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Influence of mastication on the concentrations of aroma volatiles — some aspects of flavour release and flavour perception

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Abstract

General aspects of flavour perception are discussed with regard to the relationships between odour concentration and odour intensity and effects on odour thresholds occurring in mixtures of odourants. Factors modifying flavour release during eating are emphasised, such as the influence of human physiology, the structure and the concentration of the odourants or the duration of mastication. Recent investigations performed by our group in terms of COST action 96 "Interaction of food matrix with small ligands influencing flavour and texture" concerning this topic are summarised. © 2000 Elsevier Science Ltd. All rights reserved.

1. Introduction

Besides texture and colour, the aroma is a key factor for the acceptance of food by the consumer. The optimum composition of food aroma compounds and their modifications induced by changes in the food matrix formulation are, therefore, of general interest for the food industry. This interest is enhanced by the market pressure to develop new products. Aroma perception, however, is not a simple stimulus — response process but is much more complex. Each food aroma is characterised by distinct compositions of a certain number of key odourants. Their release in mouth during consumption evokes the characteristic aroma impressions which we associate with a certain food. These impressions can change significantly when the characteristic balance is altered, e.g. by matrix effects.

To understand flavour release and factors affecting the release patterns during eating, it is a prerequisite that the important odourants of a food aroma and their mutual influence in flavour perception have been elucidated. In 1963, Rothe and Thomas proposed a term to measure the contribution of an odourant to a certain food aroma, called the "aroma value" [cf. Eq. (1)]. They assumed that only those odourants contribute to a food aroma whose concentrations in the food material exceed their odour thresholds.

$$AV_x = \frac{c_x}{a_x} \tag{1}$$

AV_x aroma value of an odourant in a food matrix
c_x Concentration of an odourant in a food matrix
a_x odour threshold of the odourant in a matrix similar to the food

Until now, the validity of the aroma value (AV) concept for the characterisation of the key food odourants in the multitude of volatile compounds has proven to be useful many times (e.g., Buettner & Schieberle, 2000a; Schieberle, Gassenmeier, Guth, Sen & Grosch, 1993; Teranishi, Buttery, Stern & Takeoka, 1991).

However, the aroma value concept cannot exactly describe the real odour intensity perceived at a given odourant concentration. First, it has been criticized that the aroma value is directly related to a specific odour threshold which can vary significantly due to the different techniques used for determination (Frijters, 1979). Second, flavour perception is not linearly correlated with the increase of odourant concentration, as it is proposed by the AV concept. It is characterised by much more complex patterns. Subsequently, many attempts have been made to find rules describing flavour perception as a function of odourant concentration. One mathematical

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description of flavour perception as a function of the odourant concentration was proposed by Stevens (1957):

$$R = c \cdot (I - I_0)^n \quad (2)$$

R: intensity of the odourant
I: concentration of the odourant
I_0: odour threshold of the odourant
c, n : specific constants of the odourant.

To show the correlation of concentration versus intensity, three model graphs resulting for three different exponential factors n are displayed in Fig. 1. The straight line with $n = 1$ corresponds to the aroma value resulting in a linear increase with increasing concentration. Since, in general, Stevens coefficients n of odourants published in the literature are below 1, the odour intensity of an odourant should follow a hyperbolic curve, showing a more or less linear increase of odour intensity at lower concentrations, but resulting in a plateau at infinity. The existence of a saturation plateau in flavour perception is well in line with our daily experience. However, phenomena like the depression or complete deletion of flavour perception, e.g. due to adaptation in very high odour concentrations, cannot be explained with this mathematical approach.

Following, a survey of investigations, being presented during COST Action 96, will be detailed. These studies were based on the theoretical premises given above and were aimed at elucidating an array of factors influencing ortho- and retronasal flavour perception. On one hand, the difficulties in predicting odour intensities and odour qualities of odourant mixtures will be discussed. On the other hand, effects on concentration changes of flavour compounds in mouth, e.g. the structure and the concentration of the odourant or the duration of mastication, will be described.

2. Materials and methods

Acetaldehyde 99%, decanal 95%, diacetyl 97%, ethyl butanoate 99%, ethyl hexanoate 99%+, ethyl 3-hydroxyhexanoate 98%+, ethyl 2-methylpropanoate 99%, ethyl 2-methylbutanoate 99%, ethyl 3-methylbutanoate 98%, ethyl octanoate 99%+, hexanal 98%, 4-hydroxy-2,5-dimethyl-3(2H)-furanone, methyl 2-methylbutanoate 99% and octanal 99% were obtained from Aldrich (Steinheim, Germany). The compounds were purified prior to analysis according to known procedures using either destillation in vacuo or crystallisation, respectively. (*Z*)-3-Hexenal was prepared according to the literature (Fielder & Rowan, 1995).

2.1. Sensory evaluation

Sensory evaluations were performed in an isolated sensory panel room as described previously (Guth & Grosch, 1993). Ten assessors were recruited from the Deutsche Forschungsanstalt fuer Lebensmittelchemie and were trained to evaluate odour qualities and odour

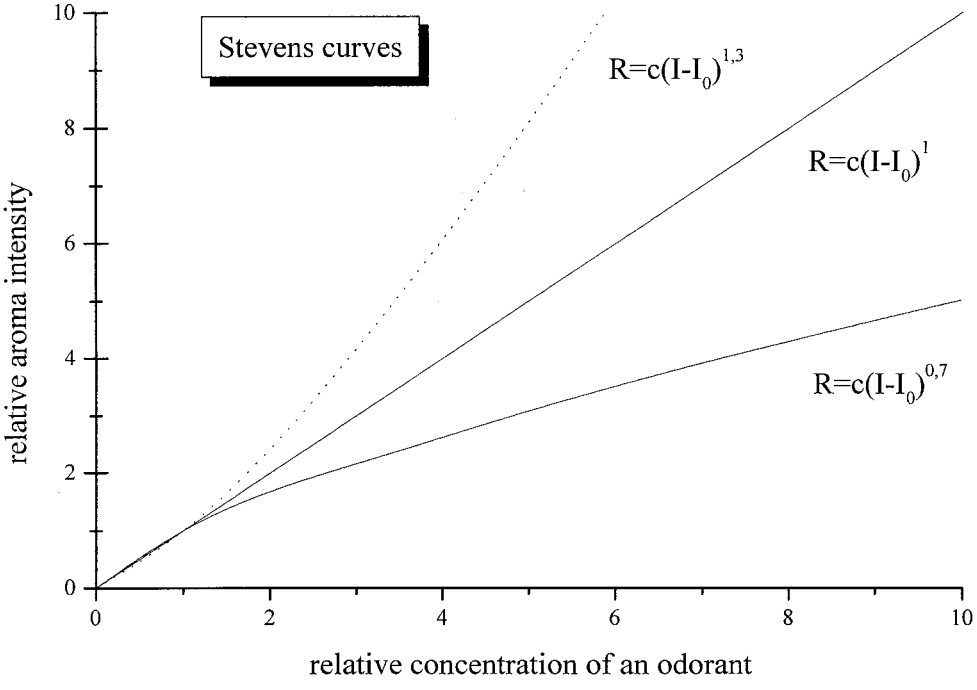

Fig. 1. Stevens curves resulting for three different exponential factors ($n = 1.3$; $n = 1$; $n = 0.7$).

intensities of about 40 defined odourous chemicals in different concentrations.

2.2. Orthonasal evaluation of single odourants

For the evaluation of the odour intensities of single odourants (methyl butanoate, ethyl butanoate, methyl 2-methylbutanoate, ethyl 3-methylbutanoate, ethyl 2-methylpropanoate), aqueous solutions from stock solutions in ethanol 100 mg odourant/1°ml ethanol) were prepared in concentration steps increasing by a factor of five using tap water starting from each odour threshold concentration. The sensory panel was asked to evaluate the odour intensities of the aqueous solutions of five fruity smelling esters presented in different concentrations. The assessors should select the solutions of the different esters eliciting the same odour intensity. An aqueous solution of ethyl butanoate containing 400 μg ethyl butanoate/l water was presented as the reference. The samples were presented in parallels as a duo-test according to the §35 LMBG of the German Health Organization (1993; method 00.90-8). The minimum of concurring answers for a significance-level $\alpha[\%] \leqslant 0.05$ is given by the following term:

$$X \geqslant \frac{n+1}{2} + k\sqrt{n}$$

$X =$ minimum of concurring answers
$k =$ 0.98 for $\alpha[\%] \leqslant 0.05$
$n =$ number of panelists

That means, in our experiments nine concurring answers out of a group of 10 people were required for a significant determination of similar odour intensities of two different ester solutions [significance $\alpha[\%] \leqslant 0.05$]. All tests were performed in duplicates.

2.3. Determination of odour thresholds in odourant mixtures

Odour thresholds of 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone, butane-2,3-dione and (*Z*)-hex-3-enal in tap water and in tap water containing one of these odourants in concentrations corresponding to an odour activity value of 200 (OAV 200), respectively, were determined according to §35 LMBG of the German Health Organization (1993; methods 00.90-0 and 00.90-7) by triangle tests and in parallels. The minimum of correct answers for the highest significance-level $\alpha[\%] \leqslant 0.01$ is given by the following term:

$$X = 0.4714 \cdot z \cdot \sqrt{n} + \frac{(2n+3)}{6}$$

$X =$ minimum of correct answers
$z =$ 3.10 for $\alpha[\%] \leqslant 0.01$
$n =$ number of panelists.

Therefore, in our experiments eight correct answers out of a group of 10 people were required for a significantly correct identification (significance $\alpha[\%] \leqslant 0.01$) of a sample in a triangle test. All tests were performed in duplicates.

To avoid adaptation, the samples were presented in order of increasing concentrations (1:5 dilutions).

2.4. Mastication experiments

Aqueous solutions of single reference aroma compounds (acetaldehyde, hexanal, octanal, decanal, ethyl-3-hydroxyhexanoate, ethyl butanoate, ethyl hexanoate, ethyl octanoate) at four different concentration levels (100 μg/l; 1 mg/l; 10 mg/l; 100 mg/l) in tap water were made from ethanolic stock solutions (100 mg odourant/1 ml ethanol). 25 ml of each model solution were rinsed in the mouth, limiting the actual amount of odourants in the mouth to 2.5 μg of odourant for the most diluted model solution applied.

2.5. Determination of flavour compounds in solvent extracts by stable isotope dilution assays

After rinsing in the mouth for a certain period of time (1 min or 5 s, respectively), the solutions and saliva were spitted into diethylether, spiked with stable isotope labeled standards and stirred for equilibration (20 min). The following labeled standards were applied: [$^2H_4$]-hexanal, [$^2H_4$]-octanal, [$^2H_2$]-decanal, [$^2H_5$]-ethyl-3-hydroxyhexanoate, [$^2H_3$]-ethylbutanoate, [$^2H_3$]-ethyl hexanoate, [$^2H_3$]-ethyl octanoate (Buettner, Hofmann & Schieberle, 2000). The etheral phase was removed, the solution extracted again with diethylether (30 ml, five times, total volume 150 ml) and the combined organic phases were dried over anhydrous Na_2SO_4. The volatile fraction was subsequently isolated by high vacuum transfer and the obtained aroma extract concentrated by careful distillation (Hinterholzer & Schieberle, 1998). For each experiment, four replicates were performed. Quantification of the volatiles was done by multidimensional gas chromatography (MD-HRGC) with the mass spectrometric system ITD-800 running in the chemical ionisation-mode using methanol as the reagent gas (Guth, 1997).

2.6. Determination of acetaldehyde by static headspace analysis

Because of its high volatility acetaldehyde can not be determined by the solvent extraction procedure described above. For this reason, acetaldehyde was determined by static headspace analysis using [1,2-$^{13}C_2$]-acetaldehyde as the internal standard. The masticated material was spitted into a vessel, sealed immediately with a septum and spiked with known amounts of [1,2-$^{13}C_2$]-acetaldehyde. After stirring for 30 min to

reach equilibration aliquots of the headspace were drawn with a gastight syringe and injected for MS-analysis using the system described previously (Guth & Grosch, 1994).

3. Results and discussion

3.1. Orthonasal flavour perception

3.1.1. Single odourants

As outlined in the introduction, the divergence between calculated aroma values and real odour intensity of flavour compounds was demonstrated by the following experiment: the evaluation of the odour intensities of aqueous solutions of five fruity smelling esters showed that the aroma values of different ester solutions differed significantly despite their similar perceived odour intensities (Table 1). This result corroborates the idea of Stevens law that no linear correlation does exist between concentration and odour activity in higher concentrations. However, the OAV concept should be more or less valid for lower concentrations of an odourant (near to its odour threshold), where the slope of the Stevens curve shows a linear increase. From a theoretical point of view, matrix influences should have a major effect in this region than in higher concentrations when the Stevens curve becomes flat. These sensory parameters have to be considered when matrix effects on flavour release, that means flavour binding or flavour liberation, shall be evaluated.

3.1.2. Mixtures of odourants

When looking at mixtures of odourants the situation becomes more complicated. Numerous previous studies were aimed at the evaluation of the overall quality and intensity of odorant mixtures. Different groups generally agreed that the perceived intensity of an odorant mixture is almost always less than the sum of the intensities of the single compounds (Jones & Woskow, 1964; Laing et al., 1984). Also, the odour quality of odorant mixtures has been evaluated for several compounds and it has been demonstrated that the odour quality of a less intense odorant can be completely suppressed in the presence of a compound eliciting a stronger odour intensity (Cain, 1975; Laing et al., 1984). On the other hand, it was stated that the single qualities of the compounds can be perceived also in odourant mixtures when the mixture contains odours with equal unmixed intensities. It was also shown that the odour quality of odourant mixtures can differ significantly from the odour qualities of the single compounds. This phenomenon was first observed by Laing and Willcox (1983) when studying mixtures of (*E*)-dec-2-enal and (*E*)-hex-2-enal. Recently, Masanetz et al. (1998) showed that a distinct mixture of the geraniumleaf-like smelling (*Z*)-octa-1,5-dien-3-one and methional having an odour of cooked potato, can elicit a characteristic fishy note and, therefore, can cause a fishy off-odour in foods such as dry spinach.

Table 1
Concentrations and odour activity values of esters causing the same odour intensity (Buettner et al., 1998)

Odourant	Concentration (μg/l water)	Odour activity value [a]
Methyl butanoate	5000	1000
Ethyl butanoate	400	400
Methyl 2-methylbutanoate	2500	10 000
Ethyl 3-methylbutanoate	400	2000
Ethyl 2-methylpropanoate	1000	10 000

[a] The odour activity values were calculated by dividing the concentrations of the odourants by their nasally determined detection thresholds in water.

However, the influence of one odourant on the odour threshold of another odourant in different media has, up to now, not been studied systematically. Recent experiments performed in our group have shown that odour thresholds are significantly influenced when a second odourant is present (Buettner, Hoch & Schieberle, 1998). In these studies one odourant was dissolved in water (in concentrations corresponding to an OAV of 200) and was used as the "solvent". The odour threshold of a second odourant was determined therein and was compared to the corresponding threshold in pure water. A significant increase of the odour threshold of 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone was observed (its odour intensity decreased) when a solution of butane-2,3-dione or (*Z*)-hex-3-enal in water was used as the solvent (cf. Table 2). In contrast, the odour thresholds of butane-2,3-dione or (*Z*)-hex-3-enal were not affected significantly by the presence of the additional odourant. Up to now, such effects observable in odourant mixtures cannot be seized by any theoretical model. Sensory findings like these more and more corroborate the idea that not only the reduction or increase of single odourants by matrix–flavour interactions has to be regarded when studying flavour release but also the effect of their reduction or increase on the perception of other odorants present in the food aroma. This agrees with the findings of sensory experiments performed in our group. It was shown that the omission of a certain odourant in a certain concentration from a model food aroma, e.g. orange aroma, can lead to significant flavour changes, while the omission of the same odourant in the same concentration from another aroma model, e.g. apple aroma, may not influence the overall aroma at all (unpublished data). So, changes in an overall flavour always depend on the composition of the food flavour which first has to be evaluated as an ensemble. This requires precise methods for the determination of the contributing odourants and their concentrations.

Table 2
Odour thresholds of selected odourants in water and in solutions containing another odourant in concentrations corresponding to the odour activity value (OAV) 200 (Buettner et al., 1998)

Odourant	Odour threshold (μg/l water or μg/l water containing another odourant)			
	In water [a]	+ 4-Hydroxy-2,5-dimethyl-3(2H)-furanone [b]	+ Butane-2,3-dione [b]	+ (Z)-Hex-3-enal [b]
4-Hydroxy-2,5-dimethyl-3(2*H*)-furanone	10	–	180	560
Butane-2,3-dione	3	4	–	3
(*Z*)-Hex-3-enal	0.3	0.4	0.4	–

[a] Odour threshold in water as reported in the literature (Rychlik, Schieberle, & Grosch et al., 1998).
[b] Aqueous solutions of the following odourants with concentrations corresponding to the odour activity value (OAV) 200 were used as solvent (concentrations are given in parentheses: 4-hydroxy-2,5-dimethyl-3(2*H*)-furanone (2000 μg/l water), butane-2,3-dione (600 μg/l water), (*Z*)-hex-3-enal (50 μg/l water).

3.2. Retronasal flavour perception — influence of physiology

Retronasal flavour perception is a very complex process which is strongly related to the human physiology. The odour stimuli, released from the food material during the chewing process, are influenced by mouth temperature, presence of saliva and possible adsorption or even resorption by the mouth mucosa. Previously, several techniques were applied to follow the release of volatile compounds during eating like the trapping of exhaled volatiles on adsorptive materials (Delahunty, Piggott, Conner & Paterson, 1996) or "real-time" measurements using mass spectrometric techniques (Soeting & Heidema, 1988; Taylor & Linforth, 1997). During mastication of the food, normal respiration continues thus showing that the oral cavity is mainly closed off from the trachea by the soft palate (Kahle, Leonhardt & Platzer, 1984). Recent investigations in our group showed that, in fact, the mouth is a closed system. Assuming that no swallowing at all occurs (even of saliva) and no air from the oral cavity is forced by, e.g. distinct mouth movements to pass the border (formed by the tongue and the soft palate above the tongue), this is also valid for highly volatile gases (Buettner & Schieberle, 2000b). This border prevents the eating person of the ingression of food material into the trachea. Land (1994) showed that the act of swallowing is always immediately followed by the expiration of a 5 to 15 ml volume of air. This pulse of air consists of the gas phase above the food material in the oral cavity enriched with volatile compounds released during mastication. The pulse takes only place at the moment when the epiglottis has momentarily closed the trachea between two breaths and, therefore, this air should contain the main amount of odourants evoking the retronasally perceived odour impression.

Based on the physiological prerequisites, the decrease of key odourants of orange juice during mastication was determined recently by application of a novel methodology called "SOOM-technique" (spit-off odourant measurement) (Buettner et al., 2000; Buettner & Schieberle, in press). The key odourants remaining in the food material were quantified using stable isotope dilution assays. Consequently, losses caused by swallowing and exhaling of odourants through the nose were excluded. By application of this technique exact data can be obtained on the amounts of odourants being retained in the mouth. Only these odourants can be effective in flavour perception.

3.3. Influence of the structure of the odourant on the retardation in mouth

By application of the SOOM-methodology the influence of the chemical structure of the odourants on their release in mouth was recently shown (Buettner et al., 2000; Buettner & Schieberle, in press). Aqueous model solutions of reference odourants were masticated for 1 min and the remaining amounts determined after mastication. The results obtained for homologous series of aldehydes and esters in the concentration 100 μg/l water are displayed in Fig. 2. The investigations showed clearly a discrimination of the odourants in mouth according to their polarity. With increasing chain length an increasing lingering effect in mouth was observed in both substance groups. Furthermore, the influence of the polarity was also demonstrated by comparison of the decrease of ethyl hexanoate and ethyl 3-hydroxyhexanoate in mouth. The hydroxyester was significantly retained in the aqueous phase obviously due to the hydroxygroup.

Generally, it was shown that an increase in the polarity of an odourant results in a lower retardation in the mouth.

3.4. Influence of the concentration of the odourant on the retardation in mouth

Many investigations in the field of flavour release were carried out at unrealistically high odourant concentrations mainly due to insufficient sensitivity of the

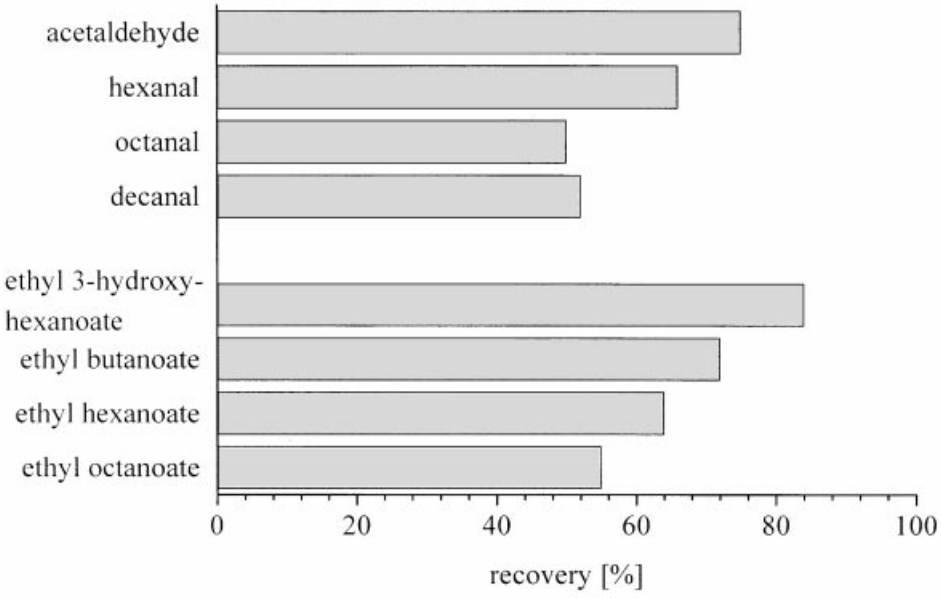

Fig. 2. Remaining quantity of odourants in spitted-off aqueous solutions after mastication of model solutions (100 μg/l water) for 1 min in the mouth (Buettner et al., 2000; Buettner & Schieberle, in press).

applied methodologies. However, many key odourants of food aromas are characterised by their high odour activity which goes along with relatively low concentrations of these compounds in the food material. For example it was shown recently that the two key odourants of grapefruit aroma, 4-mercapto-4-methyl-pentan-2-one and 1-*p*-menthene-8-thiol (previously identified by Demole et al., 1982), are found in the juice in concentrations as low as in the ppt-range (Buettner & Schieberle, 2000a).

Data on the influence of the odourant concentration on flavour retardation or even flavour release during mastication under real-mouth conditions could therefore not easily obtained. Recently, it could be shown that the relative amounts of odourants retained or released in the mouth during mastication are strongly influenced by the initial concentrations of the odourants applied (Buettner et al., 2000; Buettner & Schieberle, in press). Mastication of model aroma solutions showed that the percentage of odourants remaining in the mouth were significantly higher when lower concentrations of the odourants were applied. These findings were consistent for different substance groups of odourants, e.g. esters, aldehydes and terpenes. Some examples are given in Fig. 3. That means that against the assumption of many investigations the release of odourants in mouth does not strictly follow the premises of Henry's law and that therefore the amount of a released odourant and the amount of the odourant remaining in the food material is not constant in different concentration ranges. Up to now, this effect cannot be fully understood. However, the findings give way to the idea that the mucous membrane of the mouth could have a major effect by possibly adsorbing or even resorbing distinct amounts of odourants. The high resorptive potency of the mouth mucosa in regard to pharmaceuticals is already well known in medicine. Effects like these on odourants are possible and even probable but have not yet been proofed.

3.5. Influence of the duration of mastication on the retardation of odourants in mouth

Depending on the structure of the food material, the time of the food remaining in the mouth can vary significantly. For example, orange slices are chewed for a distinct period of time while orange juice stays in contact with the oral cavity just for a few seconds. Complete equilibration of the odourants between the food material and the air and saliva of the mouth during chewing can not be achieved immediately after the food is introduced into the mouth. That means that the amounts of odourants being released from the food material depend directly on the duration of mastication and the release rates of each single odourant. Consequently, the composition of the odourants released from the food material can change significantly during mastication not only resulting in higher amounts of the odourants with longer chewing but also in a different flavour profile. An example of this effect is given in Fig. 4 (Buettner et al., 2000; Buettner and Schieberle, in press). Mastication of model solutions containing hexanal, octanal and decanal, respectively, revealed not only lower amounts of the three aldehydes being retained in the mouth after the short-time mastication. It was also clearly demonstrated that the flavour profile changed significantly with increasing duration of mastication resulting in a much more pronounced effect of

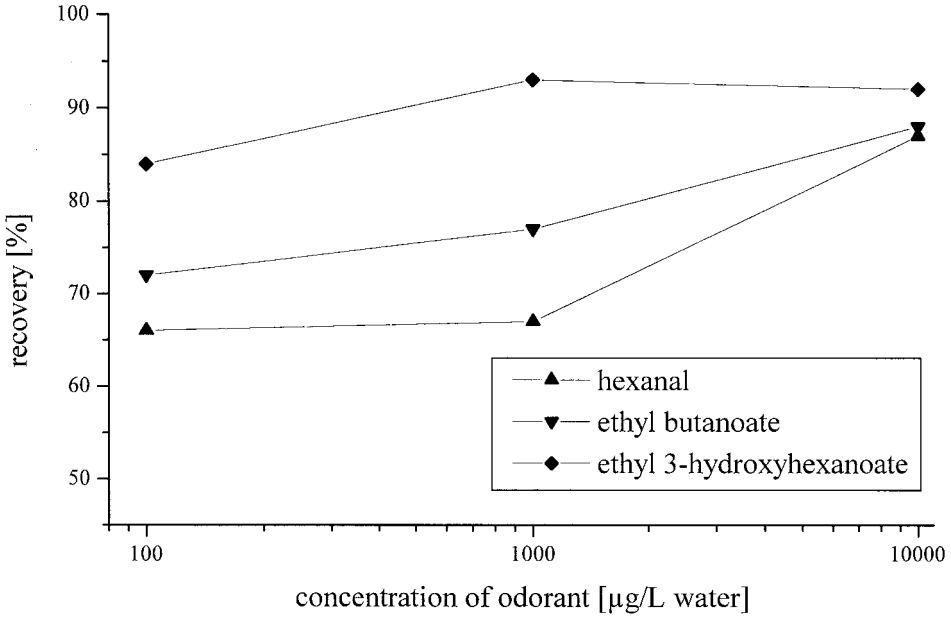

Fig. 3. Remaining quantities of hexanal, ethyl butanoate and ethyl 3-hydroxyhexanoate in spitted-off aqueous solutions after 1 min of mastication (Buettner et al., 2000; Buettner and Schieberle, in press).

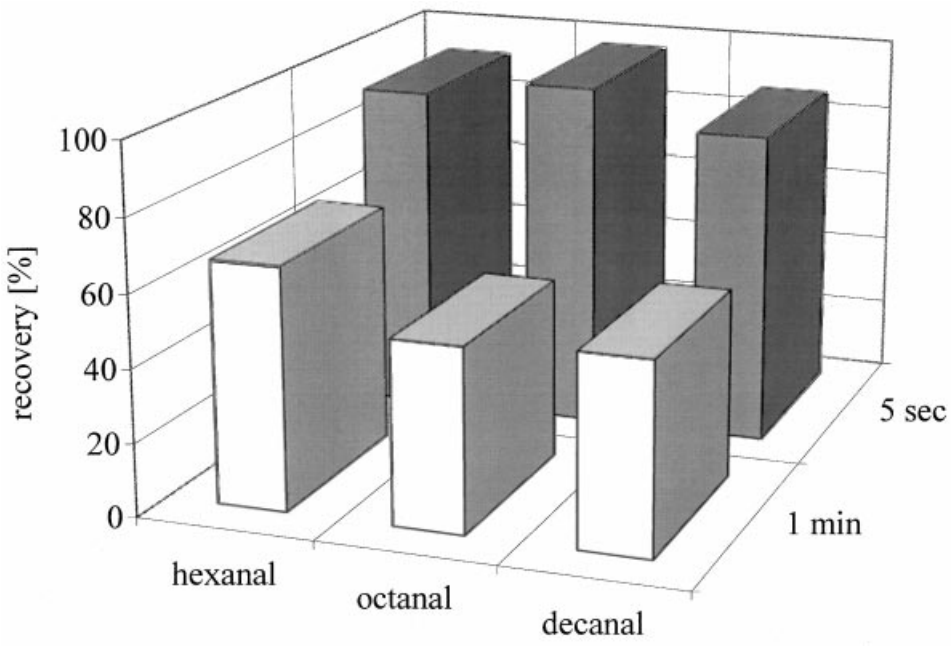

Fig. 4. Influence of the duration of mastication (1 min, 5 s) on the remaining quantities of aldehydes in spitted-off aqueous solutions (initial concentration 100 µg/l water) (Buettner et al., 2000; Buettner and Schieberle, in press).

the polarity, as discussed above. That means that the relative amount of the longer-chain aldehydes increased with longer mastication.

These experiments demonstrate that flavour release is a dynamic process which is directly related to the release rates of the odourants which on the other hand are significantly influenced by the matrix compounds present in the food material. These dynamic effects can be exactly determined even for odour-active, low-concentrated volatiles by application of the SOOM-technique.

4. Summary

Human sensory perception of an overall food aroma can be regarded as to be composed of several kinds of

sensations and flavour qualities, respectively, each present at a different intensity. Undoubtedly, the food matrix influences significantly the amounts of odourants released during consumption. However, the cited examples indicate that not only the composition of the food material itself, that means its physico-chemical properties, influences the pattern of flavour release and therefore flavour perception but also the structure and concentration of the odorant, their composition as a mixture and the eating patterns. One future main target in the investigation of flavour release will be to determine the amounts of odourants being released in mouth and to clarify the destiny of the released odourants afterwards. This requires the exact determination of the amounts of odourants being adsorbed or resorbed by the mouth mucosa and the knowledge of the quantities to be transferred retronasally to the olfactory epithelium for being effective in flavour perception.

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