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Food Chemistry 71 (2000) 339–345

Food Chemistry

www.elsevier.com/locate/foodchem

Influence of mastication and saliva on aroma release in a model mouth system

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Received 4 January 2000; received in revised form 25 April 2000; accepted 2 May 2000

Abstract

The influence of mastication, saliva composition and saliva volume on aroma release from rehydrated diced bell peppers and French beans was studied in a model mouth system. Released volatile compounds were analysed by gas chromatography combined with sniffing port and flame ionisation detection. Compounds were identified by gas chromatography/mass spectrometry, resulting in more than 40 compounds to be identified in each vegetable. Mastication increased release from bell peppers significantly and increased the number of volatile compounds with detectable odours in sniffing port analysis from six to 10 compounds. Addition of artificial or human saliva resulted in the same aroma profile for bell peppers. The amylase activity of artificial saliva consisting of human or porcine α-amylase was in the same range as amylase activity in human saliva at 37°C. Bacterial α-amylase had lower activity. Human and porcine α-amylase in artificial saliva added to rehydrated French beans did not differ significantly in starch breakdown and affected aroma release similarly. Increase in saliva volume decreased release of aroma compounds from rehydrated French beans significantly. The artificial saliva with porcine or human α-amylase sufficiently simulated human saliva with regard to aroma release. The three parameters mastication, saliva composition and saliva volume were shown to be important factors in aroma release from rehydrated vegetables. © 2000 Elsevier Science Ltd. All rights reserved.

1. Introduction

The flavour of foods perceived during eating, is determined by the rate and extent of flavour release in the mouth (McNulty, 1987). For many foods, considerable physico-chemical changes occur in the mouth during eating, which affect the release of volatile compounds from the food. Chewing will generally increase the surface area exposed to the air in the mouth, which increases the release of volatiles (Haring, 1990; van Ruth, Roozen & Cozijnsen, 1994). The physical form of foods affects the chewing and therefore the volatile release during eating. Disruption of plant or animal tissue during chewing might result in in-mouth (enzymatic) generation of volatiles. Hydration/dilution of foods by saliva will affect the partitioning of the volatile compounds over the food, saliva and vapour phase (Taylor, 1996). Proteins present in saliva can bind specific volatile compounds, and saliva volume can effect the hydration of the food and dilution of the volatiles. Furthermore, saliva volume is of importance and has been reported before as variable in models for flavour release (de Roos & Wolswinkel, 1994; Harrison, 1998; McNulty).

Saliva is secreted into the mouth by three major glands and functions in digestion, dentition protection, mucosal protection and protection through pH maintenance. Secretion of saliva from the salivary glands is under both sympathetic and parasympathetic control. The latter has control over the volume of saliva produces, whereas the former has greater control over certain proteins released (Beidler, 1995). Without stimulation, flow rates are about 0.5 ml per min, whereas maximal secretion averages 7.4 ml per min after rinsing of the mouth with 0.5 M citric acid (Davenport, 1977). Changes in the intensity and duration of gland stimulation cause salivary flow rate to vary widely. Concomitant with the change in flow rate, intensity and duration also causes changes in salivary composition (Young & Schneyer, 1981). It is estimated that over 200 different proteins and peptides are present in the saliva of humans (Beidler,

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PII: S0308-8146(00)00186-2

1995). Salivary composition and flow rate are affected by the degree of hydration, body position, exposure to light, olfaction, smoking, (previous) stimulation, climatological circumstances and circadian and circannual rhythms (Dawes, 1987; Wisniewski, Epstein & Caggiula, 1992). All these factors are responsible for large variations within a subject on a day and day-to-day basis, and also for a wide among-subject variation (Pangborn & Lundgren, 1978).

Amylase is a major protein component of human saliva and it initiates the breakdown of starch in the mouth (Beidler, 1995). In addition, other enzymes are present in human saliva, such as lingual lipase. High-molecular-weight mucins are the primary contributors to the saliva's viscosity. These and other proline-rich glycoproteins of saliva serve to lubricate hard surfaces. The saliva components can influence aroma release by their influence on partitioning and mass transfer (proteins) and through enhanced release of compounds by starch degradation (enzymes).

Summarising, mastication, saliva composition and volume are considered to be important parameters in aroma release in the mouth. The aim of the present work was to study the influence of these three parameters on aroma release from rehydrated diced bell peppers and French beans under mouth conditions.

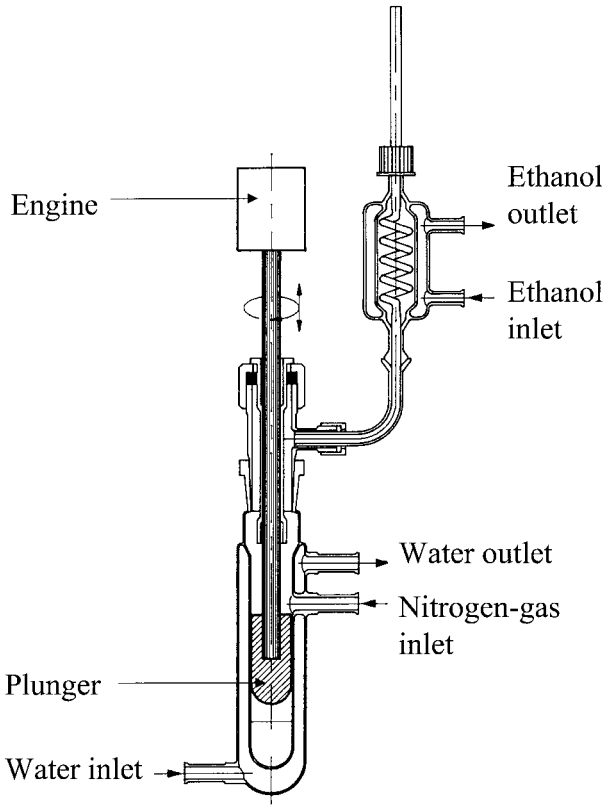

Fig. 1. Schematic diagram of the model mouth system.

2. Materials and methods

2.1. Materials

Commercially dried bell peppers (*Capsicum annuum*) and French beans (*Phaseolus vulgaris*) were supplied by Top Foods b.v. (Elburg, The Netherlands). Bell peppers were grown in Hungary, French beans in the Netherlands and both were blanched and diced prior to drying. After drying, the vegetables were packed in glass jars (720 ml) and stored at 4°C in absence of light until sampling.

2.2. Isolation of volatile compounds

For rehydration, 10 ml of distilled water was added to the dried vegetables (1.2 g) and the flask (50 ml) was placed in a waterbath at 100°C for 10 min. The vegetables were subsequently cooled down in a waterbath at 25°C for 4 min. The sample material was transferred into the sample flask (70 ml) of the model mouth system (Fig. 1), which was kept at 37°C by water circling through a double wall, and then 4 ml of artificial saliva were added. The artificial saliva consisted of distilled water, potassium phosphate dibasic trihydrate, sodium chloride, potassium chloride, calcium chloride dihydrate, sodium nitrate, sodium bicarbonate, mucin and α-amylase (Van Ruth, Roozen & Legger-Huysman, 1997). A purified nitrogen gas flow (100 ml min^{-1}) flushed the headspace of the vegetables/saliva mixture for 1 h, while volatiles were trapped in 0.10 g Tenax TA (35/60 mesh, Alltech Nederland b.v., Zwijndrecht, The Netherlands), positioned in a glass tube, 3 mm i.d. and 10 cm long. During isolation mastication could be applied by means of a plunger making up and down screwing movements.

2.3. Analysis of volatile compounds

2.3.1. Gas chromatography/flame ionisation detection analysis

Volatile compounds were thermally desorbed from the Tenax trap (200°C, 10 min) and cryo-focused on a cold trap (−100°C; Carlo Erba TDAS 5000, Interscience b.v., Breda, The Netherlands). Volatile compounds were analysed on a Carlo Erba MEGA 5300 gas chromatograph (GC; Interscience b.v., Breda, The Netherlands) equipped with a Supelcowax 10 capillary column, 60 m length, 0.25 mm i.d., 0.25 μm film thickness and a flame ionisation detector (FID) at 275°C. An initial temperature of 40°C for 4 min was used, followed by a rate of 2°C min^{-1} to 92°C and then by 6°C min^{-1} to 272°C. Six replicates of each vegetable sample were analysed.

2.3.2. Gas chromatography/sniffing port analysis

GC/sniffing analyses (GC/SP) were conducted using the same type of gas chromatograph, thermal desorption/cold trap device, analytical column and oven tem-

perature program as for GC/FID analyses. However, the effluent at the end of the analytical column was split 1:2:2 for FID (275°C), sniffing port 1 and sniffing port 2, respectively. Twelve assessors were selected and trained on the technique of sniffing. In preliminary sessions aroma descriptors were generated and clustered after group sessions of the panel, resulting in a list of 20 descriptors for the rehydrated vegetables. This list of descriptors included bell pepper, burned/rubber, butter, caramel, chocolate, citrus, coffee, cooked vegetables, fish, fresh vegetables, fruity, grassy/green, mushrooms, onion/leek, plastic/chemical, rotten, sickly, sour, spicy, sweet. Assessors used laptop computers and a program in Pascal for data collection. When an odour was detected, the assessors pressed a number key on the keyboard and released it when the odour had disappeared. Subsequently, a menu with the odour descriptors was presented on the screen and one of the descriptors or 'other/I do not know' had to be chosen, using a letter key. The data files of the twelve assessors were combined in a sniffing chromatogram, presenting the number of assessors detecting an odour simultaneously vs. retention time. The signal-to-noise level of the group of assessors was determined by assessment of dummy samples, i.e. Tenax tubes without adsorbed volatile compounds. Two assessors sniffed the volatiles isolated on a Tenax tube and FID response was recorded simultaneously, therefore one vegetable sample required six sniffing sessions and resulted in six replicate FID responses.

2.3.3. Gas chromatography/mass spectrometry analysis

The volatile compounds trapped in Tenax TA were identified by combined GC (Varian 3400, Varian, Walnut Creek, CA, USA) and mass spectrometry (MS; Finnigan MAT 95, Finnigan MAT, Bremen, Germany). The GC was equipped with a thermal desorption/cold trap device (TCT injector 16200, Chrompack b.v., Middelburg, The Netherlands). Analytical column and oven temperature program were identical to those used in GC/FID and GC/SP analyses. Mass spectra were obtained with 70 eV electron impact ionisation, while the mass spectrometer was continuously scanning from m/z 24 to 400 at a scan speed of 0.7 s decade^{-1} (cycle time 1.05 s).

2.4. Determination of starch content and starch degradation

2.4.1. Determination of starch content

Starch contents were determined in 1.2 g dried French beans in quadruplicate after rehydration, as described in Paragraph 2.2, using a Boehringer starch test-kit (no. 207748, Boehringer, Mannheim, Germany).

2.4.2. Determination of starch degradation

A Dionex Bio-LC system (Sunnyvale, CA, USA) equipped with a Dionex CarboPac PA-100 (4×250 mm) and a Dionex pulsed electrochemical detector in the pulsed amperometric detection mode were used for high-performance anion-exchange chromatography (HPAEC). Starch degradation products (di-, tri-, tetra-, penta- and hexamers of glucose) were analysed in 1.2 g dried French beans in duplicate after isolation of volatiles. A gradient of sodium acetate in 100 mM NaOH was applied: 0 to 30 min, 0 to 200 mM; 30 to 45 min, 200 to 600 mM; 45 to 50 min, 1000 mM; 50 to 65 min, 0 mM.

2.5. Determination of salivary amylase activity

Human saliva of four volunteers (aged 20–50) was collected in glass jars, without stimulation of the glands. The basic artificial saliva consisted of water, salts, and mucin (Van Ruth et al., 1997). Different types of α-amylase were added (200 units ml^{-1}): bacterial α-amylase (no 1329, Merck, Darmstadt, Germany), porcine α-amylase (Merck, Darmstadt, Germany) and α-amylase from human saliva (Sigma, St Louis, MO, USA). A modified method of Moore (1898) was used to determine amylase activity, in order to compare values with literature. A potato starch dispersion (1% w/w) was stirred and boiled. The gelatinised starch solution (10 ml) was diluted with 90 ml distilled water and warmed to 37°C. Artificial or human saliva (1 ml) was added to the 100 ml starch solution. The mixture was tested at 10 s intervals by mixing 0.3 ml solution with 0.3 ml of iodine (0.5% v/v) until no blue tinge was produced (the achromic point). The activity of amylase (D) is defined as $D = 10/v*5/n$, where v is the saliva volume in ml and n the time to reach the achromic point in min. Amylase activity was determined in quadruplicate in each type of saliva.

2.6. Statistical analyses

FID peak areas and amylase activity values were subjected to Student's t-tests. Friedman two-factor ranked analysis of variance were applied on the numbers of assessors detecting volatile compounds in GC/SP to determine significant differences, as well as in starch degradation experiments. For the different aroma compounds saliva volume effects were examined by analysis of variance. A significance level of $P < 0.05$ was used throughout the study.

3. Results and discussion

3.1. Effect of mastication

The effect of mastication under mouth conditions on aroma release from rehydrated diced bell peppers was studied in the model mouth system. The isolated volatile compounds were analysed by GC/SP, and identified by GC/MS and their retention times. Furthermore, they

were characterised by their odour descriptors, FID peak areas and number of assessors detecting the compounds (Table 1). When dummy samples were examined, maximal three assessors detected an odour simultaneously, consequently a perception of four or more assessors is considered to be above noise level. Six and ten volatile compounds were detected by the sniffing panel in GC/SP for the non-masticated and masticated sample, respectively. These compounds are considered to be major contributors to the aroma of the vegetable. However, larger numbers of volatile compounds were detected by GC/FID and GC/MS, which is in agreement with previous work that showed the release of 46 volatile compounds from rehydrated bell peppers in the model mouth system (Van Ruth & Roozen, 1994). 2-Methoxy-3-isobutyl pyrazine is the only compound with a distinct bell pepper odour. Mastication increased the release of each of the aroma compounds (Student's *t*-test, $P < 0.05$; Table 1). Increased release influenced also odour intensity of the compounds, as they were perceived by a significantly larger number of assessors at the sniffing port (Friedman two factor analysis of variance, $P < 0.05$). Brown and Wilson (1996) showed in vivo a similar mastication effect: greater mastication efficiency resulted in increased salivary concentration of flavour compounds. Burdach and Doty (1987) demonstrated the importance of oral movements in perceived retronasal olfactory intensity.

3.2. Comparison of human and artificial saliva

The release of aroma compounds from rehydrated diced bell peppers was studied in the model mouth system with human and artificial saliva. The human saliva was a mixture of the saliva of four volunteers and the artificial saliva was composed of water, salts, mucin and human α-amylase. The influence of human and artificial saliva was compared for six compounds that possessed detectable odours in GC/SP (Fig. 2). No significant differences in aroma release were determined (Student's *t*-tests, $P < 0.05$), indicating that this type of artificial saliva is suitable for simulating human saliva in this aroma experiment focused on rehydrated bell peppers. These bell peppers consisted of 0.5% starch only, therefore no enzymatic effect of α-amylase could be expected. However, this enzyme might be important for other food products.

The enzymatic efficiency of α-amylase in human and artificial saliva was compared by measurement of amylase activity in the saliva of four volunteers, and in artificial saliva with bacterial, porcine or human α-amylase (Table 2). The four volunteers showed activity in the 18–208 D units ml^{-1} range, which agrees with values (20–250 D units ml^{-1}) reported by Spector (1956), based on the same analysis. Both the latter author and the present results demonstrated a large variability in amylase activity between subjects. For the artificial salivas with porcine and human α-amylase similar activities were observed as for the human saliva. However, the activity of the bacterial α-amylase was very low (10 D units ml^{-1}). This is due to the differences in optimum temperatures with respect to activity for the three amylases. Porcine and human α-amylase show optimum activity at 40°C, whereas bacterial α-amylase shows highest activity at ca 70°C. From these experiments can be concluded that both porcine and human α-amylase are suitable as components in artificial saliva, with regard to amylase activity.

Table 1
Aroma compounds of rehydrated diced red bell peppers isolated in the model mouth system with and without mastication, odour descriptors, flame ionisation detector response (FID) and number of assessors perceiving the compounds in gas chromatography/sniffing port analysis[a,b]

Compound	Odour descriptors	Without mastication		With mastication	
		FID response (V s)	Number of assessors[c]	FID response (V s)	Number of assessors[c]
2-Methylpropanal	Chocolate	0.63x	4	5.31y	8
2/3-Methylbutanal	Chocolate	3.94x	9	24.91y	12
Diacetyl	Caramel, butter	0.65x	10	4.75y	12
1-Pentene-3-one	Plastic/chemical	0.06x	–[d]	0.77y	11
Hexanal	Grassy/green	4.41x	–	49.13y	12
Heptanal	Fruity, rotten, sour	0.22x	–	1.26y	7
Unknown	Fish, sickly, rotten	0.09x	–	0.60y	11
1-Octen-3-one	Mushroom, citrus	0.03x	7	0.28y	11
Dimethyl trisulphide	Rotten, onion/leek	–[e]	5	–	9
2-Methoxy-3-isobutyl pyrazine	Bell peppers	0.009x	10	0.055y	12
CV[%]		59		26	

[a] Average coefficients of variance: CV [%]; $n = 6$.
[b] Values with different letters within a row are significantly different, Student's *t*-test, $P < 0.05$.
[c] Numbers of assessors are significantly different for samples with and without mastication, Friedman two-factor ranked analysis of variance, $P < 0.05$.
[d] Below detection: perceived by three assessors or less.
[e] Peak area < 0.01 V s.

3.3. Saliva composition

Amylases can influence aroma release in food products with high starch contents. Therefore, the impact of porcine α-amylase and human α-amylase on aroma release from rehydrated diced French beans (starch content 20.4±1.3%) was studied in comparison with addition of artificial saliva without α-amylase. Focus was on both degradation of starch and aroma release.

Starch degradation was examined by determination of breakdown products of starch in rehydrated French beans after the volatile compounds had been isolated (Fig. 3). Artificial saliva's containing porcine α-amylase, human α-amylase, or no amylase were compared. Based on the quantities and range of quantifiable maltodextrins, the presence of porcine or human α-amylase increased the degradation of starch (Friedman two factor analysis of variance, $P < 0.05$). Porcine and human α-amylase showed similar breakdown patterns.

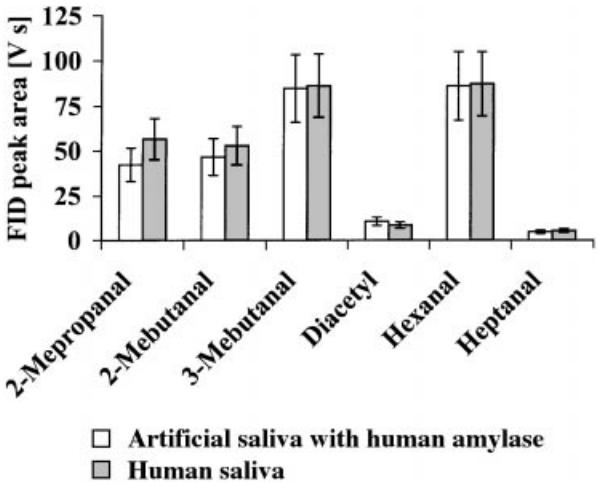

Fig. 2. Flame ionisation detection peak areas of aroma compounds released from rehydrated diced bell peppers in the model mouth system with addition of human saliva and artificial saliva containing human α-amylase ($n = 6$).

Table 2
Amylase activity (D value per ml) of human saliva and artificial saliva and average coefficients of variance[a]

Human saliva[b]	Amylase activity	Artificial saliva[c]	Amylase activity
A	18	Saliva with bacterial amylase	10
B	42	Saliva with porcine amylase	56
C	101	Saliva with human amylase	49
D	208		
CV [%]	26	CV [%]	8

[a] CV [%]; $n = 4$.
[b] Saliva of volunteers A, B, C and D.
[c] Artificial saliva consisting of water, salts, mucin and either bacterial, porcine or human α-amylase.

Volatile compounds, which possessed detectable odours in GC/SP in previous work (Van Ruth, Roozen, Cozijnsen & Posthumus, 1995) and could be quantified, were selected for comparison of the artificial salivas (Table 3). Release of 2-methylpropanal, 2-methylbutanal and 3-methylbutanal was significantly increased when porcine or human α-amylase was present in the saliva (Student's t-test, $P < 0.05$). This is likely to be due to release of these compounds from starch by activity of the amylases. The amylose fraction of starch can form helical structures in which the hydroxyl groups are oriented to the outside of the coil. Consequently hydrophobic regions exist in the inside of the polymer, in

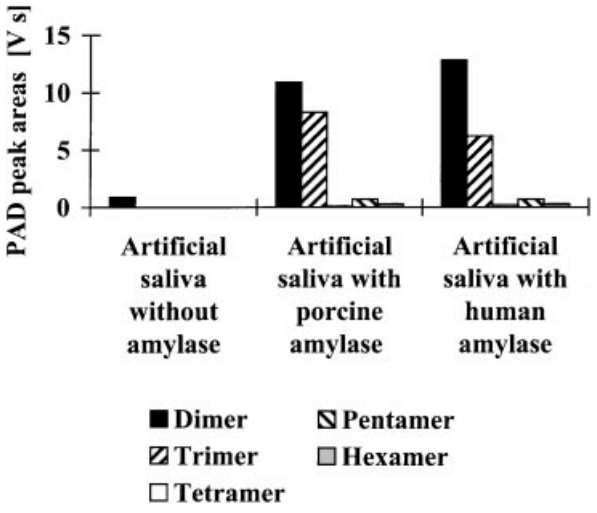

Fig. 3. Pulsed amperometric detection peak areas of starch degradation products in rehydrated diced French beans after addition of artificial saliva containing no α-amylase, porcine α-amylase or human α-amylase. Degradation products include di-, tri-, tetra-, penta- and hexamers of glucose ($n = 2$).

Table 3
Flame ionisation detector peak areas (V s) and average coefficients of variance (CV [%]) of aroma compounds released from rehydrated diced French beans in the model mouth system with addition of different types of artificial saliva ($n = 6$)[a]

Compound	Artificial saliva without amylase	Artificial saliva with porcine amylase	Artificial saliva with human amylase
2-Methylpropanal	7.25x	8.66y	8.23y
2-Methylbutanal	8.60x	9.83y	9.27y
3-Methylbutanal	9.90x	10.49x	10.79x
Pentanal	2.70x	2.66x	2.73x
Hexanal	3.25x	3.10x	3.16x
Octanal	0.04x	0.03x	–[b]
1-Octen-3-ol	0.07y	0.05x	0.04x
CV [%]	17.6	15.7	11.0

[a] Values with different letters within a row are significantly different, Student's t-test, $P < 0.05$.
[b] Peak area < 0.01 V s.

which flavour can be retained. The heating and cooling steps in the drying process are ideal conditions for the formation of these inclusion complexes (Osman-Ismail & Solms, 1973). The degradation of the complexes, which are present in dried French beans, by α-amylase can therefore result in increased release.

1-Octen-3-ol was released in significant greater amounts when saliva without α-amylase was added in comparison with the artificial saliva's containing α-amylase. This could be due to the fact that α-amylase can affect aroma release as protein, besides the enzymatic effects mentioned above. Many studies have shown that proteins diminish aroma release (Le Thanh, Thibeaudeau, Thibaut & Voilley, 1992; O'Keefe, Wilson, Resurrecion & Murphy, 1991; Plug & Haring, 1994).

The effects of α-amylase as an enzyme and as a protein did not differ significantly between the saliva with either porcine α-amylase or human α-amylase (Friedman two-factor analysis of variance, $P < 0.05$). Therefore, porcine α-amylase is a suitable replacer of expensive human α-amylase in artificial saliva with respect to aroma release.

3.4. Saliva volume

The volume of saliva is of importance for aroma release, in addition to its composition. The influence of saliva volume on aroma release from rehydrated diced French beans was studied in the model mouth system with addition of the artificial saliva containing porcine α-amylase. Fig. 4 represents the FID peak areas of 2-methylbutanal, 3-methylbutanal, hexanal and octanal, which contribute to the aroma of the beans. Release of the compounds decreased with increase of saliva volume for each of the compounds. Analysis of variance showed that the compounds differed significantly in absolute [$F(3,30) = 46.2$, $P < 0.05$] and relative [$F(3,25) = 102.2$, $P < 0.05$] change in release. This decrease agrees with the flavour release model of Harrison (1998) which predicted decreased release with higher saliva flow rates. Odake, Roozen and Burger (1998) reported lower release of diacetyl from emulsions and dressings with increased saliva volumes in the same model mouth system used in the present work. Dilution plays an important role in the changes in aroma release observed, however, also the change in overall composition of the mixture and the effect of mastication might have influenced the release. It is generally known that saliva flow rates differ widely among subjects. Bonnans and Noble (1995) as well as Fischer, Boulton and Noble (1994) have reported the influence of salivary flow on the perception of gustatory stimuli. The differences in aroma release resulting from varying saliva volumes shown in the present experiments, could have similar effects on perception.

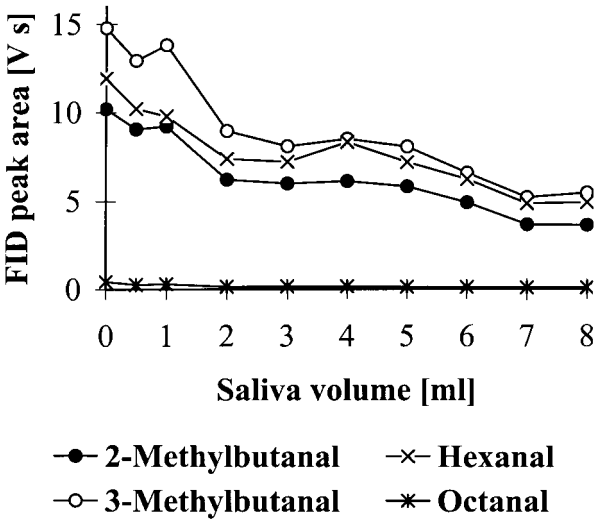

Fig. 4. Flame ionisation detection peak areas of four aroma compounds released from rehydrated diced French beans in the model mouth system with addition of various volumes of artificial saliva ($n = 6$).

4. Conclusions

Mastication, saliva composition and saliva volume influenced aroma release from rehydrated bell peppers and French beans markedly. Artificial saliva consisting of water, salts, mucin and α-amylase simulated human saliva sufficiently with respect to aroma release and starch degradation. Alteration of artificial saliva composition regarding replacement of human α-amylase by porcine α-amylase did not change aroma release.

Acknowledgements

The authors wish to thank Maarten Posthumus for identification of the aroma compounds and Jan Cozijnsen for excellent technical assistance. Both are at the Wageningen Agricultural University.

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