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Volatile flavour retention in food technology and during consumption: Juice and custard examples

Saskia M. van Ruth [a,*], Johannes Frasnelli [b], Leire Carbonell [c]

[a] RIKILT – Institute of Food Safety, Wageningen UR, Authenticity and Identity Department, P.O. Box 230, 6700 AE Wageningen, The Netherlands
[b] Smell and Taste Clinic, Department of Otorhinolaryngology, University of Dresden Medical School, Fetscherstrasse 74, Dresden, Germany
[c] Instituto de Agroquímica y Tecnología de Alimentos (IATA-CSIC), P.O. Box 73, Burjassot, Valencia, Spain

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Abstract

In this study two aspects of the influence of water on flavour retention were evaluated. The first part of the study was focused on the influence of dehydration and subsequent reconstitution of mandarin juices, which was examined by headspace Proton Transfer Reaction Mass Spectrometry. The different treatments were discriminated by their mass spectra with help of Principal Component Analysis. The second part of the study concerned intranasal volatile flavour retention during food consumption. Volatile flavour concentrations were measured at four intranasal locations in nine subjects during consumption of custard desserts. Differences between the locations indicated various degrees of retention of volatile flavour compounds by the watery mucous in the nasal tract.

Keywords: Aroma; Clemenules; Mandarin juice; Water; PTR-MS

1. Introduction

Flavour can be considered as volatile components that are sensed in the nose (aroma), non-volatile components that are sensed on the tongue (taste) along with compounds and structures that are perceived in the mouth as mouthfeel and/or texture. The aroma stimulus depends upon the concentrations of volatile flavour compounds in the region of the olfactory epithelium. Their concentrations are affected by the release rates of the compounds from the food in the mouth.

Food composition affects volatile flavour release as volatile compounds may be dissolved, adsorbed, bound, entrapped, encapsulated or diffusion-limited by other food components. The relative importance of each of these mechanisms with respect to volatile flavour release varies with the properties of the volatile compounds and the physical and chemical properties of the components in the food (Kinsella, 1988). Knowledge of binding behaviour of flavour compounds in relation to various food components and their rates of partitioning between different phases is of great practical importance for the flavouring of foods, in determining their retention/release during processing and storage, as well as their availability for perception during consumption. These types of physicochemical interactions have been thoroughly reviewed (Bakker, 1995; Druaux & Voilley, 1997).

Thermodynamics determine the retention and release of volatile flavour compounds under equilibrium conditions. Equilibrium between gas phase and food product may come close under relatively stable conditions, such as during storage. Under equilibrium conditions and in a near ideal state of infinite dilution, Henry's law prevails. In that case, the partial pressure of the volatile in the gas phase above the solution is proportional to the volatile concentration in the liquid phase of the food (Taylor, 1998). The gas/product partition coefficient of a compound is affected by interactions between the volatile flavour compound and other food components, such as water. Due

* Corresponding author. Tel.: +31 317 475414; fax: +31 317 417717.
E-mail address: saskia.vanruth@wur.nl (S.M. van Ruth).

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to the relatively non-polar character of most flavour compounds, they are relatively incompatible with a highly polar, aqueous solution in terms of intermolecular forces. Their interactions with water and other food components depend on e.g. molecular size, functional groups, shape, and volatility (Kinsella, 1988).

Under dynamic conditions, flavour release is not only determined by thermodynamics but also by kinetic factors. Kinetic factors determine the rate at which equilibrium is achieved. Mass transfer and resistance to mass transfer determine the volatile flavour concentrations in the gas phase. Under dynamic gas flow conditions, the measured volatile concentrations in the headspace are very different to those expected from air/product partition data. This is due to the limited amount of the sample which is involved in volatile partitioning and restricted delivery of molecules from the bulk interior over time. Compounds with high air/product partition coefficients are relatively quickly depleted at the product/gas interface. Therefore, a larger proportion of molecules has to be transferred when they try to restore equilibrium. They cross the interface faster than that they can be replaced by diffusion and convection from the bulk phase (De Roos, 2000; Linforth & Taylor, 2006).

During eating, most foods undergo considerable physicochemical changes in the mouth. Chewing increases the surface area exposed to the air in the mouth, which in turn enhances the release of volatiles (Van Ruth & Buhr, 2003). Mechanical deformation of food material can also result in in-mouth generation of volatile compounds. On the other hand, water plays a role by the hydration of foods by saliva. The water in saliva has a diluting effect on the flavour compounds, whereas proteins in saliva have the potential of binding flavour compounds (De Wijk & Prinz, 2005; Harrison, 1998). Saliva can also have an indirect effect on flavour release by its influence on the physical properties of a food, and subsequently on chewing rate and force (Van Ruth & Roozen, 2000a). An additional effect of water on in vivo flavour concentrations is the potential retention of flavour compounds by mucous in the respiratory tract. This aspect will be further evaluated in the present study.

In the present study an example of the retention of volatile flavour in food technology was examined. The effect of juice concentration and reconstitution on the volatile profiles of mandarin juices was evaluated. The volatile profiles of mandarin juice in their original form were compared with dehydrated juice after reconstitution with water and after reconstitution with a combination of water and juice (cut-back) using fingerprint Proton Transfer Reaction Mass Spectrometry (PTR-MS).

The importance of water on in vivo flavour concentrations is shown by its impact on flavour retention in the nasal cavity during eating. Volatile flavour concentrations of two types of strawberry flavoured custards were measured real-time at four locations in the nasal cavity of nine subjects. Differences between locations indicate retention of volatile compounds by the mucous in the nasal tract.

2. Materials and methods

2.1. Food technology: mandarin juices

2.1.1. Plant materials and juice manufacture

Clementine mandarins (*Citrus reticulata*, cv. Nules) were harvested in an orchard in Lliria (Valencia, Spain) between October 2003–April 2004 and immediately used for juice manufacture.

The fruits were washed and the juice extracted in a conventional in-line extractor (Exzel, lent by Luzzysa, El Puig, Valencia, Spain). After extraction the raw juice was sieved in a screw finisher (diameter sieve holes: 0.5 mm). The juice was thermally treated at 85 °C for 10 s and subsequently cooled at 7 °C in a plate heat exchanger (APV Iberica S.A.; Madrid, Spain). A part of the juice was aseptically packed in 1 l jars, which had been steam sterilised. Jars were stored at −20 °C until sampling. A second part of the juice was concentrated at 37 °C until 65 °Brix in a scraped surface vacuum evaporator (Model L 127, Luwa AG, Zürich, Switzerland). The concentrate obtained was either diluted with water until 10.8 °Brix, or alternatively, diluted with the pasteurized juice until 45 °Brix, and subsequently diluted to 10.8 °Brix (cut-back juice). The diluted juices were bottled in 1 l glass jars and stored at −20 °C until sampling.

2.1.2. Instrumental analysis

The bottles of frozen juice were immersed in a water bath at 15 °C, 3 h prior to the PTR-MS analysis. Five ml of a juice was transferred into a 100 ml glass vial. Three replicates were prepared for each juice. The juices equilibrated at 20 °C for 1 h. Samples were analysed according to the method described by Lindinger, Hansel, and Jordan (1998). The headspace was drawn at a rate of 20 ml/min, 15 ml/min of which was led into the PTR-MS (Ionicon, Innsbruck, Austria). A constant drift voltage of 600 V was employed. MS data were collected over the mass range m/z 20-220 using a dwell time of 0.5 s per mass. The average of the 3rd–5th cycle for each replicate sample was used for data analysis. Headspace concentrations were calculated as described elsewhere and included background and transmission corrections (Lindinger et al., 1998). Mass spectral data were subjected to Principal Component Analysis.

2.2. In vivo flavour retention: custard

2.2.1. Materials and custard preparation

Full-fat milk (3.5% fat) and sucrose (Siucra; Irish Sugar Ltd, Carlow, Ireland) were purchased in a local supermarket. Carboxymethyl cellulose (C-5678) was obtained from Sigma–Aldrich Chemie (Steinheim, Germany). A commercial strawberry flavour mixture was kindly provided by Givaudan (Duebendorf, Switzerland). It was composed of 15 volatile flavour compounds and the solvent triacetin. Full composition was specified previously (Van Ruth, de

Witte, & Rey Uriarte, 2004). Ethyl butyrate and ethyl hexanoate were present at the concentration 90 and 20 mg/g, respectively. Diacetyl (Sigma–Aldrich) was added to the flavour mixture (75 μl/ml flavour mixture) to allow evaluation of a hydrophilic compound.

A soft custard and a firm custard were prepared as described previously (Van Ruth et al., 2004), which contained 0.1% (w/w) CMC or 1.0% (w/w) CMC, respectively. After preparation, 40 g of the custard was placed in a 100 ml glass bottle, 14 μl of the flavour mixture was injected in the custard, and the bottle was sealed. The custard was stirred for another 5 min and stored at 4 °C for 24 h prior to analysis. Three batches of custard were prepared per subject (nine subjects). Each batch of custard was evaluated at the four nasal positions. At least two batches were analysed per nasal position.

2.2.2. Instrumental analysis

Intranasal flavour concentrations were measured during consumption of soft and firm custards in nine subjects (three male, six female). All were in excellent health. The nasal cavities of the subjects were examined by endoscopy. A Teflon tube (polytetrafluoro-ethylene; KronLab, Sinsheim, Germany; inner diameter: 0.75 mm, outer diameter: 1.6 mm) was placed in the respective position under endoscopic control as described previously (Frasnelli, van Ruth, Kriukova, & Hummel, 2005). In each subject, the intranasal flavour concentrations were measured in the nasopharynx (back of the throat), the nostril, in front of the middle turbinate (between nostril and site near olfactory epithelium), and in the area of the olfactory cleft (near the olfactory epithelium). Air was drawn from the tubing into the heated transfer line and further to the PTR-MS

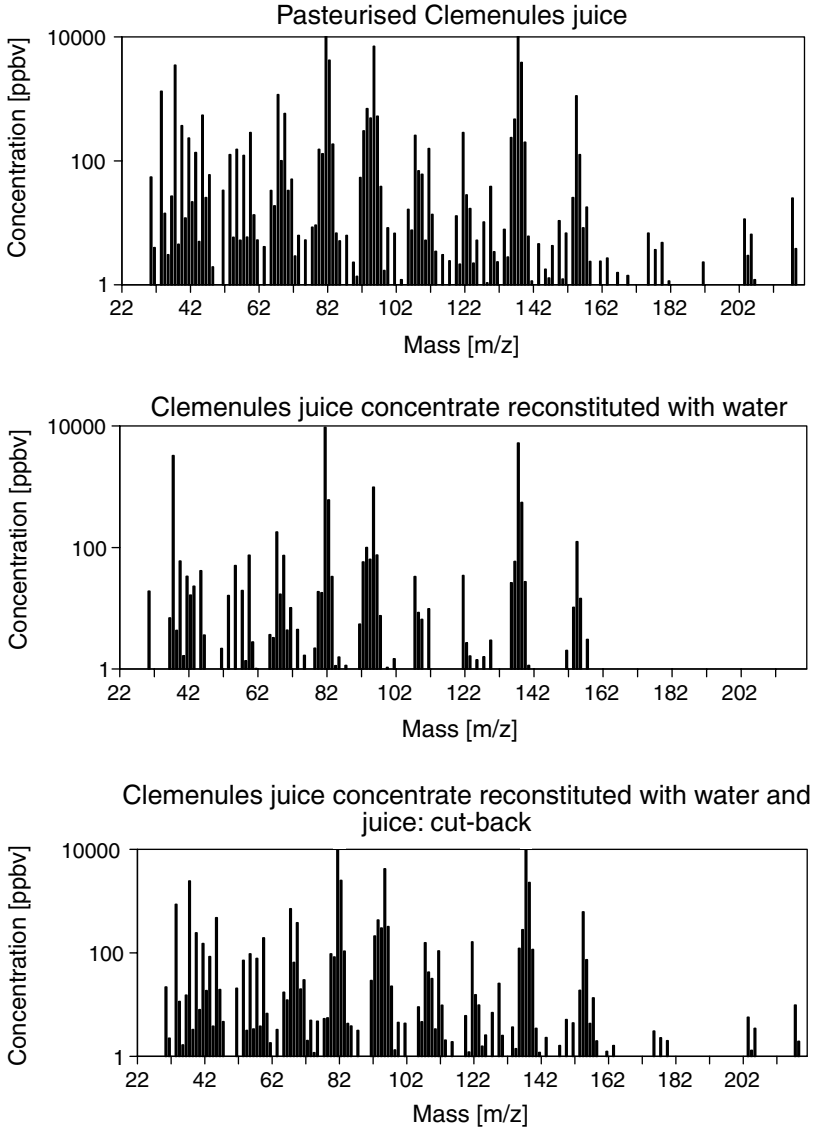

Fig. 1. Mass spectra of mandarin juices determined by headspace Proton Transfer Reaction Mass Spectrometry analysis: pasteurised juice, concentrated juice reconstituted with water, and concentrated juice partially reconstituted with juice (cut-back) (mean of three replicate measurements).

at a rate of 100 ml/min, 15 ml/min of which was directed into the PTR-MS. The samples were analysed according to Lindinger et al. (1998). Preliminary experiments showed that the masses m/z 87, 117, and 145 could be exclusively assigned to diacetyl, ethyl butyrate, and ethyl hexanoate, respectively. Spectra were background and transmission corrected. From the individual curves, maximum intensities (I_{max}) and time to maximum intensity (T_{max}) were calculated. Analysis of variance (ANOVA) was carried out to determine differences between custards, intranasal positions, and subjects. Post hoc Least Significant Difference tests (LSD) were applied where appropriate. A significance level of $P < 0.05$ was used throughout the study.

3. Results and discussion

3.1. Food technology: mandarin juices

The pasteurized mandarin juice, and the two reconstituted mandarin concentrates were subjected to headspace PTR-MS analysis in order to evaluate their volatile profiles. The mass spectra of the three juices are presented in Fig. 1. Masses measured in highest concentrations were m/z 45, 67, 81, 82, 95, 137, 138 and 154. Mass m/z 45 is characteristic for ethanol. Mass m/z 67, 81, 82, 95, 137, and 138 are characteristic for terpenes. Studies of Tani, Hayward, and Hewitt (2003) have shown that terpenes such as α- and β-pinene, 3-carene, limonene, and p-cymene produce fragment ions at masses 67, 81, 82, 95, 137, and 138, among others. The presence of terpenes as predominant compounds are in agreement with studies of Pérez, Luaces, Oliva, Ríos, and Sanz (2005), who reported the volatile composition of Henandina mandarins after intermittent curing procedures. The most abundant volatile compounds in their study were aceetaldehyde, ethanol, α-pinene, d-limonene, linalool, and myrcene (concentrations > 200 ng/ml juice).

Highest concentrations of volatiles were observed for the pasteurized juice, followed by the cut-back juice (concentrate reconstituted with water and juice). The juice concentrate reconstituted with water only presented generally lowest concentrations.

The mass spectral data of the juices were subjected to PCA, the first two dimensions of which are presented in Fig. 2. It is immediately apparent from these results that this analysis leads to a clear distinction in this plot between the pasteurised juice and the two reconstituted juices. It is interesting to note that in case of the cut-back juice the points are relatively close to each other. This may indicate a better homogeneity of the cutback juices. On the other side, the pasteurised juice with highest flavour concentrations is affected by heterogenity of the samples, the points being more scattered than the other two samples. Apart from the interesting advantage of the PCA plot that it visualises the similarity or dissimilarity of the samples, it also provides information on the mass peaks which are characteristic for the different samples. The pasteurised juice correlates with nearly all the mass peaks, which indicates that highest concentrations were determined for this type of juice. The cut-back juice, which was composed of concentrate, water and juice, was simply between the two other types of juice in terms of flavour concentrations. This means that considerable amounts of volatiles were lost together with the water during the concentration processing of the juice. From the PCA it appears that no mass peaks correlated with the two reconstituted samples. As a consequence there is no evidence for significant formation of volatile flavour compounds during the concentration step. It is obvious that the removal of water from the mandarin juice had large consequences for its volatile profile. Present results are in agreement with other studies which

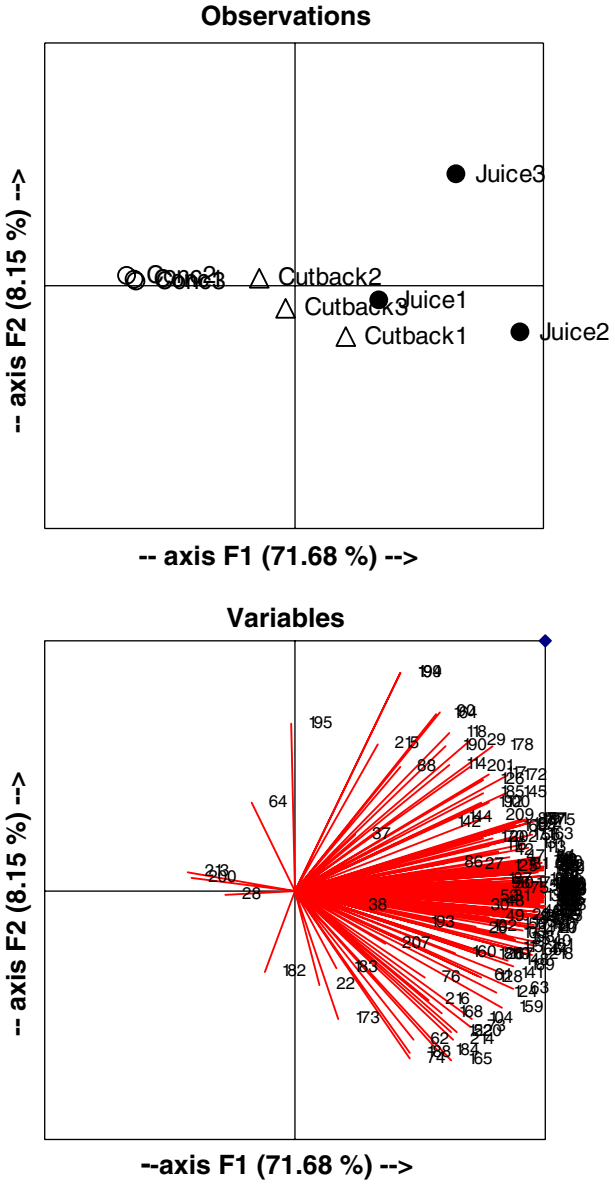

Fig. 2. First two dimensions of Principal Component Analysis on the mass spectral data of mandarin juices: pasteurised juice (juice 1, 2, 3), concentrated juice diluted with water (conc 1, 2, 3) and concentrated juice diluted with water and juice (cutback 1, 2, 3).

showed that mandarin flavour is easily affected by heat treatments (Hagenmeier & Shaw, 2002; Shaw, Moshonas, & Nisperos-Carriedo, 1992). Mandarin juices have been difficult to market because of certain off-flavours as well as changes that occur during storage (Moshonas & Shaw, 1997). Biasioli et al. (2003) showed an application of fingerprint PTR-MS analysis for discriminating orange juices which underwent heat treatments and pressure treatments. Their results showed correlation between PTR-MS data and sensory results. Therefore, the present change in volatile profile due to concentration and reconstitution of the juice may have significant effects on their sensory properties.

3.2. In vivo flavour retention: custard

In-nose analysis was carried out with nine subjects during consumption of a soft and a firm custard at four intranasal locations using PTR-MS. An example of a real-time intranasal ethyl butyrate concentration curve is shown in Fig. 3 for the nostril and nasopharynx locations. I_{max} values were calculated from the individual replicate analyses. Mean values were calculated for the soft and the firm custard, for the three volatile compounds, and the four locations (Table 1). I_{max} values were standardised to the I_{max} concentration in the nasopharynx and are displayed for the soft custard in Fig. 4. I_{max} depended significantly on the location of the measurement (ANOVA, $P < 0.05$). In addition, a significant interaction between the compounds and the positions was observed, indicating that the concentration of the compounds not only altered during their transport in the nasal cavity, but that the shift was compound-dependent. Highest I_{max} concentrations were measured in the nasopharynx for all compounds and both custards. Lowest concentrations were measured near the olfactory cleft. The three compounds differed in hydrophobicity. Diacetyl is a relatively hydrophilic compounds, whereas ethyl butyrate and ethyl hexanoate are more hydrophobic. The standardised I_{max} values (Fig. 4) show that the decrease in concentration between nasopharynx and olfactory cleft was more severe for diacetyl than it was for the other two compounds. Therefore, not only the quantities changed, but also the balance of the compounds.

Table 1
Maximum in-nose flavour concentrations (ppbv) measured during consumption of a soft and firm custard at four intranasal locations determined by Proton Transfer Reaction Mass Spectrometry (means of nine subjects)[a]

	Nasopharynx	Nostril	Middle turbinate	Olfactory cleft
Soft custard				
Diacetyl	11.9[a]	8.0[b]	5.3[bc]	3.2[c]
Ethyl butyrate	42.8[a]	29.5[b]	28.8[b]	18.5[c]
Ethyl hexanoate	3.6[a]	2.6[b]	2.2[b]	1.8[b]
Firm custard				
Diacetyl	8.7[a]	6.9[a]	2.8[b]	2.0[b]
Ethyl butyrate	60.2[a]	49.8[a]	20.9[b]	14.2[b]
Ethyl hexanoate	4.2[a]	3.1[ab]	1.9[bc]	1.4[c]

[a] Different superscripts in a row indicate significant differences (LSD test, $P < 0.05$).

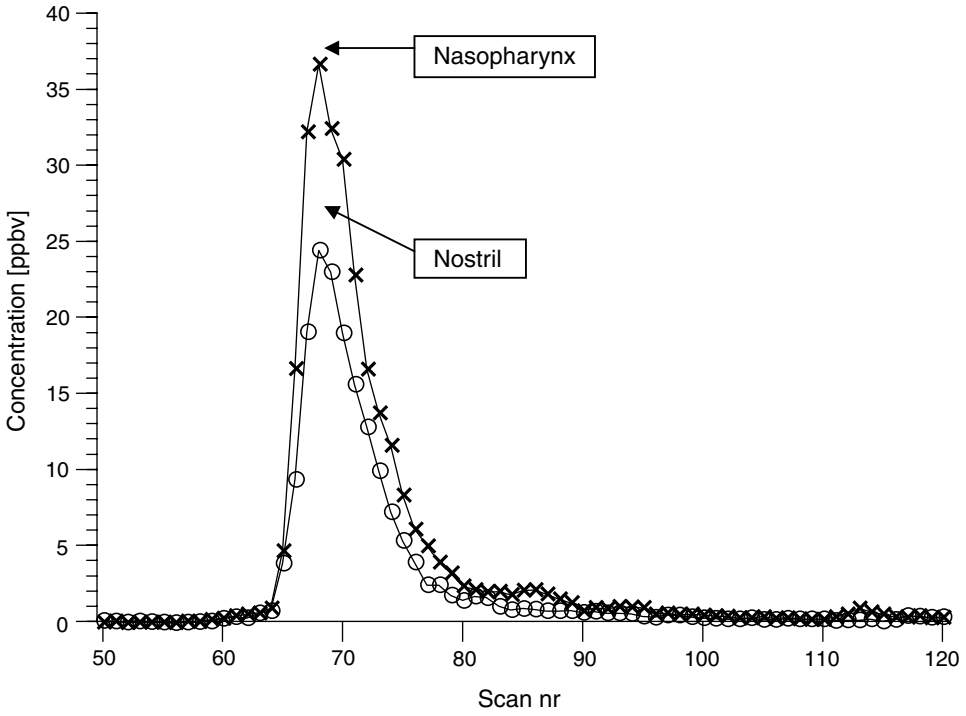

Fig. 3. Real-time in-nose concentrations of ethyl butyrate measured during consumption of a soft custard at two intranasal locations by Proton Transfer Reaction Mass Spectrometry (means of nine subjects).

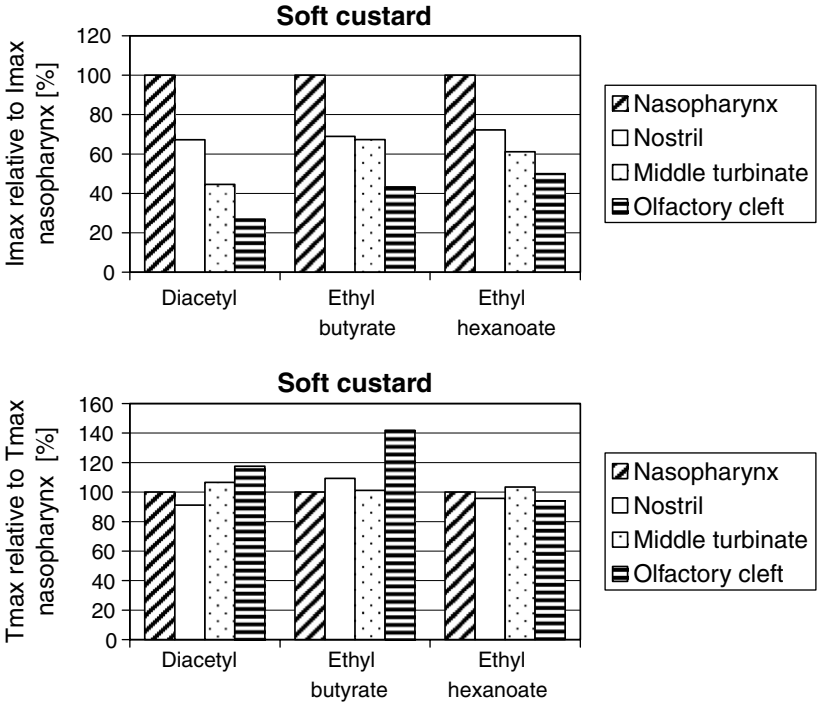

Fig. 4. Relative in-nose flavour parameters measured during consumption of a soft custard at four intranasal locations by Proton Transfer Reaction Mass Spectrometry: relative maximum flavour concentrations (I_{max}: upper diagram) and relative time to maximum flavour concentrations (T_{max}: lower diagram). Means of nine subjects.

It is remarkable that only a small portion (20–50%) of the volatile flavour compounds present in the nasopharynx would reach the area near the olfactory cleft. The largest part of the airstream flows through the lower portions of the nose (Keyhani, Scherer, & Mozell, 1995). However, this should not affect the flavour concentration in the airstream. As the decrease in intranasal concentration followed the hydrophobicity of the compounds, this indicates that selective absorption of the compounds in the watery mucous in the nasal cavity may play a role.

The soft and firm custard were compared for their flavour release properties by standardising I_{max} values to the I_{max} in the soft custard (Fig. 5). Diacetyl showed significant lower in-nose concentrations for the firm custard, compared to the soft custard (ANOVA, $P < 0.05$). Ethyl butyrate and ethyl hexanoate did not show overall significant differences between the two types of custard. Higher affinity of diacetyl for the custard with higher CMC concentrations may have resulted in a change in thermodynamic properties. However, it is also possible that diacetyl, which has a relatively high vapour pressure, is more affected by the restricted delivery of molecules from the bulk interior to the interface due to the higher viscosity (Linforth & Taylor, 2006; Van Ruth & Roozen, 2000b). It is remarkable that the firm custard always showed similar concentrations for the nasopharynx and nostril locations. Furthermore, compared to these two locations a decrease for the middle turbinate and olfactory cleft areas was observed. Differences between the locations can not directly originate from differences between the custards in flavour release in the mouth. This change in flavour concentration may indicate a change in airstream flow patterns and subsequently retention of volatile compounds by the mucous in the various areas in the nasal cavity. The change in flow pattern may originate from the differences in oral processing of soft and firm food products. The effect, a more turbulent flow in the nose, is also observed with sniffing and it has shown to increase olfactory acuity (Churchill, Shackelford, Georgi, & Black, 2004). These results imply that oral processing would not only affect the release of compounds in the mouth, but would also affect airstream flows and with it flavour concentrations near the olfactory epithelium.

T_{max} values were calculated from the individual replicate analyses and mean values calculated for the soft and the firm custard, for the three volatile compounds, and the four locations (Table 2). T_{max} values were standardised to the T_{max} concentration in the nasopharynx and presented for the soft custard in Fig. 4. Few significant differences were observed between the three intranasal locations (ANOVA, $P < 0.05$). A significant difference was only observed for diacetyl in the soft custard and ethyl hexanoate in the firm custard. Both compounds showed lower T_{max} values for the nasopharynx and higher T_{max} values for the other locations. Overall, a significant higher T_{max} was observed for the firmer custard (ANOVA, $P < 0.05$). Previous studies on the influence of oral processing on in vivo flavour release on these custards has shown that T_{max} correlated significantly with the time to swallowing (Aprea, Biasioli, Gasperi, Märk, & van Ruth, 2006).

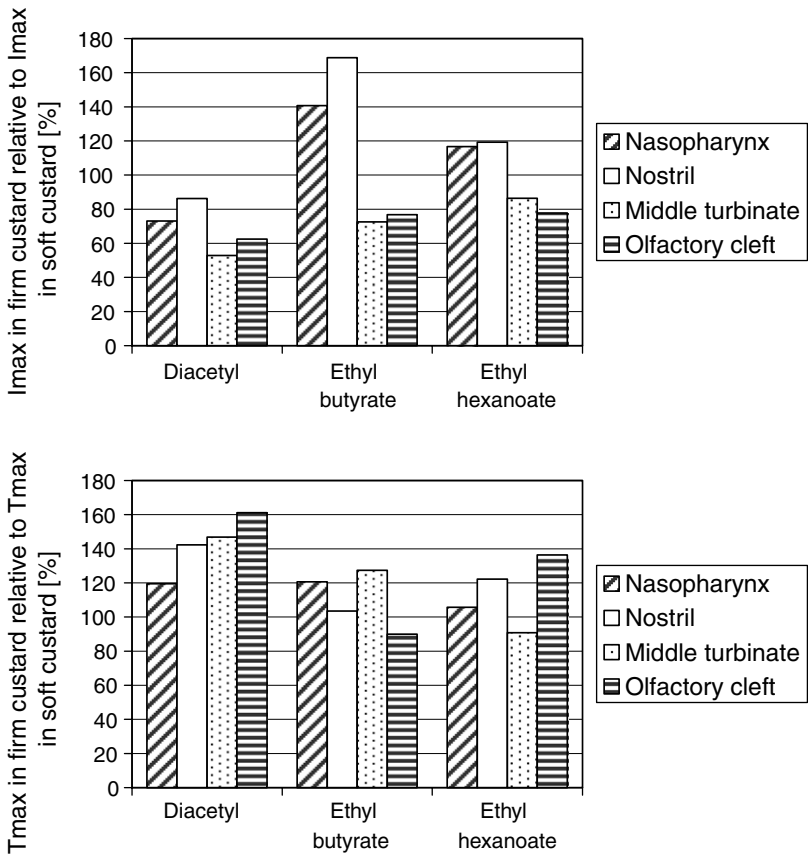

Fig. 5. Relative in-nose flavour parameters measured during consumption of custard at four intranasal locations by Proton Transfer Reaction Mass Spectrometry: maximum flavour concentrations for firm versus soft custard (I_{max}: upper diagram) and time to maximum flavour concentrations for firm versus soft custard (T_{max}: lower diagram). Means of nine subjects.

Table 2
Time to maximum in-nose flavour concentrations measured during consumption of a soft and firm custard at four intranasal locations determined by Proton Transfer Reaction Mass Spectrometry (means of nine subjects)[a]

	Nasopharynx	Nostril	Middle turbinate	Olfactory cleft
Soft custard				
Diacetyl	16.4^{ab}	14.9^{b}	17.5^{ab}	19.3^{a}
Ethyl butyrate	15.5^{a}	16.9^{a}	15.7^{a}	22.0^{a}
Ethyl hexanoate	21.1^{a}	20.2^{a}	28.3^{a}	19.8^{a}
Firm custard				
Diacetyl	19.6^{a}	21.2^{a}	25.7^{a}	31.1^{a}
Ethyl butyrate	18.7^{a}	17.5^{a}	20.0^{a}	19.8^{a}
Ethyl hexanoate	22.3^{b}	24.7^{ab}	25.7^{ab}	27.0^{a}

[a] Different superscripts in a row indicate significant differences (LSD test, $P < 0.05$).

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