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RAPID COMMUNICATION

Gas chromatography/sniffing port analysis evaluated for aroma release from rehydrated French beans (*Phaseolus vulgaris*)

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The technique of gas chromatography/sniffing port analysis was evaluated for studying the release of aroma compounds from rehydrated diced French beans. The aroma compounds 2-/3-methylbutanal and hexanal were released at a constant rate over time. An identical selection of odour active compounds was obtained at different sampling times. Mutual proportions were remained among the sampling times, although the total number of perceptions of assessors were different. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Over the last few decades a major part of flavour research has dealt with analysis of volatile compounds. This is not surprising as the nose is capable of discerning hundreds of different odours, whereas the taste-buds are able to differentiate four stimuli only (Hoff *et al.*, 1978). Recently, it was shown that aroma compounds contribute considerably to the flavour of rehydrated French beans (van Ruth *et al.*, 1995*a*).

An instrumental approach to characterise aroma can be divided into two phases. The first phase deals with representative isolation of volatile compounds, as gas chromatographic (GC) profiles are largely influenced by isolation procedures (Linforth *et al.*, 1994; van Ruth *et al.*, 1995*b*; Roberts & Acree, 1996). The second phase involves selection of volatile compounds contributing to the flavour. This implies correct determination of the relevant aroma compounds from the whole range of volatiles present in a food (Dirinck & De Winne, 1994). An interesting approach is sniffing the gas chromatographic (GC) effluent in order to associate odour activity with the eluting compounds. These GC techniques can be classified into four categories.

1. Dilution analysis methods, e.g. CHARM (Acree *et al.*, 1984) and aroma extraction dilution analysis (Ullrich & Grosch, 1987).
2. Time-intensity methods, e.g. OSME (Sanchez *et al.*, 1992).
3. Posterior intensity methods (Casimir & Whitfield, 1978).
4. Response interval methods for recording time duration of perceived odours and the number of assessors with an odour perception (Linssen *et al.*, 1993).

Recently, the authors reported a dynamic headspace model system (van Ruth *et al.*, 1994), in which release of volatiles from three vegetables did not differ significantly from their release in the mouths of 12 assessors (van Ruth *et al.*, 1995*b*). In the present work, response interval sniffing port analysis was performed in order to study aroma release from rehydrated French beans at different time periods, using the model system mentioned.

MATERIALS AND METHODS

Plant material

Commercially air-dried French beans (*Phaseolus vulgaris*) were supplied by Top Foods b.v. (Elburg, The Netherlands). The beans were packed in glass jars and stored at 4°C in the absence of light until sampling. 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.

Instrumental analysis

The rehydrated beans were transferred into the sample flask of the mouth model system with a mastication device (van Ruth *et al.*, 1994). Artificial saliva (4 ml) was added and the headspace flushed with nitrogen gas in order to trap volatile compounds in Tenax TA for 1, 1.5, 3 and 12 min. Gas chromatography/sniffing port analysis (GC/SP) was performed and flavour descriptors were generated by seven experienced assessors (aged 20–50 years) as described in an earlier report (van Ruth *et al.*, 1995*c*). The descriptors used in the final experiments were: burned; caramel; chemical; chocolate; citrus; French beans; fruity; grassy, metal; mushroom; musty; rancid; rotten; sour; spicy and sweet. Tenax tubes without adsorbed volatile compounds were used as dummy samples to determine the signal-to-noise level of the group of assessors. The volatile compounds trapped in Tenax TA were identified by combined GC/mass spectrometry (GC/MS) as described previously (van Ruth *et al.*, 1995*b*).

Statistical evaluation

The number of assessors detecting an odour simultaneously during GC/SP analysis were subjected to a Friedman analysis of variance by ranks. This enabled the determination of consistent differences in the number of odour perceptions of individual aroma compounds. A significance level of $p < 0.05$ was used.

RESULTS AND DISCUSSION

Volatile compounds of rehydrated diced French beans were isolated in the mouth model system for different time periods and analysed by GC/SP. Figure 1 represents the sniffing chromatograms of aroma compounds isolated from French beans for 1 and 12 min, respectively. The aroma compounds were identified by GC/MS and their retention times, and characterised by their flame ionisation detection (FID) peak areas and the odours described by the assessors of the sniffing panel (Table 1). GC sniffing of dummy samples showed that detection of an odour at the sniffing port by one out of seven assessors can be considered as 'noise'.

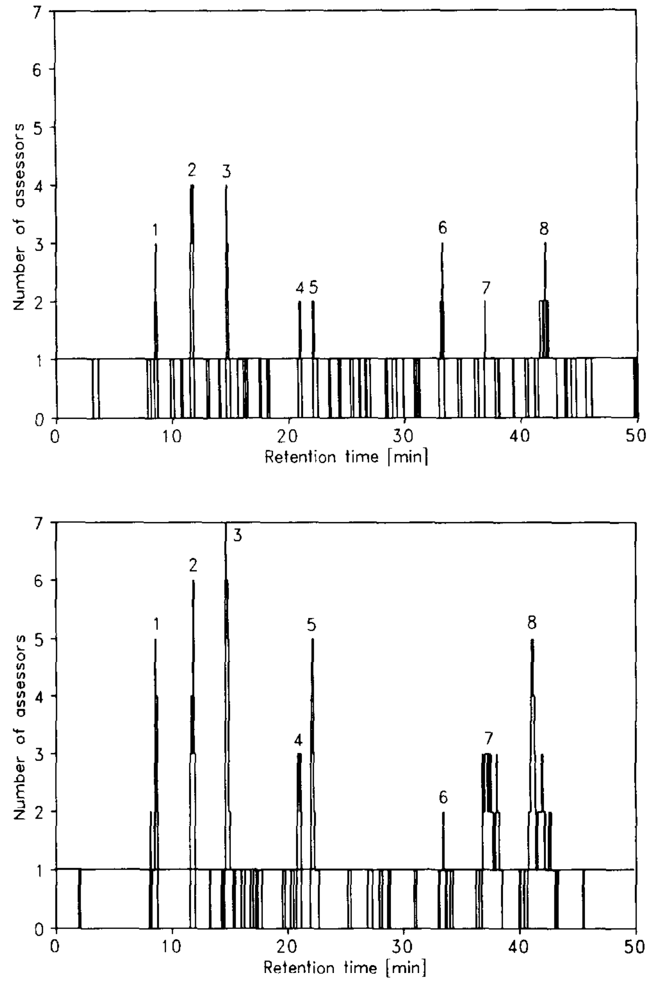

Fig. 1. Sniffing chromatograms of volatile compounds released from rehydrated diced French beans in a mouth model system during (a) 1 min and (b) 12 min. Numbers on the chromatograms refer to compounds in Table 1.

GC/SP revealed eight compounds possessing detectable odours (Table 1). Each of the detected odour active compounds were previously shown to be present as an aroma compound in rehydrated diced French beans, bell peppers and leeks (van Ruth *et al.*, 1995*c*). 2-Methylpropanal and 2-/3-metylbutanal were present among the odour active compounds. The presence of the latter compounds is typical for dried vegetables (Luning *et al.*, 1995; van Ruth *et al.*, 1995*c*). These

Table 1. Odour active compounds released from rehydrated French beans in a mouth model system during 1, 1.5, 3 and 12 min, their odour descriptions and number of assessors perceiving an odour at the sniffing port

Peak no.	Compound	Odour description	Time of isolation (min)			
			1	1.5	3	12
1	2-Methylpropanal	Chocolate	3	5	5	5
2	2-/3-Methylbutanal	Chocolate, sweet	4	4	6	6
3	2,3-Butanedione	Caramel	4	4	4	7
4	Hexanal	Grassy	2	1	2	3
5	2-Methyl-2-butenal	Sweet, chemical	2	3	4	5
6	Unknown	Rancid, chemical	3	2	2	2
7	1-Octen-3-one	Mushroom, metal	2	2	3	3
8	Dimethyl trisulphide	Rotten, metal	3	4	3	5

odour active compounds can be formed by Strecker degradation of valine and (iso)leucine during the drying process. Most of the other aroma compounds can be derived in lipid oxidation reactions (Tressl *et al.*, 1981; Whitfield, 1992).

Despite a difference in isolation time (1–12 min), the GC/SP analysis resulted in an identical selection of odour active compounds. Although the number of odour perceptions accumulated per compound differed between the sampling times, the mutual proportions of the number of assessors perceiving odour active compounds were retained (Friedman analysis of variance by ranks, $p < 0.05$). These results demonstrated that the method is quite robust and adequate even when short isolation times are used.

A disadvantage of some of the other sniffing methods (Acree *et al.*, 1984; Ullrich & Grosch, 1987; Sanchez *et al.*, 1992) is that the sniffing is performed by one or two sniffers only. In some methods (Acree *et al.*, 1984; Ullrich & Grosch, 1987), this selection is done repeatedly and at several dilutions, which probably suits the aim to analyse the most potent odourants of a product. However, as thresholds of individuals differ markedly, it should be taken into account that some of the less potent odour active compounds could be missed during selection. In addition, it should be considered when selecting assessors that 12% of the population suffer from partial (specific) or total anosmia (Doty *et al.*, 1984). Figure 2 represents the FID peak areas of the aroma compounds 2- and 3-methylbutanal, and hexanal. 2- and 3-Methylbutanal were separated sufficiently for FID peak area determination, although the assessors of the sniffing panel were not able to separate both compounds. This could be due to the fact that these compounds possess similar odours (chocolate), as was shown in training sessions. The aroma compounds 2- and 3-methylbutanal, and hexanal were released at a constant rate over time in the mouth model system. High correlation coefficients between peak area and isolation time were obtained for 2-methylbutanal (0.999), 3-methylbutanal (0.999) and hexanal (0.996). These results are in agreement with studies of Roozen & Legger-Huysman (1995), which showed a linear release of 2-/3-methylbutanal from chocolate flakes in the oral vapour of assessors over time in *in vivo* measurements. In preliminary experiments using on-column injections, it was shown that the FID response of 2-/3-methylbutanal and hexanal were 10, 10 and 13 V.sg, respectively. Using these data, it was calculated that 0.21 ng 2-methylbutanal was detected by the FID after sampling for 1 min, as well as 0.40 ng 3-methylbutanal and 0.18 ng hexanal. In the GC/SP technique performed, 20% of the effluent will pass the FID, and 40% will flow through each of the sniffing ports. The amounts of the three odour active compounds left for each sniffing port are consequently 0.42, 0.80 and 0.36 ng, respectively, present in ca. 0.1 ml GC effluent and 4 ml suppletion of helium and air. The detection concentration estimated for hexanal (0.09 ng/ml) is in agreement with studies of Hall & Andersson (1983), who determined an odour threshold of 0.07 ng/ml nitrogen gas for hexanal by olfactometry.

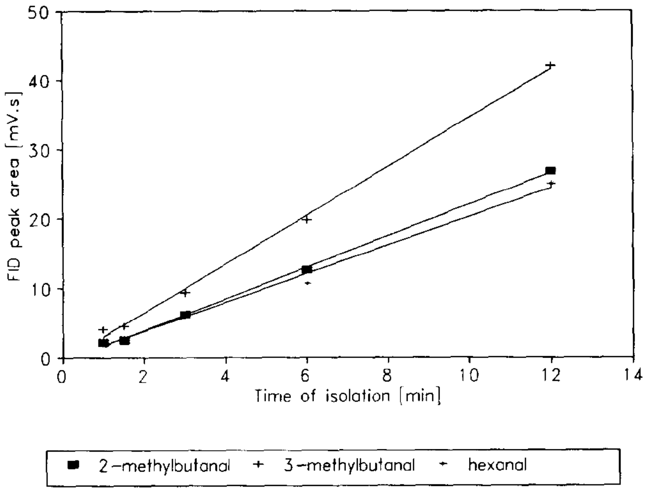

Fig. 2. FID peak areas of 2-/3-methylbutanal and hexanal released from rehydrated diced French beans in a mouth model system vs isolation time.

The FID responses of 2-/3-methylbutanal and hexanal after 1 min isolation were sufficient for calculations. However, the other aroma compounds were hard to detect by FID at this concentration level. This implies that, if the FID responses per gram of these aroma compounds are in the same range as 2-/3-methylbutanal and hexanal, their thresholds are probably markedly lower than those of the latter compounds. The thresholds of 2-/3-methylbutanal and hexanal in water are respectively 1, 1 and 5 µg/g, according to the studies of Leffingwell & Leffingwell (1991). One of the odour active compounds, 1-octen-3-one, is expected to provide similar FID responses as 2-/3-methylbutanal and hexanal. A threshold of 0.005 µg/g in water for 1-octen-3-one was reported by the same authors, which confirms our suggestion. The other compounds bring on more difficulties, as thresholds are unknown (e.g. 2-methyl-2-butenal), or because they have no comparable FID response (e.g. dimethyl trisulphide), or they elute simultaneously with other volatile compounds (e.g. 2,3-butanedione with pentanal and 2-methylpropanal with acetone).

In Fig. 3, the number of perceptions at the sniffing port, accumulated for all odour active compounds, are plotted against the time of isolation. This number of odour perceptions is shown to be log linear related to the isolation time (correlation coefficient 0.997). As volatile compounds were linearly released over time (Fig. 2), the number of odour perceptions is log linear related to the amount of aroma compounds released as well. In the present work, the number of assessors perceiving an odour at the sniffing port, which was shown previously to be linearly related to the sensory intensity of aroma compounds (van Ruth *et al.*, 1996), is log linear related to the isolation time, which in turn corresponds with the aroma release. These results are in agreement with Fechner's law, which shows the sensory intensity to be log linearly related to the physical

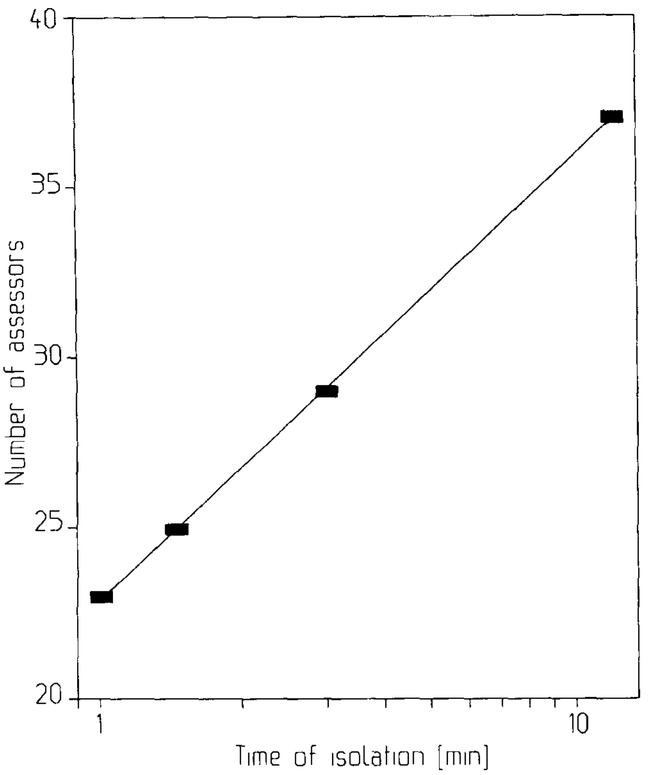

Fig. 3. Total number of odour perceptions at the sniffing port of aroma compounds of rehydrated diced French beans released in a mouth model system vs log isolation time.

concentration (Meilgaard *et al.*, 1991). This linear relationship is retained for the sampling periods studied, and it should be kept in mind that the linearity is limited by the maxima of possible odour perceptions.

In conclusion, aroma compounds of rehydrated French beans were released at a constant rate over time in the mouth model system. Different sampling times resulted in an identical selection of odour active compounds. Mutual proportions were retained, although the total number of perceptions differed among the sampling times.

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