simultaneously, to investigate the unimodal effect of aroma concentration and aroma exposure time on *ad libitum* intake. The soup base and aroma had to be of perceptually matching qualities, i.e. congruent, in order to be perceived as tomato soup. The soup base consisted of 5 g Maggi Bouillon (Nestlé), 4 g Cup-a-Soup – Tomato Crème (Unilever), 30 g modified starch 'Honig allesbinder' (Heinz) and 561 g cooked water. The soup base contained 96 kJ/100 g energy. Batches of 600 g soup base were kept at 60 °C using a water-bath. The soup was consumed at a temperature between 50 and 55 °C. The aroma used was a mixture of three flavours (Givaudan) dissolved in water: 6 g tomato (RB-329-620-8) + 0·15 g pizza herb (UN-981-546-3) + 0·2 g soup greens (CT-722-418-3) per 100 g solution.

Development of retronasal aroma release profiles

The 'natural' aroma release during regular soup consumption was measured *in vivo*, with atmospheric-pressure chemical ionisation–MS as described previously by Ruijschop *et al.*[18]. During the full scan, the response of all compounds with molar masses between 50 and 250 g/mol was determined.

The ions with molar masses 80, 100 and 148 g/mol gave the highest response during the 'full scan' and were selected for measuring aroma release. Aroma profiles that were presented retronasally during soup consumption experiments were based on these measured aroma release profiles.

Using a computer-controlled four-channel olfactometer based on air-dilution olfactometry (OM4; Burghart), four different retronasal aroma release profiles were generated. This allowed full control of the aroma release profiles independently of food properties and human characteristics. The aroma release profiles differed in concentration and length and were coded as 'low-short', 'low-long', 'high-short' and 'high-long'. The chosen exposure times were either 3 s (short) or 18 s (long). Moreover, these profiles were derived from the measured aroma release profiles by decreasing their concentration to create the 'low'-aroma release profiles and by increasing their concentration to create the 'high'-aroma release profiles. Differences in concentrations were chosen in such a way that four colleagues at NIZO food research perceived the lowest concentration at a weak intensity and the highest concentration as strong but not unpleasant or unnatural. This was done to maximise the effects of aroma concentration on intake within the limits of natural soup aroma compositions. The differences in concentrations were achieved by varying the duration of the aroma pulses that were initiated every second. Accordingly, the aroma pulse of the low aroma concentration was five times shorter than that of the high aroma concentration, but the pulse patterns over time of 'low' and 'high' concentrations were the same. At the chosen olfactometer flow rate and pulsation frequency of 1 Hz, the odour pulses blend into a continuous percept that has an intensity proportional to the average aroma concentration.

The olfactometer was set at a constant dilution rate by mixing 0·5 litres/min of odorised air with 7·5 litres/min clean humidified air, resulting in a constant aromatised air flow of 8 litres/min. Odour pulses were generated by switching between aromatised and non-aromatised air while keeping the overall flow rate constant. At the chosen flow rate, this resulted in a stimulus rise time < 20 ms. The aroma solution was refreshed every 2 min (after every fourth sip) to reduce the depletion of volatiles from the olfactometer's odour vessel (Fig. 1(b)). Subsequently, the four aroma release profiles, as presented to the respondents, were verified by connecting the olfactometer to the atmospheric-pressure chemical ionisation–MS equipment. For each condition, twelve aroma release profiles were measured (Fig. 1).

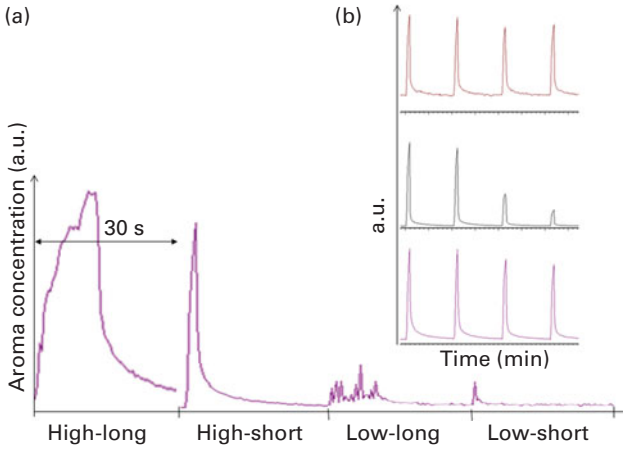

Fig. 1. Aroma release profiles measured with atmospheric-pressure chemical ionisation–MS. (a) Aroma release curves for the four test conditions, measured with molar mass 80 g/mol. (b) Aroma release curves of the 'high-short' condition showing depletion during four sips, measured with compounds with molar masses 80, 100 and 148 g/mol. a.u., Arbitrary units. (A colour version of this figure can be found online at http://www.journals.cambridge.org/bjn)

Experimental design

We used a randomised 2 × 2 within-subject design, investigating the effects of aroma exposure time (3 and 18 s) and aroma concentration (low and high). In brief, the participants visited the test location on five separate days, with a washout period of at least 5 d. Before the actual experiment, on a separate day, the participants were tested on odour sensitivity and informed individually about the experimental procedure. On the other 4 d, the participants were exposed to one of the four aroma release profiles. The order of the conditions was randomised over the participants in such a way that the conditions were spread over test days and sessions as much as possible.

Although Ruijschop *et al.*[18,37,46] did not observe any effect of sessions on the results in similar previous experiments, results of the present study indicated that the participants had to get accustomed to the experimental setting. Therefore, the results obtained for the first session were not used in the data analysis and the session was considered a training session.

Procedure

The participants were instructed to consume the same breakfast on all the test days and record their food intake in a diary to standardise the individual state of hunger. To ensure that they arrived in a hungry state, they were not allowed to eat or drink, except for the consumption of beverages containing no energy, the last 3 h before the start of a test session and nothing at all 1 h before the start of a session. The participants were tested between 10.40 and 14.20 hours.

After arrival, a medically trained person inserted a silicon suction catheter with a total length of 20 cm (CH 10; D-Care B.V.; further on referred to as 'retronasal tube') into the lower meatus of one of the two nasal cavities with the outlet positioned at the epipharynx of the soft palate, approximately 7·5 cm from the naris[41]. The retronasal tube was then connected to the olfactometer. The participants could breathe normally. Furthermore, it was desirable to have enough time in between aroma stimulation to keep adaptation as low as possible, while also a normal eating rate was preferred. The time in between aroma stimulation was set to either 27 or 12 s, depending on the test condition (3 or 18 s exposure time, respectively). In this way, the amount of odour adaptation due to frequent exposure could be reduced. This resulted in an eating rate that was four times lower than the average eating rate for soup[47].

Every 30 s, the participants swallowed one sip (10 (SD 0·04) g) of soup base. During soup consumption, the participants received instructions on a computer screen and heard beeps notifying them when a sip of soup would come into their mouth, when to swallow and when to complete the appetite questionnaires. Software that has been described previously[43] steered the olfactometer, the peristaltic pump, the beeps and the instruction screen. The participants received the soup base into their mouth through a silicon tube (diameter 4·8 mm, Rubber B.V.) by means of an electric peristaltic pump (Watson-Marlow). To ensure temporal synchronisation of oral and nasal stimuli to facilitate sensory integration of the oral soup stimulus and the retronasal aroma[43,48], the participants were subjected to aroma exposure just before or at the instructed moment of swallowing. This resulted in a realistic impression of consuming tomato soup.

The participants were instructed to consume tomato soup until they felt comfortably full. At that moment, they had to inform the experimenter, who would stop the system. All the participants had to stay in the test set-up with the retronasal tube in the nose for a minimum of 25 min to prevent meal termination due to inconvenience or boredom. After meal termination or 25 min, the retronasal tube was removed.

Data collection

The computer recorded the number of sips to determine the *ad libitum* soup intake. Furthermore, ratings of hunger, satiation, fullness, desire to eat, appetite for something savoury, appetite for something sweet and thirst were recorded on 100 mm VAS (anchored from not at all to very much) before (baseline), during and after food intake. Participants completed the appetite questions during and after food intake at ten fixed time points (4, 8, 12, 16, 20, 25, 30, 35, 40 and 50 min after the start of food intake) and one directly after finishing consumption. The pleasantness of the soup was evaluated at the same time points during soup consumption. Additionally, after three sips, the participants gave an initial judgement of the soup by rating pleasantness (not at all–very much), overall flavour intensity (not intense–very intense) and length of aftertaste (not long–very long) on 100 mm VAS. Intensity was measured with VAS to collect normally distributed data[49]. All questionnaires were filled out on paper and scanned using TeleForm (v 10.1; Cardiff).

Data analysis

Statistical analyses were carried out using SAS (version 9.1.3; SAS Institute, Inc.). Unless stated otherwise, two-sided tests were used. A P value $<0{\cdot}05$ was considered significant. Raw data are presented as means and standard deviations and model results as least-squares means and standard errors of the least-square means. The latter are estimated means, based on a mixed model adjusted for covariates and random effects and further on referred to as means and standard errors. We considered the first session as a training session and excluded data obtained in this session from the analysis. A slightly unbalanced dataset was obtained.

Differences in *ad libitum* intake between the test conditions were compared using a mixed model fitted by restricted maximum likelihood (proc mixed, SAS). Mixed models can handle missing and unbalanced data[50,51]. The *ad libitum* intake was the dependent variable with treatment factors concentration and exposure_time, order (=session) as a block factor with a fixed effect, maximum pleasantness as a covariable and subject as a random variable. Order was included in the model, because food intake tended to increase with the number of completed sessions. The maximum rated pleasantness was included, because people tend to consume more when given more-pleasant foods. The error variances were allowed to be different between the sessions, because the participants tended to become accustomed to the set-up, resulting in decreasing variances over time. We first tested for overall differences among the four test conditions. Subsequently, we split results into main and interaction effects of concentration and exposure_time. Differences in intake due to test conditions were compared using *post hoc t* tests with Bonferroni correction. One-sided tests were used for comparison between the test conditions, because we expected a lower food intake during a longer exposure time and/or a higher concentration[18]. Some of the participants gave exceptionally low pleasantness scores for the soups. To evaluate the influence of these data on the outcome of the study, we re-analysed the data after removal of all the data with maximum pleasantness scores <45.

The number of appetite and pleasantness questionnaires that the participants filled in during soup consumption varied among the participants and test conditions, because they stopped eating at different moments. At baseline ($t=0$), there were 118 observations with complete appetite questionnaires (initial ratings). Of the 118 observations, 113 were left out 8 min after the start of *ad libitum* intake, while ninety-eight were left out after 12 min and seventy-one after 16 min. The change in appetite and change in pleasantness were calculated by subtracting the initial ratings from the ratings after 12 min of consumption. Differences in 'change scores' between the test conditions were compared using a mixed model. The change scores of appetite and pleasantness ratings were the dependent variables with treatment factors concentration and exposure_time, order as a block factor with a fixed effect, initial ratings as a covariate and participant as a random variable. The error variances were allowed to be different between the sessions.

Results

Dataset

Data obtained in the first session were removed before data analysis. The dataset contained data from six participants in two test conditions, thirty participants in three test conditions and six participants in four test conditions. Split up per condition the dataset contains data from thirty participants in the 'low-short' condition, thirty-two participants in the 'low-long' condition, twenty-nine participants in the 'high-short' condition

and twenty-seven participants in the 'high-long' condition. A slightly unbalanced dataset with repeated measurements on thirty-eight participants and in total 118 observations was collected.

Aroma release profiles

For each condition, twelve aroma release profiles were generated by the olfactometer and measured using atmospheric-pressure chemical ionisation–MS. The average maximum concentrations of the aroma release profiles in the four conditions were determined, which were greater in the 'high' than in the 'low' aroma release profiles. The difference in maximum concentration between the 'high' and 'low' aroma release profiles was sixteen times for components with molar mass 80 g/mol, fourteen times for components with molar mass 100 g/mol and six times for components with molar mass 148 g/mol. The duration of the 'long' conditions was indeed longer than that of the 'short' conditions (Fig. 1(a)). Furthermore, the concentration decreased over time due to the depletion of the aroma solution (Fig. 1(b)), but this was not the same for the three different volatiles that were measured. Between the first and the fourth sip, the average depletion was 0 % for compounds with molar mass 148 g/mol, 14 % for compounds with molar mass 80 g/mol and 74 % for compounds with molar mass 100 g/mol.

Over all the conditions, the mean intensity was 54 (SD 20) and the mean aftertaste was 45 (SD 20). Neither rated intensity nor rated aftertaste was affected by exposure_time, concentration, or the interaction between exposure_time and concentration (all $P > 0{\cdot}05$).

Ad libitum *intake*

The mean *ad libitum* intake was 388 (SD 175) g of soup with the 'low-short' aroma release profile, 368 (SD 154) g of soup with the 'low-long' profile, 350 (SD 135) g of soup with the 'high-short' profile and 333 (SD 144) g of soup with the 'high-long' profile. Fig. 2 shows the mean *ad libitum* intake and standard error per test condition with covariates maximum pleasantness and order. Exposure_time ($F_{1,73} = 3{\cdot}59$; $P = 0{\cdot}062$), concentration ($F_{1,73} = 3{\cdot}90$; $P = 0{\cdot}052$), and the interaction between concentration and exposure_time ($F_{1,73} = 2{\cdot}87$; $P = 0{\cdot}095$) were not significant, although an overall effect of test conditions ($F_{3,73} = 2{\cdot}96$; $P = 0{\cdot}0379$) was found. Both maximum pleasantness ($F_{1,73} = 5{\cdot}16$; $P = 0{\cdot}026$) and order ($F_{1,73} = 11{\cdot}12$; $P < 0{\cdot}0001$) contributed significantly to the statistical model. The standard deviation due to interperson variability alone was equal to 132 g.

Although the main and interaction effects were not significant, the overall *F* test showed that there were differences between the test conditions. Therefore, we carried out *post hoc t* tests with Bonferroni correction. Results showed that the participants consumed less in the 'high-long' condition than in the other three conditions. The relative decreases in intake as calculated from the mixed model results were 9·1 % ($P = 0{\cdot}044$; one-tailed) between the 'high-short' and 'high-long' conditions, 9·3 % ($P = 0{\cdot}035$; one-tailed) between the 'low-long' and 'high-long' conditions, and 9·4 % ($P = 0{\cdot}029$; one-tailed) between the 'low-short' and 'high-long' conditions. No differences in intake were found between the 'low-short' and 'high-short' conditions ($P = 1{\cdot}0$) or between the 'low-short' and 'low-long' conditions ($P = 1{\cdot}0$).

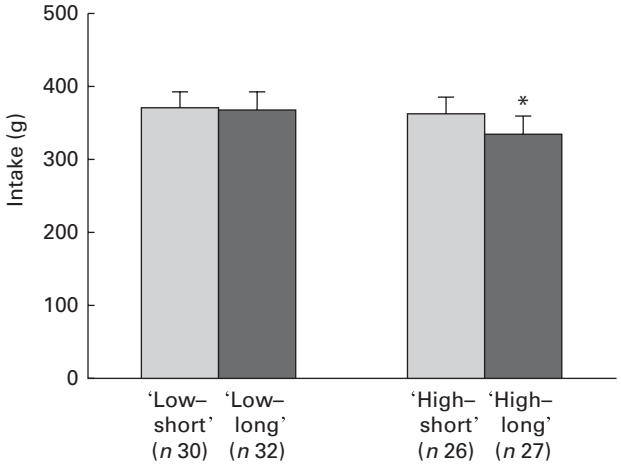

Fig. 2. *Ad libitum* intake during all the test conditions. Values are means, with their standard errors represented by vertical bars. *Mean value was significantly different from those of the other three conditions ($P < 0{\cdot}05$).

We checked whether low pleasantness ratings for the soup influenced the outcome of the study, by removing the data with pleasantness scores < 45 from the dataset. The removal these data did not change the *ad libitum* intake outcome of the present study.

Appetite and pleasantness ratings

There were no differences in appetite ratings between the test conditions at baseline ($t = 0$; all $P > 0{\cdot}05$). During *ad libitum* intake, the appetite ratings showed, as expected, a decrease in hunger, desire to eat, appetite for something sweet and appetite for something savoury, while fullness and satiation increased (Table 1; all $P < 0{\cdot}001$). Appetite for something savoury decreased more than that for something sweet ($P < 0{\cdot}001$). Change scores were calculated by subtracting the initial ratings from ratings after 12 min of consumption, which equals 240 g of soup intake due to the constant eating rate of 10 g/30 s. After 12 min, the dataset contained data from two participants in one condition, three participants in two conditions, twenty-six participants in three conditions and three participants in four test conditions. The change scores of appetite ratings were not affected by exposure_time, concentration, or the interaction between exposure_time and concentration, measured after 240 g intake (all $P > 0{\cdot}05$) and just after meal consumption (all $P > 0{\cdot}05$).

The mean maximum pleasantness scores of the four soups were 70 (SD 17) for the 'low-short' condition, 69 (SD 19) for the 'low-long' condition, 60 (SD 19) for the 'high-short' condition and 65 (SD 20) for the 'high-long' condition. Soups with a high concentration were rated as more pleasant than those with a low concentration ($F_{1,74} = 4{\cdot}61$; $P = 0{\cdot}035$).

British Journal of Nutrition

Table 1. Initial appetite and pleasantness scores per test condition, measured on 100 mm visual analogue scales, and changes in appetite and pleasantness after 12 min of soup consumption

(Mean values with their standard errors)

	Hunger		Satiation		Fullness		Desire to eat		Appetite for something sweet		Appetite for something savoury		Thirst		Pleasant-ness	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Initial scores*																
Low-short (*n* 30)	68	3·8	*22*	3·4	19	3·1	73	3·5	55	4·6	66	3·4	68	3·7	56	3·5
Low-long (*n* 32)	65	3·7	25	3·3	22	3·0	73	3·3	51	4·4	64	3·2	64	3·5	63	3·2
High-short (*n* 29)	68	3·7	23	3·4	23	3·1	74	3·4	58	4·6	65	3·3	67	3·6	58	3·4
High-long (*n* 27)	65	3·8	29	3·4	22	3·2	73	3·5	58	4·7	66	3·5	64	3·8	63	3·6
Change after 12 min†																
Low-short (*n* 27)	−27	3·9	28	4·2	30	4·2	−26	4·0	−10	4·1	−25	4·5	−15	3·6	−17	2·7
Low-long (*n* 26)	−24	3·8	26	4·0	31	4·0	−27	3·9	−10	3·9	−21	4·3	−13	3·5	− 9	2·4
High-short (*n* 24)	−28	3·9	31	4·2	32	4·1	−28	4·0	−13	4·0	−25	4·4	−11	3·7	−15	2·7
High-long (*n* 21)	−26	4·0	24	4·5	33	4·4	−27	4·2	−12	4·3	−25	4·6	−15	3·9	−15	3·0

* Initial scores are means corrected for order with their standard errors; the total number of observations was 118.
† Change scores (initial score−score after 12 min) are means corrected for order and initial scores with their standard errors; the total number of observations was 98.

Discussion

The present study shows that the amount of aroma exposure affects *ad libitum* intake. Since all other sensory factors that can influence food intake were standardised, the differences in *ad libitum* intake that were found in the present study should be attributed to changes in the aroma release profile alone. Taste and mouthfeel were the same in all the conditions, because the participants received the same soup base in the mouth. Moreover, eating rate, bite size and time that the product stayed in the mouth were kept the same by using a computerised system with a peristaltic pump and auditory beeps.

A 9% lower food intake was observed when the participants were exposed to the 'high-long' condition with 18 s exposure time and high concentration than when exposed to the other three conditions (Fig. 2). These results indicate that *ad libitum* food intake depends on a combination of both aroma exposure time and aroma concentration. An effect of exposure time on food intake was found only at high concentrations. The two aroma concentrations used in the present experiment centred on the release concentrations observed during regular tomato soup consumption. The total aroma stimulation during the 'low' conditions may have been too small to allow aroma exposure time to exert its effects on food intake. Furthermore, aroma concentration affected food intake only when the exposure time was long (18 s). Similarly, the total aroma stimulation during the 3 s of aroma exposure may have been too little to demonstrate an effect of aroma concentration on food intake. Possibly, the total amount of aroma volatiles is more important for the development of sensory satiation and the subsequent lower food intake, than the separate factors aroma concentration and aroma exposure time.

The 9% decrease in food intake due to an increase in exposure time that was found in the present study is in line with the results of previous studies[13,17]. These studies have reported decreases in food intake between 9 and 20% after an increase in sensory exposure time. Kissileff *et al.*[17] showed that participants consumed 20% less yogurt shake when the eating rate was 70 g/min than when it was 140 g/min. Lowering the eating rate increases the sensory exposure time per bite. Zijlstra *et al.*[13] found a decrease of 9–18% in the *ad libitum* intake of chocolate custard when the sensory exposure time was increased from 3 to 9 s. The decrease in *ad libitum* intake was 9% when the bite size was large (15 g) and 18% when it was small (5 g). Similar to that observed in the set-up of the present study, the participants consumed the food through a tube that was connected to a pump, while beeps signalled when to swallow, controlling the time the food is in the mouth. Ruijschop *et al.*[18] found no differences in *ad libitum* intake. We assume that they used a measure that was less sensitive than the one used in the present study. Ruijschop *et al.* measured *ad libitum* intake 10 min after the preload with the retronasal aroma delivery, whereas we measured *ad libitum* intake during the retronasal aroma delivery. Also in a more 'natural' setting, people consume less when foods need longer processing in the mouth[47]. For example, the *ad libitum* intake of liquids is greater than the *ad libitum* intake of solid foods. In all the studies mentioned above, taste and mouthfeel may have contributed to the effect of sensory exposure time on food intake, while in the present study, the effects resulted from differences in aroma alone.

In contrast to our expectations, we did not find any differences in subjective appetite ratings, while Ruijschop *et al.*[18] found an increase in subjective satiation after eight sips (equal to 8 min) with an aroma exposure time of 43 s/sip than after eight sips of 14 s/sip. They used a technique similar to that used in the present study: a strawberry aroma was delivered retronasally after each sip from a sweetened milk drink. Rolls *et al.*[52] and Zijlstra *et al.*[53] also found an increase in subjective fullness when the sensory exposure time to a fixed preload was longer due to, respectively, air incorporation or increase in viscosity. Although subjective appetite ratings have been shown to predict food intake[54], some studies, including the present study, have reported no effect on appetite ratings even though an effect on food intake was found[12,14–16,23]. In most of these studies, however, the appetite ratings were recorded after an *ad libitum* intake,

while the results of appetite ratings are more comparable with each other after consumption of a fixed amount of food. We measured the appetite ratings after consumption of a fixed amount of soup, but found no effect of aroma release profile on appetite ratings. In two studies, Zijlstra *et al.* investigated the effect of consuming foods with different viscosities on fullness after a fixed amount of food[53] and on *ad libitum* intake[14]. The difference in fullness was small (8 mm on 100 mm VAS), while the difference in intake was large (30%). If a 30 % difference in intake is accompanied with only small changes in appetite ratings, then no difference in appetite ratings can be expected with a 9 % difference in intake, as was found in the present study.

The rated intensity of the soups in the four conditions did not differ between the conditions. This may be caused by a dominant role of taste in flavour intensity; taste intensity was the same in all the conditions. Furthermore, the washout time of at least 5 d in between sessions made it impossible for the participants to compare the four soups used in the present study against each other. They were probably compared against the prototypes of well-known soups, making it more difficult to detect small differences. During the pre-tests, the participants were exposed to the conditions one after the other with a pause of circa 10 s. Possibly, the perceptual differences in intensity were emphasised by a contrast effect[55].

The participants reported that they had no idea as to how much they had consumed. In the experimental set-up used in the present study, they were not able to see how much they had eaten during *ad libitum* food intake, because they received the soup base via a tube in the mouth. We believe that this is an advantage when studying *ad libitum* intake, because visual cues play an important role in the development of satiation and the selection of portion sizes[56,57]. We observed an increase in pleasantness and food intake over the sessions, which was largest between the first and the second session. An increase in pleasantness has also been observed in other studies when the participants were unfamiliar with the stimuli[58,59]. Some participants told us that the soup was somewhat odd, although they believed that they had consumed tomato soup. Probably, the participants of the present study had to get accustomed to either the experimental setting or the soup and the aroma. The participants received a retronasal tube in their nose, felt air blowing into their nose, ate from a tube and swallowed when they heard a beep. Therefore, we considered the first session as a training session, resulting in an incomplete design. It is unlikely that the outcomes of the present analysis are artifacts of this incomplete design. The missing values were from randomly chosen conditions and random effects for participants corrected for differences between the participants in the statistical model. Furthermore, we used 1 Hz aroma pulses differing in duration to adjust aroma concentration. In this way, the depletion of aroma volatiles was the same in all the conditions. These pulses can be measured with atmospheric-pressure chemical ionisation–MS, as can be seen in the profile 'low-long' in Fig. 1(a), but were perceptually not noticed by the participants during pre-tests. After leaving the outlet of the olfactometer, the aroma volatiles travelled for 20 cm through the retronasal tubes before arriving to the nose of the participants. The odour pulses blended into a continuous percept that had an intensity proportional to the average aroma concentration.

In the present study, all factors that may influence food intake were standardised as much as possible. Under normal circumstances, the physical properties of foods affect the extent of retronasal aroma release during consumption[31–35]. Designing food products that release a large quantity of retronasal aroma may contribute to a decrease in food intake, but other factors should also be taken into account. In our daily life, many factors other than aroma influence food intake. Possibly, small effects of aroma on food intake are overruled by major factors such as food palatability.

We hypothesised that an increase in both aroma concentration and aroma exposure time increases SSS, which in turn increases subjective satiation and decreases *ad libitum* food intake. In line with our hypothesis, an increase in aroma concentration and aroma exposure time decreased food intake by 9 %. The subjective appetite ratings were not affected. Overall, we conclude that it is likely that both aroma concentration and aroma exposure time play a role in the development of satiation. Possibly, the inconsistency of the data on food intake and subjective appetite ratings reflects the small effect size.

Acknowledgements

The authors thank Givaudan for providing the flavours. They also thank all the participants and Kees de Graaf, Nynke van der Laan, Leonie Hermans, Dione Bouchaut and Monica Mars for their help during and after the study.

The present study was financially supported by the Dutch Technology Foundation STW (grant 07 438). STW had no role in the design and analysis of the study or in writing of this article.

The authors' contributions are as follows: M. G. R. designed and executed the experiment, analysed the data and wrote the manuscript; P. A. L., R. M. A. J. R. and C. M. M. L. designed the experiment and wrote the manuscript; G. G. provided significant advice on the data analysis and wrote the manuscript; J. H. F. B. helped to set up the study and reviewed the manuscript; M. A. J. S. v. B. provided significant advice on the design of the experiment and the writing of the manuscript.

None of the authors had a personal or financial conflict of interest.

References

1. Hetherington MM (1996) Sensory-specific satiety and its importance in meal termination. *Neurosci Biobehav Rev* **20**, 113–117.
2. Cecil JE, Francis J & Read NW (1999) Comparison of the effects of a high-fat and high-carbohydrate soup delivered orally and intragastrically on gastric emptying, appetite, and eating behaviour. *Physiol Behav* **67**, 299–306.
3. French SJ & Cecil JE (2001) Oral, gastric and intestinal influences on human feeding. *Physiol Behav* **74**, 729–734.

4. Hetherington MM & Boyland E (2007) Short-term effects of chewing gum on snack intake and appetite. *Appetite* **48**, 397–401.
5. Blundell JE, Rogers PJ & Hill AJ (1987) Evaluating the satiating power of foods: implications for acceptance and consumption. In *Food Acceptance and Nutrition*, pp. 205–219 [J Solms, DA Booth, RM Pangbourne and O Raunhardt, editors]. London: Academic Press.
6. Brunstrom JM (2007) Associative learning and the control of human dietary behavior. *Appetite* **49**, 268–271.
7. Fay SH, Ferriday D, Hinton EC, *et al.* (2011) What determines real-world meal size? Evidence for pre-meal planning. *Appetite* **56**, 284–289.
8. Guinard JX & Brun P (1998) Sensory-specific satiety: comparison of taste and texture effects. *Appetite* **31**, 141–157.
9. Rolls BJ, Rolls ET, Rowe EA, *et al.* (1981) Sensory specific satiety in man. *Physiol Behav* **27**, 137–142.
10. Rolls BJ, Rowe EA, Rolls ET, *et al.* (1981) Variety in a meal enhances food-intake in man. *Physiol Behav* **26**, 215–221.
11. Rolls BJ, Rowe EA & Rolls ET (1982) How sensory properties of foods affect human feeding behavior. *Physiol Behav* **29**, 409–417.
12. Weijzen PLG, Liem DG, Zandstra EH, *et al.* (2008) Sensory specific satiety and intake: the difference between nibble- and bar-size snacks. *Appetite* **50**, 435–442.
13. Zijlstra N, de Wijk RA, Mars M, *et al.* (2009) Effect of bite size and oral processing time of a semisolid food on satiation. *Am J Clin Nutr* **90**, 269–275.
14. Zijlstra N, Mars M, de Wijk RA, *et al.* (2008) The effect of viscosity on *ad libitum* food intake. *Int J Obes* **32**, 676–683.
15. Weijzen PLG, Smeets PAM & de Graaf C (2009) Sip size of orangeade: effects on intake and sensory-specific satiation. *Br J Nutr* **102**, 1091–1097.
16. Lavin JH, French SJ, Ruxton CHS, *et al.* (2002) An investigation of the role of oro-sensory stimulation in sugar satiety. *Int J Obes* **26**, 384–388.
17. Kissileff HR, Zimmerli EJ, Torres MI, *et al.* (2008) Effect of eating rate on binge size in bulimia nervosa. *Physiol Behav* **93**, 481–485.
18. Ruijschop RMAJ, Boelrijk AEM, de Ru JA, *et al.* (2008) Effects of retro-nasal aroma release on satiation. *Br J Nutr* **99**, 1140–1148.
19. Vickers Z, Holton E & Wang J (2001) Effect of ideal-relative sweetness on yogurt consumption. *Food Qual Prefer* **12**, 521–526.
20. Lucas F & Bellisle F (1987) The measurement of food preferences in humans – do taste-and-spit tests predict consumption. *Physiol Behav* **39**, 739–743.
21. Weijzen PLG, Zandstra EH, Alfieri C, *et al.* (2008) Effects of complexity and intensity on sensory specific satiety and food acceptance after repeated consumption. *Food Qual Prefer* **19**, 349–359.
22. Zandstra EH, de Graaf C, van Trijp HCM, *et al.* (1999) Laboratory hedonic ratings as predictors of consumption. *Food Qual Prefer* **10**, 411–418.
23. Vickers Z & Holton E (1998) A comparison of taste test ratings, repeated consumption, and postconsumption ratings of different strengths of iced tea. *J Sens Stud* **13**, 199–212.
24. Maier A, Vickers Z & Jeffrey Inman J (2007) Sensory-specific satiety, its crossovers, and subsequent choice of potato chip flavors. *Appetite* **49**, 419–428.
25. Vickers Z & Holton E (1996) I-26. Flavor intensity does not influence sensory specific satiety or consumption of cheddar cheese. *Food Qual Prefer* **7**, 332–473.
26. Hollis JH & Henry CJK (2007) Sensory-specific satiety and flavor amplification of foods. *J Sens Stud* **22**, 367–376.
27. Havermans RC, Geschwind N, Filla S, *et al.* (2009) Sensory-specific satiety is unaffected by manipulations of flavour intensity. *Physiol Behav* **97**, 327–333.
28. Chung S-J & Vickers Z (2007) Influence of sweetness on the sensory-specific satiety and long-term acceptability of tea. *Food Qual Prefer* **18**, 256–264.
29. Vickers Z, Holton E & Wang J (1998) Effect of yogurt sweetness on sensory specific satiety. *J Sens Stud* **13**, 377–388.
30. Perez C, Dalix AM, Guygrand B, *et al.* (1994) Human responses to 5 concentrations of sucrose in a dairy product – immediate and delayed palatability effects. *Appetite* **23**, 165–178.
31. de Roos KB (2003) Effect of texture and microstructure on flavour retention and release. *Int Dairy J* **13**, 593–605.
32. Guichard E (2006) Flavour retention and release from protein solutions. *Biotechnol Adv* **24**, 226–229.
33. Gonzalez-Tomas L, Bayarri S, Taylor AJ, *et al.* (2007) Flavour release and perception from model dairy custards. *Food Res Int* **40**, 520–528.
34. Seuvre AM, Diaz M, Cayot P, *et al.* (2004) Influence of the composition and the structure of different media on the release of aroma compounds. *Lait* **84**, 305–316.
35. Tarrega A, Yven C, Semon E, *et al.* (2008) Aroma release and chewing activity during eating different model cheeses. *Int Dairy J* **18**, 849–857.
36. Salles C, Chagnon MC, Feron G, *et al.* (2011) In-mouth mechanisms leading to flavor release and perception. *Crit Rev Food Sci Nutr* **51**, 67–90.
37. Ruijschop R, Burgering MJM, Jacobs MA, *et al.* (2009) Retronasal aroma release depends on both subject and product differences: a link to food intake regulation? *Chem Senses* **34**, 395–403.
38. Blissett A, Hort J & Taylor AJ (2006) Influence of chewing and swallowing behavior on volatile release in two confectionery systems. *J Texture Stud* **37**, 476–496.
39. Small DM, Gerber JC, Mak YE, *et al.* (2005) Differential neural responses evoked by orthonasal versus retronasal odorant perception in humans. *Neuron* **47**, 593–605.
40. Negoias S, Visschers R, Boelrijk A, *et al.* (2008) New ways to understand aroma perception. *Food Chem* **108**, 1247–1254.
41. Heilmann S & Hummel T (2004) A new method for comparing orthonasal and retronasal olfaction. *Behav Neurosci* **118**, 412–419.
42. Hummel T, Heilmann S, Landis BN, *et al.* (2006) Perceptual differences between chemical stimuli presented through the ortho- or retronasal route. *Flavour Fragr J* **21**, 42–47.
43. Bult JHF, de Wijk RA & Hummel T (2007) Investigations on multimodal sensory integration: texture, taste, and ortho- and retronasal olfactory stimuli in concert. *Neurosci Lett* **411**, 6–10.
44. Van Strien T (2005) *Dutch Eating Behavior Questionnaire Manual (Nederlandse Vragenlijst voor eetgedrag Handleiding)*. Amsterdam: Boom Test Publishers.
45. Hummel T, Kobal G, Gudziol H, *et al.* (2007) Normative data for the "Sniffin' Sticks" including tests of odor identification, odor discrimination, and olfactory thresholds: an upgrade based on a group of more than 3,000 subjects. *Eur Arch Otorhinolaryngol* **264**, 237–243.
46. Ruijschop R, Boelrijk AEM, Burgering MJM, *et al.* (2010) Acute effects of complexity in aroma composition on satiation and food intake. *Chem Senses* **35**, 91–100.
47. van Dongen MV, van den Akker S & de Graaf K (2010) Eating rate in relation to *ad libitum* food intake of different food products. *Appetite* **55**, 169–169.
48. von Bekesy G (1964) Olfactory analogue to directional hearing. *J Appl Physiol* **19**, 369–373.

British Journal of Nutrition

49. Hayes JE, Allen AL & Bennett SM (2013) Direct comparison of the generalized visual analog scale (gVAS) and general labeled magnitude scale (gLMS). *Food Qual Prefer* **28**, 36–44.
50. Littell RC, Milliken GA, Stroup WW, *et al.* (2006) *SAS for Mixed Models*, 2nd ed. Cary, NC: SAS Institute.
51. Brown H & Prescott R (2006) *Applied Mixed Models in Medicine*. Wiley: West Sussex.
52. Rolls BJ, Bell EA & Waugh BA (2000) Increasing the volume of a food by incorporating air affects satiety in men. *Am J Clin Nutr* **72**, 361–368.
53. Zijlstra N, Mars M, de Wijk RA, *et al.* (2009) Effect of viscosity on appetite and gastro-intestinal hormones. *Physiol Behav* **97**, 68–75.
54. de Graaf C, Blom WAM, Smeets PAM, *et al.* (2004) Biomarkers of satiation and satiety. *Am J Clin Nutr* **79**, 946–961.
55. Lawless HT & Heymann H (1998) *Sensory Evaluation of Food: Principles and Practices*. New York, NY: Chapman & Hall.
56. van Dongen MV, de Graaf C, Siebelink E, *et al.* (2009) Hidden fat facilitates passive overconsumption. *J Nutr* **139**, 394–399.
57. Wansink B, Painter JE & North J (2005) Bottomless bowls: why visual cues of portion size may influence intake. *Obes Res* **13**, 93–100.
58. Russell K & Delahunty C (2004) The effect of viscosity and volume on pleasantness and satiating power of rice milk. *Food Qual Prefer* **15**, 743–750.
59. Sulmont-Rosse C, Chabanet C, Issanchou S, *et al.* (2008) Impact of the arousal potential of uncommon drinks on the repeated exposure effect. *Food Qual Prefer* **19**, 412–420.