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Flavour release from rehydrated French beans (*Phaseolus vulgaris*) influenced by composition and volume of artificial saliva

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Abstract Influence of saliva composition and volume on flavour release from rehydrated French beans (*Phaseolus vulgaris*) was studied in three types of mouth model systems; dynamic headspace (DH), dynamic headspace and mastication (DHM) and a purge-and-trap (PT) model system. Volatile compounds were analysed by gas chromatography, using flame ionization detection (FID), mass spectrometry and sniffing port detection. Areas of FID peaks were largest in the PT system, followed by those of the DHM and DH systems, respectively. Saliva composition as well as volume influenced the release of volatile compounds from rehydrated French beans. Generally, FID data showed a decrease in release by the saliva component mucin, because of interactions between volatile compounds and protein, and an increase in release by its α-amylase, probably due to degradation of inclusion complexes of starch. The decrease in flavour release by the enlarged saliva volume was evaluated by a model study. Sniffing patterns of odour active compounds were barely influenced by either saliva composition or volume.

Key words Flavour release · Saliva-flavour – relationship · French beans

Introduction

Stimulation of the olfactory receptors by foods via the retronasal route is a primary determinant of their flavour. Persons who have lost their sense of smell frequently perceive the loss as one of taste, rather than one of smell [1–3]. To elicit an olfactory response, a flavour compound must achieve a sufficient concentration in the vapour phase [4]. The rate of volatilization of a compound depends upon the partition coefficient of the compound, molecular interactions between flavour compounds, the ambient temperature, the composition and viscosity of the food material and of the binding to components of the food [4–8]. The binding of volatile compounds to food ingredients can have major effects on the perception of the total food product, as it may result in a distortion of the composition of volatile compounds [9].

The breakdown of the food matrix through mastication enhances flavour release [6, 10] as well as retronasal odour perception [11]. The physical form of foods affects the profile of volatile compounds and it changes during consumption, due to hydration and dilution of foods by saliva. Saliva plays a prominent role in perception of the physical and chemical properties of oral stimuli [12]. Substances secreted in saliva, including α-amylase which functions in the initial digestion of starch, could alter the perception of the flavour of the food [13]. Previously, the influence of saliva components on the flavour release was shown in rehydrated bell peppers [14]. Beside factors influencing the amount and composition of saliva, such as the size of the food particles and variations within a day and day-to-day, there is a wide among-subject variation [12].

Extraction and headspace analysis are the main methods for isolation of volatile compounds from vegetables [15–18]. The factors influencing flavour release under those conditions in the mouth may create significant differences between the classical headspace profile of volatile compounds and the actual profile in the mouth [19]. Recently the authors introduced a dynamic headspace (DHM) model system for isolation of volatile compounds from vegetables under conditions found in the mouth, such as volume, temperature, salivation and mastication [10].

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The present study deals with the influence of artificial saliva components, salts, mucin and α-amylase, on the flavour release from rehydrated diced French beans in three types of the mouth model system. A mechanistic model was developed for changes in release of odour active compounds by saliva volume.

Materials and methods

Plant material. Commercially air-dried French beans (*Phaseolus vulgaris*) were supplied in pieces by Top Foods (Elburg, The Netherlands). The beans were blanched prior to drying. The vegetables were packed in glass jars and stored at 4 °C in the absence of light until sampling.

Sample preparation. After storage, the dried beans (1.2 g) were rehydrated prior to analysis, by adding 10 ml distilled water, followed by heating in a waterbath at 100 °C for 10 min and cooling down in a waterbath at 25 °C for 4 min.

The diced French beans were transferred into the sample flask of the following mouth model systems: DH, "dynamic headspace" (DH) and "purge-and-trap" (PT) as described previously [10] and 4 ml of one of the artificial salivas was added in all saliva composition experiments. Saliva W consisted of distilled water only. Saliva WS consisted of 5.208 g $NaHCO_3$, 1.369 g $K_2HPO_4 \cdot 3H_2O$, 0.877 g NaCl, 0.500 g NaN_3, 0.477 g KCl and 0.441 g $CaCl_2 \cdot 2H_2O$ in 1 l distilled water (adjusted to pH 7). The composition of saliva WSM was identical to WS, but 2.160 g mucin (Sigma, St Louis, Mo., USA) was added. Saliva WSMA was identical to saliva WSM, but 200 000 units α-amylase (Merck, Darmstadt, Germany) was added. In the saliva volume experiments 0, 0.5, 1, 2, 3, 4, 5, 6, 7 or 8 ml of saliva WSMA was added to the rehydrated French beans. The headspace of the model systems DH and DHM was flushed with purified nitrogen gas in order to trap volatile compounds in Tenax TA, as described previously [14]. The plunger of the DHM model system made about four up and down screwing movements per min in order to simulate mastication during isolation of the volatile compounds. In the PT model nitrogen gas was purged through the vegetable/saliva mixture.

Instrumental flavour analysis. Desorption of volatile compounds from Tenax TA was performed by a thermal desorption/cold trap device. The compounds were analysed by gas chromatography with flame ionization detection (GC/FID) as reported previously [20].

GC/sniffing port analysis (GC/SP) was carried out as described previously [21]. In preliminary GC/SP experiments ten assessors (aged 20–50 years) generated odour descriptors for the volatile compounds of rehydrated French beans, which were clustered during group sessions of the panel. These descriptors included bell pepper, burned, caramel, chemical, chocolate, citrus, cooked vegetables, cucumber, detergent, fatty, French bean, fruity, garlic, grassy, herbal, leek, metal, mushroom, onion, rancid, rotten, sickly/musty, sour, spicy and sweet. Completed with "other/I do not know", one of these descriptors had to be chosen for each compound detected by the assessors at the sniffing port. Tenax TA tubes without adsorbed volatile compounds were used as dummy samples for determining the signal-to-noise level of the measurements made by the group of assessors.

The volatile compounds trapped in Tenax TA were identified by combined GC/mass spectrometry (GC/MS) as reported previously [21].

Starch determination. Starch contents were determined in 1.2 g dried French beans and 1.2 g dried red bell peppers after rehydration in duplicate, using a Boehringer Starch test-kit no. 207748 (Boehringer, Mannheim, Germany).

Starch degradation determination. High-performance anion-exchange chromatography (HPAEC) was performed using a Dionex Bio-LC system (Sunnyvale, Calif., USA) equipped with a Dionex CarboPac PA-100 (4 × 250 mm) and a Dionex pulsed electrochemical detector in the pulsed amperometric detection mode. In this system, degradation products of starch (mono-, di-, tri-, tetra-, penta- and hexamers of glucose) were analysed in duplicate in 1.2 g dried French beans after rehydration and isolation of volatile compounds, using a gradient of sodium acetate in 100 mM NaOH as follows: 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.

Results and discussion

Saliva composition

Volatile compounds of rehydrated French beans were isolated in three mouth model systems (DH/DHM/PT). Model systems DH and PT were studied as they are frequently used in instrumental flavour analysis. Previously it was shown that release from rehydrated French beans in model system DHM did not differ from release in the mouths of 12 assessors [22]. Four different compositions of artificial saliva were added (W/WS/WSM/WSMA). Figure 1 represent the SP chromatograms of diced rehydrated French beans, to which salivas W or WSMA was added in model system DHM. The volatile compounds were identified by GC/MS and their retention times. They were further characterized by the areas of their peaks and the odour descriptors provided by the assessors at the sniffing port (Tables 1 and 2). GC/SP of dummy samples revealed that detection of an odour at the sniffing port by three or fewer out of ten assessors can be considered as "noise".

Qualitatively, similar volatile compounds were present in the three mouth model systems and saliva compositions (Table 1). Large differences in areas of peaks of volatile compounds between the model systems are obvious. For all salivas, model PT showed the largest areas of peaks, followed by DHM and DH, respectively (sign rests, $P < 0.05$). This is in agreement with previous results, which showed similar differences between the model systems in flavour release from rehydrated bell peppers [10]. In particular the addition of mucin decreased the release of volatile compounds in model system DH (sign tests, $P < 0.05$). This effect of protein agrees with previous results concerning the flavour release from rehydrated bell peppers [14] and with studies of Gremli [23], Damodaran and Kinsella [24], Kim and Min [25] and O'Keefe [26]. The effect of mucin was observed to a lesser extent in model systems DHM and PT, which could be due to disturbance of interactions between volatile compounds and proteins by mechanical forces during sampling.

The presence of α-amylase in artificial saliva (WSMA) in model system DHM increased the area of the peak of many volatile compounds of French beans

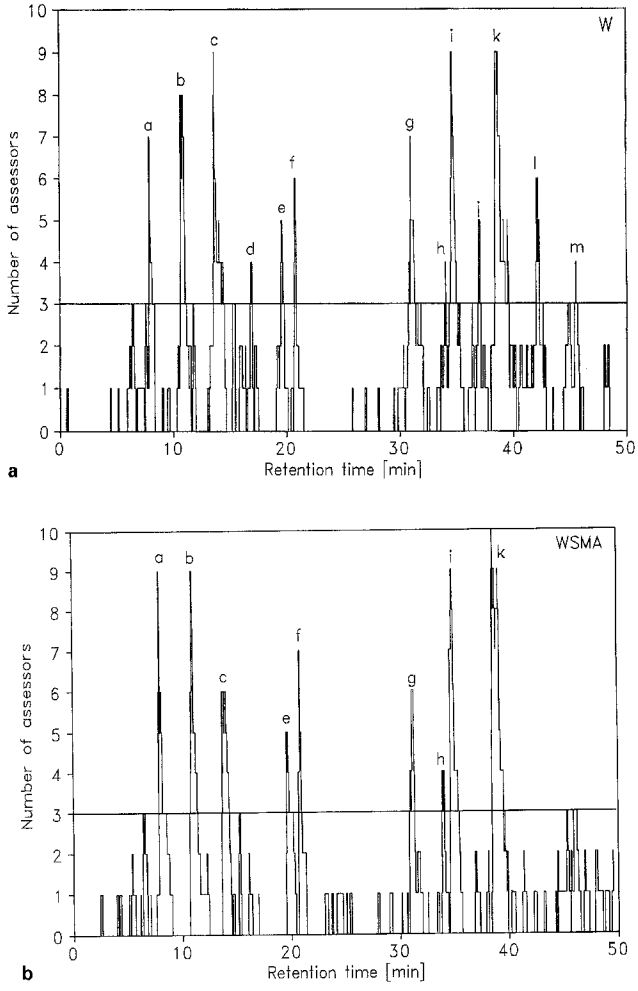

Fig. 1a, b Chromatograms of volatile compounds of rehydrated French beans in model system dynamic headspace with mastication (DHM) with addition of saliva W (*top*) and WSMA (*bottom*) obtained by sniffing port detection. For an explanation of codes and letters see Tables 1 and 2, respectively

(Table 1). Comparison of rehydrated French beans and red bell peppers [14] revealed that 24 volatile compounds were detected in both vegetables; propanal, 2-methylpropanal, methyl acetate, butanal, 2-butanone 2- and 3-methylbutanal, pentanal, 1-penten-3-one, 2,3-pentanedione, hexanal, 1-penten-3-ol, 2-heptanone, heptanal, limonene, 2-hexenal, 1-pentanol, octanal, 2-heptenal, 6-methyl-5-hepten-2-one, dimethyl trisulphide, nonanal, 1-octen-3-ol and decanal. The presence of α-amylase in artificial saliva (WSMA) resulted in French beans generally, in increased areas of the peaks pertaining to these volatile compounds; 15 peaks showed an increase, 7 peaks a decrease and 2 peaks no change. In contrast, a general decrease in the areas of the peaks of these volatile compounds was shown in bell peppers; 6 peaks increased and 18 peaks decreased in area. Their increased release from the French beans could be caused by degradation of inclusion complexes of starch, which were formed during the drying process. The amylose fraction of starch is able to include volatile compounds. Inside the helical structure is a relatively hydrophobic area, in which several ligands (alcohols, aldehydes, terpenes and fatty acids with apolar side chains) can be included. Drying of vegetables involves heating and cooling, which are ideal conditions for the formation of inclusion complexes [27, 28]. Degradation of inclusion complexes of starch by α-amylase could result in an increase in flavour release.

The presence of α-amylase increased the degradation of starch in each of the model systems and mostly in model DHM (Table 3). This is probably due to the mechanical effect of mastication on the dried French beans, which contained a high level of starch (17.0%). In contrast, the dried bell peppers consisted of 0.5% starch. Therefore, no increase in flavour release due to starch degradation was to be expected in bell peppers. Flavour release from bell peppers was probably reduced by interactions between volatile compounds and proteins of the α-amylase. Similar interactions of α-amylase could also predominate the enzymic effect in model DH, as addition of α-amylase resulted generally in a decrease in the areas of the peaks of volatile compounds in this model system (Table 1). In contrast with DHM and PT model systems, no disturbance of these interactions by mechanical forces occurred in model system DH during sampling. Previous comparison of the three model systems revealed that model system DH demonstrated the largest decrease in flavour release from bell peppers by addition of α-amylase to the artificial saliva [14].

Model system DHM simulated well the release of flavour in the mouth [22]. Saliva composition could affect flavour perception and therefore GC/SP analyses of rehydrated French beans in model system DHM with addition of the extremes in saliva composition (W and WSMA) were performed. The SP and FID chromatograms were compared: a slight decrease in the number of assessors perceiving an odour at the sniffing port was shown in GC/SP (Fig. 1). Odour descriptions were similar for both salivas. In Table 1, the FID data showed an overall decrease in areas of the peaks of volatile compounds of the rehydrated French beans in the presence of salt, mucin and α-amylase in the artificial saliva (WSMA). Overall, saliva components seem to decrease flavour release from rehydrated French beans. Previously sniffing port analysis has correlated quite well with descriptive sensory analysis [21, 29], therefore only a slight effect of the composition of saliva on flavour perception is to be expected.

Saliva volume

The influence of saliva (WSMA) volume on flavour release from rehydrated French beans was studied in model system DHM, as mouth conditions were best simulated by this saliva and model system [22]. Table

Table 1 Volatile compounds of rehydrated diced French beans (*Phaseolus vulgaris*) isolated in three mouth model systems (DH/DHM/PT) with addition of four compositions of artificial saliva (W/WS/WSM/WSMA), retention indices, average areas of peaks (flame ionization detection) and coefficients of variance ($n = 6$)[a]

No	Ret. index	Compound	Area of the peak (V·s)											
			DH				DHM				PT			
			W	WS	WSM	WSMA	W	WS	WSM	WSMA	W	WS	WSM	WSMA
1	769	Propanal	0.33	0.23	0.16	0.10	0.79	0.72	0.46	0.66	2.14	1.22	1.17	1.22
2	810	2-Methylpropanal	4.74	3.02	2.04	1.69	8.70	8.70	7.25	8.66	26.70	19.59	21.32	21.64
3	822	Methyl acetate	0.15	0.12	0.06	0.05	0.32	0.34	0.24	0.27	1.06	0.75	0.75	0.79
4	871	Butanal	0.27	0.14	0.12	0.07	0.40	0.39	0.38	0.41	1.04	0.86	1.19	1.03
5	877	2-Methyl-2-propenal	0.06	0.08	0.04	0.04	0.36	0.19	0.14	0.24	0.75	0.07	0.01	0.04
6	902	2-Butanone	0.59	0.20	0.18	0.13	0.48	0.45	0.46	0.52	0.18	1.11	1.05	1.22
7	917	2-Methyl butanal	9.83	2.87	2.06	1.37	9.00	9.60	8.60	9.83	29.79	25.48	26.95	26.94
8	923	3-Methyl butanal	11.21	3.62	2.73	1.74	10.52	10.90	9.90	10.49	36.59	30.36	32.57	31.67
9	981	Pentanal	2.59	1.20	1.05	0.65	3.20	2.83	2.70	2.66	8.83	6.88	8.56	6.05
10	1024	1-Penten-3-one	0.03	0.04	0.01	tr	tr	0.01	0.05	0.01	0.13	0.03	0.10	0.04
11	1071	2,3-Pentane-dione	0.16	0.07	0.06	0.02	0.16	0.15	0.15	0.16	0.48	0.37	0.48	0.44
12	1074	2-Methyl-1-penten-3-one	0.35	0.20	0.22	0.08	0.37	0.66	0.68	0.54	1.00	1.26	2.80	1.54
13	1082	Butyl acetate	0.22	0.16	0.09	0.06	0.25	0.17	0.18	0.30	0.51	0.66	0.84	1.00
14	1094	Hexanal	4.08	1.40	1.17	0.67	3.65	3.34	3.25	3.10	13.82	11.04	13.56	10.97
15	1105	2-Methyl-2-butenal	0.02	0.01	tr	tr	0.03	0.02	0.04	0.03	0.10	0.10	0.10	0.10
16	1140	2-Pentenal	0.17	0.07	0.08	0.01	0.19	0.20	0.23	0.15	0.54	0.41	0.69	0.40
17	1176	1-Penten-3-ol	0.22	0.12	0.13	0.07	0.26	0.23	0.22	0.26	0.64	0.51	0.82	0.66
18	1190	2-Heptanone	0.04	0.07	tr	tr	0.01	0.05	0.02	0.04	0.23	0.21	0.18	0.17
19	1194	Heptanal	0.07	0.03	0.02	tr	0.15	0.11	0.10	0.14	0.29	0.20	0.29	0.25
20	1212	Limonène	0.10	0.05	0.01	tr	0.05	0.04	0.05	0.03	0.29	0.24	0.18	0.18
21	1225	2-Hexenal	0.47	0.32	0.13	0.04	0.34	0.30	0.23	0.23	1.41	1.16	0.92	0.89
22	1257	1-Pentanol	0.26	0.15	0.07	tr	0.19	0.16	0.14	0.13	0.83	0.69	0.55	0.51
23	1288	2-Octanone	0.03	0.04	0.01	tr	0.03	0.03	0.04	0.04	0.12	0.09	0.10	0.08
24	1293	Octanal	0.06	0.09	0.02	tr	0.04	0.06	0.04	0.03	0.19	0.15	0.13	0.12
25	1331	2-Heptenal	0.24	0.17	0.22	0.07	0.28	0.41	0.46	0.34	0.52	0.67	1.73	0.75
26	1347	6-Methyl-5-hepten-2-one	0.06	0.07	0.01	tr	0.04	0.05	0.04	0.03	0.18	0.14	0.13	0.13
27	1383	Dimethyl trisulphide	0.03	tr	tr	tr	tr	tr	tr	tr	0.14	0.10	0.13	0.16
28	1397	Nonanal	0.16	0.14	0.08	0.10	0.85	0.37	0.35	0.64	0.39	0.27	0.25	0.34
29	1456	1-Octen-3-ol	0.05	0.07	0.03	tr	0.03	0.06	0.07	0.05	0.17	0.13	0.29	0.13
30	1503	Decanal	0.17	0.08	0.09	0.01	0.15	0.27	0.24	0.92	0.33	0.15	0.73	0.41
		CV(%)	53	21	29	25	27	29	22	23	12	22	13	9

[a] DH, dynamic headspace model; DHM, dynamic headspace and mastication model; PT, purge-and-trap model; W, distilled water; WS, W + salts; WSM, WS + mucin; WSMA, WSM + α-amylase; tr, area of peak < 0.01 V·s; CV, mean coefficient of variance of individual compounds

Table 2 Odour active volatile compounds of rehydrated French beans detected by gas chromatography/sniffing port analysis and odour descriptors

Code	Odour active compound	Odour description
a	2-Methylpropanal	Chocolate, spicy
b	2- and 3-Methyl-butanal	Chocolate, spicy
c	2,3-Butanedione	Caramel, fatty
d	1-Penten-3-one	Chemical, sweet, sour, citrus, leek
e	Hexanal	Grassy, cucumber, bell pepper
f	2-Methyl-2-butenal	Chemical, fatty
g	Unknown	Chemical, rotten, rancid
h	Octanal	Chemical, citrus, fatty, sweet
i	1-Octen-3-one	Mushroom
j	Unknown	Rotten, sickly
k	Dimethyl trisulphide	Metal, rotten, garlic
l	1-Octen-3-ol	Fatty, sickly, mushroom, metal
m	Unknown	Cucumber, French bean, citrus, bell pepper

Table 4 Areas of peaks (flame ionization detection) and coefficients of variance (CV, $n = 6$) of four odour active compounds of rehydrated French beans influenced by saliva volume and parameter estimates for the description of changes in flavour release (W_r/kX_p and $1/kX_p$)[a]

Saliva volume (ml)	Area of peak (V·s) of compound:			
	2-methyl--butanal	3-methyl--butanal	Hexanal	Octanal
0	10.18	14.77	11.90	0.42
0.5	9.05	12.92	10.19	0.25
1	9.21	13.80	9.77	0.28
2	6.20	8.95	7.38	0.13
3	5.98	8.09	7.21	0.15
4	6.11	8.50	8.32	0.15
5	5.81	8.07	7.19	0.12
6	4.91	6.58	6.22	0.11
7	3.66	5.20	4.85	0.08
8	3.63	5.46	4.92	0.08
CV(%)	20	20	18	24
W_r/kX_p	0.095	0.068	0.088	3.061
$1/kX_p$	0.021	0.015	0.014	1.168

[a] The model is described in Results and discussion

4 presents the FID areas of the peaks of four odour active compounds (2- and 3-methylbutanal, hexanal and octanal) released from the French beans upon addition of different volumes of saliva WSMA. McNulty [5] and De Roos and Wolswinkel [8] modelled flavour release in the mouth. According to these studies, dilution is not expected to affect product-to-air partition coefficients of volatile compounds, as French beans possess a low fat content. The relationship between dilution factor and flavour release is supposed to be reversed proportional. The partition coefficient (P_{pg}) is defined as:

$$P_{pg} = X_g/X_p \quad (1)$$

where X_g and X_p are the quantities of flavour compounds in the gaseous and product phases, respectively.

Table 4 justifies similar modelling as was proposed by the authors mentioned above and the data were fitted to the model:

$$1/X_g = W_r/kX_p + W_s/kX_p \quad (2)$$

W_r represents the quantity of rehydration water available for dilution; W_s is the quantity of saliva and k a constant. The estimates for W_r/kX_p and $1/kX_p$ are presented in Table 4 too. Both the FID responses determined for 3-methylbutanal and the curve of the corresponding model describing the changes in flavour release by saliva volume are shown in Fig. 2. High correlation coefficients were obtained for 2-methylbutanal (0.96), 3-methylbutanal (0.96), hexanal (0.94) and octanal (0.96), which indicated that the data fit well

Table 3 Areas of peaks (pulsed amperometric detection) of starch degradation products: mono-, di-, tri-, tetra-, penta- and hexamers of glucose in rehydrated diced French beans after isolation of volatile compounds in three mouth model systems (DH/DHM/PT) with addition of four compositions of artifical saliva (W/WS/WSM/WSMA)[a]

Product	Area of peak (V·s)											
	DH				DHM				PT			
	W	WS	WSM	WSMA	W	WS	WSM	WSMA	W	WS	WSM	WSMA
Monomer	19.9	17.5	18.6	21.7	22.9	26.7	23.7	24.1	18.6	18.6	17.6	22.4
Dimer	0.8	0.9	0.8	5.8	1.0	1.2	0.9	10.9	0.7	0.9	0.8	7.1
Trimer	tr	tr	tr	4.1	tr	tr	tr	8.3	tr	tr	tr	5.1
Tetramer	tr	tr	0.1	0.1	tr	0.2	tr	0.1	tr	tr	tr	0.2
Pentamer	tr	tr	tr	0.1	tr	tr	tr	0.7	tr	tr	tr	0.3
Hexamer	tr	tr	tr	0.1	tr	tr	tr	0.3	tr	tr	tr	0.2

[a] For an explanation of codes see footnote to Table 1; tr, area of peak < 0.1 V·s

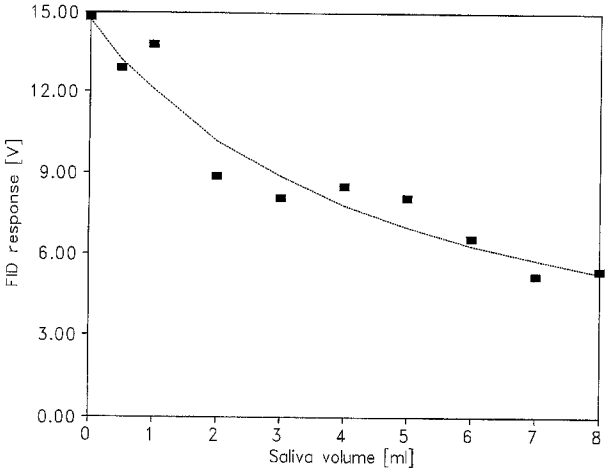

Fig. 2 Area of peaks (flame ionization detection) of 3-methylbutanal of rehydrated French beans in model system DHM influenced by saliva volume (■) and the model describing the changes in flavour release by saliva volume (. . . . .)

in the model. This implicates that the partition coefficients were hardly influenced by dilution. Saliva acted in an equivalent manner to water from a physico-chemical point of view, as was previously suggested by De Roos and Wolswinkel [8]. This confirms preliminary results which demonstrated no difference in flavour release from French beans between the addition of 8 ml saliva and the addition of 1 ml saliva diluted by 7 ml water.

A decrease in the release of flavour compounds from rehydrated French beans up to 70% by 8 ml saliva could be of interest for flavour perception. Therefore GC/SP analyses of rehydrated French beans in model system DHM were carried out, with and without addition of 8 ml saliva WSMA. Despite the 70% FID response reduction, slight changes in GC/SP patterns were observed (data not shown). These results indicate that an effect of saliva volume on flavour perception can hardly be expected.

In conclusion, saliva composition as well as volume influenced flavour release from rehydrated French beans: overall mucin decreased, α-amylase increased and dilution decreased FID responses markedly. However, GC/SP patterns were hardly influenced by either saliva composition or volume.

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