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ScienceDirect
Food Chemistry 101 (2007) 1279–1284
Food Chemistry
www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods

Chemical analysis of French beans (*Phaseolus vulgaris* L.) by headspace solid phase microextraction (HS-SPME) and simultaneous distillation/extraction (SDE)

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Received 23 March 2005; received in revised form 10 November 2005; accepted 29 December 2005

Abstract

Headspace Solid Phase Microextraction (HS-SPME) involving divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre and simultaneous distillation/extraction (SDE) techniques were applied to study the volatile and semi volatile compounds of thawed and cooked *Phaseolus vulgaris* L. A total of 104 compounds were detected by GC and GC/MS. Thereof, 76 compounds were identified for the first time in this species. The major differences between HS-SPME and SDE were found in the content of identified alcohols (23.62% SDE versus 62.20% SPME), terpenoids (39.15% SDE versus 2.45% SPME), heterocyclic compounds (13.78% SDE versus 1.21% SPME), hydrocarbons (2.22% SDE versus 13.87% SPME) and esters (0.98% SDE versus 12.98% SPME). The SPME technique was found to be useful for rapid and routine quality controls of thawed French beans, while SDE is favourable to study the entire set of flavour volatiles in the corresponding cooked samples.

Keywords: *Phaseolus vulgaris* L.; French beans; Aroma compounds; Headspace solid phase microextraction (HS-SPME); Simultaneous distillation/extraction (SDE)

1. Introduction

The flavour of fresh foods results from naturally occurring compounds and aroma chemicals produced by enzymatic degradation during harvesting and processing (De Lumen, Stone, Kazeniac, & Forsythe, 1978; Rowe, 2005). The flavour of cooked foods is due to a complex sequence of enzymatic and chemical reactions depending of the temperature (Tressl & Rewicki, 1999; Vernin & Parkanyi, 1982).

The French bean (*Phaseolus vulgaris* L.) is the most cultivated and one of the most consumed legume in France. The beans are marketed either fresh, frozen, dried or cooked. During thermal processing, such as cooking, the typical "green odour" changes significantly. Until today, only few investigations have been performed on the entire set of flavour volatiles of French beans. To evaluate the French beans volatile compounds, several techniques of extraction and analysis have been used. At first, Kermasha, Van de Voort, and Metche (1988) reported on the volatile carbonyl compounds produced by thermal or enzymatic treatment of the vegetable. Then, the high vacuum distillation (Hinterholzer, Lemos, & Schieberle, 1998) followed by gas chromatography-olfactometry and GC/MS permitted to characterise 28 odour-active volatiles in raw and cooked beans and dynamic headspace sampling coupled with GC/MS analysis (Rodriguez-Bernaldo De Quironos, Lopez-Hernandez, Gonzalez-Castro, De la Cruz-Garcia, & Simal-Lozano, 2000) allowed the identification of 27 volatile compounds. Finally, the model system mouth-gas chromatography-sniffing port analysis described by Van Ruth and Roozen (2000a, 2000b), Van Ruth, Roozen, Holliman,

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0308-8146/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2005.12.027

and Posthumus (1996, 1995a, 1995b) permitted to identify the compounds that contribute to the main flavours of dried or rehydrated French beans.

We have applied the headspace solid phase micro extraction (HS-SPME) and simultaneous distillation/extraction (SDE) to extract the aroma volatiles of French beans before analysis. Briefly, the HS-SPME is used for the extraction of volatile compounds by the use of a fused silica fibre coated with different stationary phases. This is a common technique to evaluate the flavour compounds of various foods such as vegetables, fruits, juices, soft drinks or alcoholic beverages as recently reviewed from Kataoka, Lord, and Pawliszyn (2000). To the best of our knowledge, no studies have been published on the characterization of French beans aroma compounds with SPME. The SDE is a technique based on the use of the Lickens–Nickerson apparatus (Likens & Nickerson, 1964). Chaintreau (2001) have recently reviewed on this kind of extraction that simulates food cooking: it gives a concentrated extract that contains the volatile components of a fresh food and the compounds produced by the cooking process, particularly the Maillard reaction products (Tressl & Rewicki, 1999; Vernin & Parkanyi, 1982). This latter technique was widely applied to study aroma compounds in food research (Cai, Liu, & Su, 2001; Garcia-Esteban, Asorena, Astiasaran, Martin, & Ruiz, 2004; Valeiro, Sanz, & Martinez-Castro, 2001; Valette et al., 2003), but never concerning the French beans.

In this report, HS-SPME and SDE techniques were used to study the volatile compounds of *Phaseolus vulgaris* L. The advantages and limitations of these techniques are considered. The final objective of the present study is to identify and characterise new volatile compounds in the aroma of this vegetable.

2. Materials and methods

2.1. Materials

Commercial samples of frozen French beans were purchased from a local supermarket and immediately kept at −18 °C until analysis.

2.2. HS-SPME GC and GC–MS analysis

Extraction conditions such as SPME fibre choice, time and temperature of extraction were optimised. A manual SPME device and SPME fibres were obtained from Supelco Co (Bellefonte, PA). We used a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre (50/30 μm film thickness). Before use the fibre was conditioned 1 h at 270 °C as recommended by the manufacturer. Hundred grams of congealed French beans were grinded and 10 g were placed in a 40 ml amber vial closed by a PTFE/silicone septum (Supelco). Before the extraction process, a time of 30 min at 40 °C was requested for thawing and subsequent headspace equilibration. After 1 h of fibre exposure in the sample headspace, the fibre was thermally desorbed in a GC injection port (equipped with a 0.75 mm i.d. inlet liner) for 2 min. The injector was set at 250 °C and operated in a splitless mode for 2 min.

GC and GC/MS analyses were carried out using an Agilent 6890 N gas chromatograph, equipped with a FID and coupled to a quadrupole Agilent 5973 Network mass selective detector. The gas chromatograph was equipped with two fused silica capillary column HP-1 (PDMS, 50 m × 0.2 mm i.d. film thickness = 0.33 μm). The carrier gas was helium (head pressure for both columns = 25 psi); oven temperature was programmed from 40 °C (2 min) to 200 °C at 2 °C/min and then at 15–250 °C and held isothermal for 30 min. The FID temperature was set at 250 °C and the temperatures of the ion source and the transfer line were 170 °C and 280 °C, respectively. Ionisation energy was set to 70 eV, ionisation mass range 35–350 amu. Before sampling, the fibre was reconditioned for 15 min in the GC injection port at 250 °C. A blank experiment was performed before analysis.

2.3. SDE GC and GC–MS analysis

Extraction conditions such as time of extraction, amount of vegetables, water and solvent have been optimised. SDE was realised with a Lickens–Nickerson apparatus. The French beans (300 g) were suspended in 1 l of water. Dichloromethane (50 mL) was used as the organic phase and was also added (20 ml) to fill the apparatus solvent return loop. Solvent and sample mixture were boiled for 2 h. Dichloromethane was used as it was recognised as the most useful solvent for extraction of a wide class of flavour compounds (Chaintreau, 2001). After cooling to ambient temperature for 10 min, the dichloromethane extract was collected and dried over anhydrous $MgSO_4$. The extract was carefully concentrated to 1 ml at 40 °C with a rotary evaporator at atmospheric pressure and finally evaporated under gentle nitrogen flow to 250 μl. The concentrated extract was injected (0.2 μl) directly into the GC apparatus. The GC and GC–MS conditions were the same as described for SPME except for the use of split mode (1:20) and solvent delay of 5 min for GC–MS. A blank experiment was performed before analysis.

2.4. Components identification

Identification of *Phaseolus vulgaris* L. components was based on the comparison of their mass spectra with those stored in commercial MS databases, with literature data (Adams, 1995; Garcia-Esteban et al., 2004), and with home-made mass spectra libraries built up from pure substances. Identification was also confirmed by comparison of the GC retention indices (RI) on an apolar column (determined from the retention times of a series of *n*-alkanes mixture) with GC data previously published (ESO 2000, 1999). The results were described in terms of percentage of areas of identified peaks and mean percentage values were calculated from 5 SPME and 4 SDE experiments. The results were

Table 1
Volatile compounds identified in thawed and cooked *Phaseolus vulgaris* L. using HS-SPME and SDE

KI[a]	Compound	Reliability[b]	SDE RA[c] ± SD[d]	SPME RA ± SD
472	Ethanol[e]	MS	ND[f]	0.61 ± 0.14
515	Carbon disulphide[e]	MS;KI	ND	0.52 ± 0.06
589	Ethyl acetate[e]	MS;KI	ND	1.30 ± 0.18
610	But-2-enal	MS	0.20 ± 0.02	ND
625	3-Methylbutanal	MS;KI	0.36 ± 0.05	ND
635	2-Methylbutanal	MS;KI	0.25 ± 0.07	ND
639	Butanol[e]	MS;KI	0.07 ± 0.02	ND
660	Pent-3-en-1-ol	MS;KI	0.25 ± 0.06	ND
665	Pentane-2,3-dione	MS;KI	0.38 ± 0.02	ND
665	Valeraldehyde	MS;KI	0.07 ± 0.01	0.17 ± 0.02
673	3-Hydroxybutan-2-one[e]	MS	0.69 ± 0.11	ND
676	Isopentenylmethylether[e]	MS	ND	0.12 ± 0.01
684	2-Ethylfurane[e]	MS;KI	0.16 ± 0.02	0.22 ± 0.06
697	*n*-Heptane	MS;KI	ND	0.24 ± 0.01
713	Isoamyl alcohol[e]	MS;KI	ND	0.82 ± 0.12
714	Pent-3-en-2-one[e]	MS;KI	0.16 ± 0.02	ND
715	3-Methylbut-3-en-1-ol[e]	MS	0.02 ± 0.00	ND
716	Pyridine[e]	MS;KI	10.38 ± 1.49	ND
718	3-Methylbutan-1-ol[e]	MS	1.52 ± 0.05	ND
725	Pent-2-enal[e]	MS;KI	0.32 ± 0.03	ND
744	1-Pentanol[e]	MS;KI	1.03 ± 0.02	0.96 ± 0.03
746	Toluene[e]	MS;KI	0.48 ± 0.03	2.33 ± 0.24
755	3-Methylbut-2-enal[e]	MS;KI	0.08 ± 0.01	ND
759	2-Methylheptane[e]	MS	ND	0.31 ± 0.02
767	3-Methylheptane[e]	MS	ND	0.38 ± 0.02
770	Hexanal	MS;KI	0.44 ± 0.05	2.05 ± 0.07
783	1-Octene[e]	MS;KI	ND	1.58 ± 0.14
793	3-Methylhept-3-ene[e]	MS	ND	2.51 ± 0.28
796	*n*-Octane[e]	MS;KI	0.26 ± 0.01	1.66 ± 0.15
800	(*Z*)-oct-2-ene[e]	MS;KI	ND	1.32 ± 0.10
803	3-Methylhept-2-ene[e]	MS	ND	2.22 ± 0.21
803	Furfural	MS;KI	3.24 ± 0.16	ND
807	(*E*)-oct-2-ene[e]	MS;KI	ND	1.11 ± 0.08
827	(*E*)-hex-2-enal	MS;KI	0.53 ± 0.03	ND
829	Furfurol[e]	MS	0.03 ± 0.00	ND
833	(*E*)-hex-3-enol	MS;KI	0.06 ± 0.00	tr
839	(*Z*)-hex-3-enol	MS;KI	5.59 ± 0.69	7.76 ± 0.42
852	Isoamylacetate[e]	MS;KI	ND	1.22 ± 0.1
853	Hexanol	MS;KI	2.08 ± 0.03	8.02 ± 0.91
866	3-Methylthiopropanal	MS;KI	0.05 ± 0.00	ND
870	Heptanal	MS;KI	0.07 ± 0.00	0.07 ± 0.02
921	α-Pinene[e]	MS;KI	ND	0.70 ± 0.05
930	Benzaldehyde	MS;KI	0.20 ± 0.02	ND
934	(*E*)-hept-2-enal[e]	MS;KI	0.31 ± 0.01	ND
957	3-Thiophencarboxaldehyde[e]	MS	0.26 ± 0.01	ND
959	Octan-3-one[e]	MS;KI	0.30 ± 0.01	1.08 ± 0.03
964	Oct-1-en-3-ol	MS;KI	7.98 ± 1.72	30.51 ± 0.92
974	3-Octanol	MS;KI	0.82 ± 0.09	4.37 ± 0.07
979	2-Pentylfurane[e]	MS;KI	0.83 ± 0.07	0.78 ± 0.01
982	Hepta-2,4-dienal	MS	0.69 ± 0.05	ND
986	(*Z*)-hex-3-enylacetate[e]	MS;KI	0.70 ± 0.06	8.23 ± 0.77
990	Hexyl acetate	MS;KI	ND	1.25 ± 0.1
1008	3-Ethyl-4-methylpentanol[e]	MS	2.93 ± 0.34	8.69 ± 0.44
1008	2-Ethylhexanol	MS;KI	ND	0.03 ± 0.00
1012	1,8-Cineole[e]	MS;KI	ND	0.39 ± 0.02
1022	Limonene[e]	MS;KI	0.14 ± 0.03	0.45 ± 0.04
1024	Methyl-2-ethyl-hexanoate[e]	MS	0.08 ± 0.00	0.20 ± 0.02
1033	(*E*)-Oct-3-enal	MS	0.25 ± 0.03	ND
1037	(*E*)-β-ocimene[e]	MS;KI	0.21 ± 0.03	ND
1045	Oct-2-en-1-ol[e]	MS;KI	ND	0.17 ± 0.02
1049	1-Octanol	MS;KI	ND	0.23 ± 0.03
1052	3-Thienylmethanol[e]	MS	0.49 ± 0.05	ND
1059	2-Thiophene[e]	MS	0.72 ± 0.08	ND

(*continued on next page*)

Table 1 (*continued*)

KI[a]	Compound	Reliability[b]	SDE RA[c] ± SD[d]	SPME RA ± SD
1975	(*Z*)-hex-3-enyl propionate[e]	MS;KI	ND	0.38 ± 0.06
1977	Nonanal	MS;KI	ND	0.47 ± 0.03
1079	α-terpinolene[e]	MS;KI	0.13 ± 0.01	ND
1081	2-Phenylethanol[e]	MS	0.36 ± 0.09	ND
1084	Hexyl propanoate[e]	MS;KI	ND	0.12 ± 0.05
1085	Linalool	MS;KI	15.30 ± 1.92	0.75 ± 0.02
1096	Undecane[e]	MS;KI	ND	0.07 ± 0.01
1117	Alloocimene[e]	MS;KI	tr	ND
1135	4-Ethylbenzaldehyde[e]	MS;KI	0.21 ± 0.03	ND
1151	Menthol[e]	MS;KI	tr	0.16 ± 0.04
1155	Octadienoic acid[e]	MS	0.30 ± 0.05	ND
1156	2-Isobutyl-3-methoxypyrazin	MS;KI	ND	0.21 ± 0.02
1163	(*Z*)-hex-3-enyl butyrate[e]	MS;KI	ND	0.17 ± 0.02
1175	α-terpineol[e]	MS;KI	3.82 ± 0.46	ND
1179	Decanal	MS;KI	ND	0.17 ± 0.01
1189	β-cyclocitral[e]	MS;KI	ND	0.07 ± 0.00
1185	Benzothiazole[e]	MS;KI	0.25 ± 0.03	ND
1193	Dodecane[e]	MS;KI	0.14 ± 0.02	0.10 ± 0.01
1214	Nerol[e]	MS;KI	1.10 ± 0.11	ND
1237	Geraniol[e]	MS;KI	3.37 ± 0.52	ND
1247	Nonanoic acid[e]	MS;KI	0.17 ± 0.05	ND
1284	Paravinylguaiacol[e]	MS;KI	0.91 ± 0.08	ND
1290	Deca-2,4-dienal[e]	MS;KI	0.24 ± 0.03	ND
1300	Tridecane[e]	MS;KI	ND	0.04 ± 0.00
1347	Methyl cinnamate[e]	MS,KI	0.15 ± 0.01	ND
1363	(*Z*)-jasmone[e]	MS,KI	0.14 ± 0.03	ND
1420	Geranylacetone[e]	MS,KI	0.07 ± 0.01	ND
1428	Ethyl cinnamate[e]	MS,KI	0.05 ± 0.01	ND
1439	(*E*)-β-farnesene[e]	MS,KI	0.15 ± 0.02	ND
1462	β-Ionone	MS,KI	0.17 ± 0.03	ND
1493	α-Farnesene[e]	MS;KI	0.47 ± 0.08	ND
1501	β-Bisabolene[e]	MS;KI	0.15 ± 0.02	ND
1532	Dodecanoic acid[e]	MS;KI	0.31 ± 0.07	ND
1541	Nerolidol[e]	MS;KI	11.52 ± 1.27	ND
1671	α-Bisabolol[e]	MS;KI	0.84 ± 0.09	ND
1698	Farnesol[e]	MS,KI	1.57 ± 0.34	ND
1717	Tetradecanoic acid[e]	MS;KI	3.82 ± 0.59	ND
1933	Palmitoleic acid[e]	MS;KI	0.82 ± 0.23	ND
2083	Phytol[e]	MS;KI	0.83 ± 0.15	ND
2287	Tricosane[e]	MS,KI	0.23 ± 0.01	ND
2498	Alkane c25[e]	MS;KI	0.20 ± 0.01	ND
	Total identified		93.45	97.27

Results are provided as percentage (area ± standard deviation).

[a] Kováts indices for a HP-1 capillary column. Compositional values less than 0.01% are noted as traces (tr).

[b] Reliability of identification: MS, mass spectrum identified using libraries; KI, Kovats Index in agreement with literature.

[c] RA: relative area.

[d] SD: standard deviation (SDE: 4 replicates, SPME: 5 replicates).

[e] Not previously identified in French beans.

[f] ND: not detected.

quite reproducible as shown by the standard deviations (Table 1).

3. Results and discussion

In our initial studies, we searched for the most useful technique to study the *Phaseolus vulgaris* L. aroma chemicals. HS-SPME was applied to cooked and thawed French beans. The preliminary HS-SPME assays on the cooked beans showed that most of the volatile compounds were lost during cooking, leading to a fall-off in detection. Indeed, the water cooking could induce the loss of volatile and/or hydrophilic compounds. Therefore, SDE was chosen to study the cooked French beans aroma compounds because it is a combined steam distillation and liquid–liquid extraction method (Chaintreau, 2001).

On the other hand, we optimised HS-SPME method to study the aroma chemicals of raw French beans as described in material and methods.

A total of 104 compounds were identified in *Phaseolus vulgaris* L. using the two techniques and 76 out of them have not been previously identified in this vegetable (Table 1). Using HS-SPME, 50 compounds were detected while 75 compounds were detected in SDE extracts. The major fam-

ilies of detected volatiles were alcohols (23.62% for SDE versus 62.20% for SPME), terpenoids (39.15% for SDE versus 2.45% for SPME), heterocyclic compounds (13.78% for SDE versus 1.21% for SPME), hydrocarbons (2.22% for SDE versus 13.87% for SPME), esters (0.98% for SDE versus 12.98% for SPME), aldehydes (4.45% for SDE versus 2.84% for SPME), ketones (1.53% for SDE and 1.08% for SPME) and sulphur compounds (1.49% for SDE versus 0.52% for SPME) as shown in Table 1.

SDE extracts showed higher proportions of terpenoids than the SPME extracts. On the other hand, the SPME extracts were richer in linear alcohols and esters. This finding was in accordance to the results obtained from Ceva-Antunes, Bizzo, Alves, and Antunes (2003) that found some terpenoids as major components of SDE extract of *Spondias mombin* L., while SPME extraction showed higher proportion of alcohols and esters.

Compared to SDE, SPME is more efficient for the extraction of light esters. This result could be due to the evaporation step during the SDE process, that might lead to the loss of the most volatile components (Ceva-Antunes et al., 2003; Garcia-Esteban et al., 2004). Both SPME and SDE enabled the detection of most odour active compounds in French beans, such as C5, C6 and C8 compounds derived from linoleic and linolenic acids (De Lumen et al., 1978), but their proportions depends strongly on the extraction technique. As shown in Table 1, in comparison to SDE, SPME extracts were found richer in these compounds: (*Z*)-hex-3-enol: 7.76% in HS-SPME versus 5.59% in SDE, 1-hexanol: 8.02% in HS-SPME versus 2.08% in SDE, oct-1-en-3-ol: 30.51% in HS-SPME and 7.98% in SDE, 3-octanol: 4.37% in HS-SPME and 0.82% in SDE, (*Z*)-hex-1-ene-3-yl-acetate: 8.23% in HS-SPME versus 0.70% in SDE, 3-ethyl-4-methyl-pentanol: 8.69% HS-SPME versus 2.93% in SDE. Volatile aldehydes such as but-2-enal, 3-methyl-butanal, 2-methyl-butanal, (*E*)-hex-2-enal, 3-methyl-thio-propanal, benzaldehyde, hepta-2,4-dienal, (*E*)-oct-3-enal and 4-ethylbenzaldehyde were detected using SDE but not with SPME. Aldehydes are known to derive from thermal Strecker oxidative degradation of amino acids and fatty acids (Tressl & Rewicki, 1999; Vernin & Parkanyi, 1982), however SPME successfully extracted hexanal naturally present in the French beans. The SDE process permitted also to identify heterocyclic compounds such as pyridine, furfural and thiophenes derived from sugars and amino acids (Maillard reaction) (Tressl & Rewicki, 1999; Vernin & Parkanyi, 1982).

In conclusion, SDE enables the analysis of low volatility and high molecular weigh compounds. The high temperature during distillation may led to formation of compounds not present originally, but important as keys odorant. For this reason, SDE can be useful to study the entire set of flavour volatiles in cooked French beans. On the other hand, HS-SPME allows the rapid extraction of highly volatile compounds without apparent hydrolysis and artefacts formation. Hence, this technique could be appropriate for the routine quality control analysis of *Phaseolus vulgaris* L.

The present work contributes to a better knowledge of the volatile constituents of French beans: 76 volatile and semi volatile compounds were identified for the first time and interesting differences in the volatile profiles were observed between HS-SPME and SDE.

Acknowledgement

The authors wish to thank the "Région Provence-Alpes-Cote d'Azur" for financial support (grant 2002_04847).

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