

Solid-Phase Microextraction Method for Headspace Analysis of Volatile Compounds in Bread Crumb

J. A. Ruiz,^{1,2} J. Quilez,¹ M. Mestres,³ and J. Guasch³

ABSTRACT

Cereal Chem. 80(3):255–259

A solid-phase microextraction method was developed to analyze volatile compounds in bread crumb. Three different fibers usually used to determine volatile compounds in foodstuffs were tested, and Carboxen/Polydimethylsiloxane showed the best extraction efficiency. This method can determine ≈65 compounds, although only the main compounds responsible for the crumb aroma profile (6 alcohols, 5 aldehydes, 5 acids, and 2 ketones) were evaluated quantitatively with a relative standard

deviation <20% and limits of detection were 1–18 mg/kg. All the compounds showed a good linearity in concentration ranges of interest with acceptable correlation coefficients ($r > 0.99$) and recoveries close to 100%. The optimized solid-phase microextraction (SPME) method was applied to determine aromatic compounds in some precooked frozen breads marketed in Spain (baguettes and ciabattas).

The quality of bread is judged on the basis of loaf volume, texture, color, and flavor. However, the characteristic aroma of bread is undoubtedly the most important parameter influencing consumer acceptance (Caul 1972). Bread flavor, qualitatively described as discrete and subtle, is composed of a large number of components, many of them with very distinct olfactory characteristics. The volatile and nonvolatile compounds in bread that contribute to its flavor include acids, alcohols, aldehydes, esters, ethers, furans, hydrocarbons, ketones, lactones, pyrazines, pyrroles, and sulfur compounds (Maga 1974). More than 540 compounds have been reported in the complex volatile fraction of bread (Schieberle 1996).

The flavor compounds of bread have been studied previously using different separation techniques: organic solvent extraction (Frasse et al 1993), distillation (Gassenmeier and Schieberle 1995), headspace (Luning et al 1991), and purge and trap (Seitz et al 1998). Usually, more compounds are found when organic solvent extraction or steam distillation methods are used for isolation compared with headspace methods. However, the use of solvent extraction or steam distillation allows the possibility of artifact formation or the introduction of interference from solvents. These techniques require complex systems of sample analysis and are time-consuming, cumbersome, and expensive.

Solid-phase microextraction (SPME) is a relative new sample preparation technique (Arthur and Pawliszyn 1990) that, among other applications, has been used to measure the volatile flavor profiles of foodstuff (Yang and Peppard 1994; Roberts et al 2000). SPME shows some advantages over the techniques mentioned: solvent-free and rapid sampling, low cost, and higher sensitivity (Pawliszyn 1997). SPME uses a short length of fused silica coated with an adsorbent. The coated fused silica is immersed directly into an aqueous sample or into the headspace above a liquid or solid sample. For reproducible results, some variables must be controlled during the extraction process. These include sample agitation, the sampling method (headspace vs. immersion), sample pH, ionic strength, volume, time, and temperature (Pawliszyn 1997).

According to different authors (Maga 1974; Gassenmeier and Schieberle 1995; Schieberle 1996; Seitz et al 1998), the main compounds responsible for different notes of the bread crumb aroma profile are 6 alcohols (ethanol, isobutyl alcohol, isoamyl

alcohol, 1-hexanol, benzyl alcohol, and 2-phenylethanol), 5 aldehydes (hexanal, furfural, benzaldehyde, 2-nonenal, and 2,4-decadienal), 5 acids (acetic acid, propionic acid, isobutyric acid, butyric acid, and isovaleric acid), and 2 ketones (2,3-butanedione and 3-hydroxy-2-butanone). So, the purpose of this study was to develop a SPME method to quantify these compounds. Three fibers were tested, and parameters affecting the extraction of volatile compounds on the fiber were evaluated (sampling temperature and time, salt addition, pH, amount of sample). Once the variables were optimized, analytical parameters such as linearity, limit of detection, and recoveries were examined. Finally, volatile compounds of different commercial precooked frozen breads were analyzed.

MATERIALS AND METHODS

Chemicals and Materials

The 18 volatile compounds studied were: ethanol, isobutyl alcohol, isoamyl alcohol, 1-hexanol, benzyl alcohol, 2-phenylethanol, acetic acid, propionic acid, isobutyric acid, butyric acid, isovaleric acid, hexanal, furfural, benzaldehyde, 2-nonenal, 2,4-decadienal, 2,3-butanedione and 3-hydroxy-2-butanone. In addition, 4-methyl-2-pentanol and 2-ethylbutyric acid were used as internal standards. All the standards were supplied by Sigma-Aldrich (Madrid, Spain), Fluka (Madrid, Spain), and J. T. Baker (Deventer, The Netherlands). Other chemicals used for the optimization process (HPLC water, HPLC methanol, sodium chloride, sodium hydroxide, and citric acid) were purchased from Panreac (Barcelona, Spain). Stock solutions were prepared by grouping the different compounds depending on solubility characteristics and dissolved in HPLC water or HPLC methanol. However, to prepare a global standard solution containing all the analytes with a similar flavor composition to a real sample was difficult because only some of these compounds have been quantified previously in bread by other authors (Luning et al 1991; Gassenmeier and Schieberle 1995; Schieberle 1996). So, to determine the suitable concentration of each analyte, we compared the chromatographic peak areas obtained from the extraction of bread crumb samples with those obtained from the extraction of different concentrations of standard mixes, until the areas of both chromatograms were similar. From the results of these experiments, the most suitable concentrations of the different standards in the global standard solution were 5,000 mg/L (ethanol and acetic acid), 250 mg/L (3-hydroxy-2-butanone), 25 mg/L (isoamyl alcohol, propionic acid, isobutyric acid, butyric acid, and isovaleric acid), 10 mg/L (isobutyl alcohol and 2-phenylethanol), 1 mg/L (2,3-butanedione, hexanal, 1-hexanol, benzaldehyde, benzyl alcohol), 0.25 mg/L (furfural and 2-nonenal), and 0.1 mg/L (2,4-decadienal).

The SPME device and fibers used in this study were purchased from Supelco (Bellefonte, PA). Three types of fibers were: 65 μm

¹ Europastry, S.A., Departament de Tecnologia. Crta. Sarral a Barberà de la Conca s/n. E-43424 Sarral. Tarragona, Spain.

² Corresponding author. Fax: +34977890575. E-mail: jaruiz@frida.net.

³ Departament de Química Analítica i Química Orgànica (Unitat d'Enologia, CeRTA), Facultat d'Enologia de Tarragona, Universitat Rovira i Virgili. Avda. Ramon y Cajal, 70, E-43005. Tarragona, Spain.

Polydimethylsiloxane/Divinylbenzene (PDMS/ DVB), 75 μm Carboxen/ Polydimethylsiloxane (CAR/PDMS) and 65 μm Carbowax/ Divinylbenzene (CW/DVB). All fibers were conditioned in the gas chromatograph injection port under conditions specified by the manufacturer.

Sample Preparation and Headspace SPME Procedure

Precooked frozen bread was defrosted for 15 min and baked at 190°C for 15 min in a commercial convection oven. Bread was cooled at room temperature for 30 min. Then, it was cut in slices, and crumb was separated at 1 cm from crust. Finally, crumb was frozen with liquid nitrogen and grounded with an analytical grinding device (Ika, Labortechnik, Germany) to give a powder, which was stored at -20°C until it was analyzed.

For each SPME analysis, 0.25 g of bread crumb was weighed into a 20-mL vial. Then, 9.9 mL of a 20% sodium chloride solution (pH 3 with 0.05M citric acid) and internal standard solution (0.1 mL) were added into the vial. The internal standard solution had two different analytes: 4-methyl-2-pentanol and 2-ethylbutyric acid at 0.2 and 6 ppm respectively. The most suitable internal standard was chosen to quantify each analyte, depending on the best linearity and precision parameters found. Then, the vial, previously sealed with a silicone septa, was immersed in a water bath at 50°C, and the SPME fiber was exposed to the headspace for 60 min. The sample was continuously shaken with a magnetic stirring bar during the extraction process to allow faster equilibration of analytes. When the extraction time was finished, the fiber was inserted into the gas chromatograph (GC) injector port for thermal desorption of the extracted volatiles for 5 min.

GC-MS Analysis

The GC-MS analyses were performed using a Shimadzu model GC-17A gas chromatograph coupled to a GCMS-QP5050A mass spectrometer. A TR-WAX column (Tecknokroma, Spain) with a 0.25- μm film thickness, 60 mm \times 0.25 mm i.d. was used. The GC temperature program was 35°C held for 5 min, then increased by 5°C/min to 50°C, where it was held again for 5 min, then increased by 5.5°C/min to 230°C, where it was held again for 5 min, for a total run time of 51 min. The carrier gas was helium with a column flow of 2.0 mL/min. The injector was at 300°C in splitless mode for 5 min using an inlet of 0.75 mm i.d. which improved the GC resolution. Mass spectra were recorded by electronic impact (EI) at 70 eV. The ion source and mass quadrupole temperatures were 230 and 150°C, respectively. The scan mode was used to

detect all the compounds in the range m/z 33–200. The identification of volatile compounds were verified by comparison of the mass spectral data obtained with those in the NIST62 and WILEY229 library. However, to integrate the volatile compounds, we worked with the SIM mode by using the ion m/z with the highest intensity for each analyte (Tables I and II).

RESULTS AND DISCUSSION

To develop a SPME method to analyze flavor compounds of bread crumb, we had to optimize several parameters such as selection of SPME coating, extraction temperature, salting-out effect, pH, amount of sample, and extraction time. All the experiments were performed with constant magnetic stirring, because chromatographic response is usually increased (Pawliszyn 1997).

Comparison of Different Fiber Types

Three different fiber coatings normally used to analyze volatile compounds (Brunton et al 2000; Roberts et al 2000) were tested: PDMS/DVB, CAR/PDMS, and CW/DVB. To make this comparison, 1 g of bread crumb was weighed inside a 20-mL vial. As it has been reported (Pawliszyn 1997), when solid samples were analyzed by SPME, water or other surface-active compounds should be added to improve the transport of compounds from the sample to the gaseous phase. For this reason, 10 mL of HPLC water were added into the vial together with the bread crumb. The sealed vials were immersed in a water bath at 40°C and the sample was extracted for 60 min. The chromatograms obtained with the three fibers tested are shown in Fig. 1. The CAR/PDMS fiber generally showed the highest chromatographic peaks and furthermore allowed the detection of all the compounds of interest. Therefore, CAR/PDMS fiber was selected for the method optimization.

Effect of Extraction Temperature

The SPME process is strongly influenced by temperature because the partition coefficients are temperature-dependent, and the extraction of the analytes by the fiber coating is an exothermic process (Pawliszyn 1997). To check the effect of temperature on the extraction of the different compounds, a temperature range of 40–60°C was tested. The results obtained showed that the effect of temperature on the chromatographic response was different for the volatile compounds. The chromatographic response of aldehydes, acids, and high molecular weight alcohols increased with higher temperatures, while ketones and low molecular weight

TABLE I
Major Volatile Compounds Quantified from Bread Crumb

Volatile compounds	Retention Time	Ion m/z	Linearity range ($\mu\text{g/g}$)	Limits of Detection ($\mu\text{g/g}$)
Alcohols				
Ethanol	7.50	45	0–2000	18
Isobutyl alcohol	14.61	43	0–3.5	0.05
Isoamyl alcohol	19.44	55	0–6.0	0.21
1-Hexanol	24.34	56	0–0.2	0.016
Benzyl alcohol	36.63	79	0–0.2	0.014
2-Phenylethanol	37.36	91	0–3.0	0.13
Aldehydes				
Hexanal	13.31	44	0–0.25	0.017
Furfural	27.59	95	0–0.25	0.005
Benzaldehyde	29.05	77	0–0.15	0.008
2-Nonenal	29.14	43	0–0.10	0.002
2,4-Decadienal	35.28	81	0–0.05	0.001
Acids				
Acetic acid	27.22	45	0–4500	12
Propionic acid	29.33	74	0–25	0.13
Isobutyric acid	29.97	43	0–10	0.87
Butyric acid	31.36	60	0–6.0	0.25
Isovaleric acid	32.25	60	0–10	0.18
Ketones				
2,3-Butanedione	8.87	43	0–0.30	0.005
3-Hydroxy-2-butanone	22.61	45	0–70	3.1

alcohols showed opposite behavior. After evaluating the results, an intermediate temperature of 50°C was chosen for the continuation of the optimization process.

Effect of Salt Addition

Addition of a soluble salt into the liquid sample increases the ionic strength of the solution. This makes volatile compounds less soluble, and the partition coefficients can be several times higher, increasing the extraction efficiency (Pawliszyn 1997). The effect of salt addition on the extraction was examined at 50°C by testing different concentrations of sodium chloride (0–20%). In general, the response increased on all the compounds studied when the salt concentration increased to 20%. So further experiments were performed using a solution of 20% NaCl.

pH Effect

The pH of the sample is important for slightly acidic or basic compounds because extraction is more effective when these compounds are kept undissociated. To study this effect, 20% NaCl solutions buffered with 0.05M citric acid at pH 3.0–5.0 were prepared and tested at 50°C. The highest response was found at pH 3.0–4.0, depending on the kind of compound analyzed. Acid compounds showed a higher relative response at lower pH, while most of alcohols and ketones presented higher relative areas at intermediate pH. On the other hand, the response of aldehydes was very similar at both pH. A low pH 3 was chosen because a higher pH was more detrimental to the response of acids than pH 3 to the response of aldehydes and ketones.

Effect of Amount of Bread Crumb Analyzed

Natural products contain several compounds with different affinities for the fiber and with variable concentrations, so all compounds may not be in the linear range (Roberts et al 2000). Also, it has been reported that starch, the main compound of bread crumb, can bind some aroma volatiles, decreasing the extraction efficiency (Hau et al 1994). An experiment was conducted to check the effect of the amount of bread crumb on the linear response of volatile compounds. The results obtained demonstrated that it was

necessary to analyze <0.5 g of bread crumb to obtain a linear chromatographic response of the majority of compounds studied. Therefore, 0.25 g of bread crumb were weighed for analysis.

Effect of Extraction Time

The amount of analyte extracted depended on the extraction time and on sample agitation. To extract the maximum amount of analytes, equilibrium time must be reached, but this may be too long for many compounds. Low molecular weight alcohols, ketones, hexanal, 1-hexanol, acetic acid, and 2-nonenal reached equilibrium

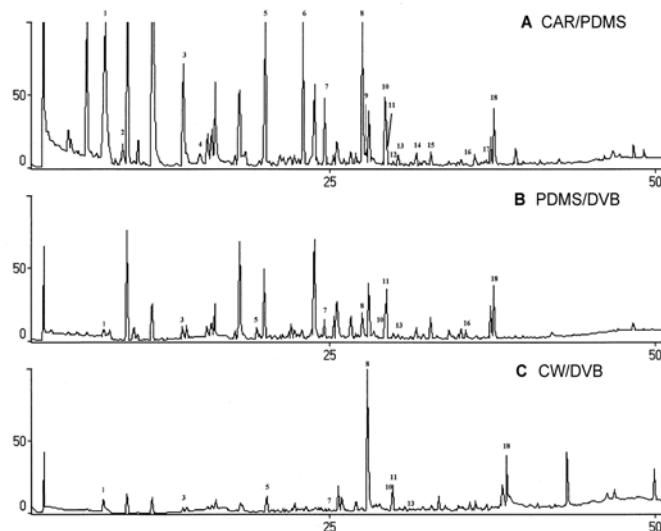


Fig. 1. Comparison of different fibers for bread crumb analysis: **A**, CAR/PDMS; **B**, PDMS/DVB; and **C**, CW/DVB. 1: ethanol, 2: 2,3-butanedione; 3: hexanal; 4: isobutyl alcohol, 5: isoamyl alcohol, 6: 3-hydroxy-2-butanone, 7: 1-hexanol, 8: acetic acid, 9: furfural, 10: benzaldehyde, 11: 2-nonenal, 12: propionic acid, 13: isobutyric acid, 14: butyric acid, 15: isovaleric acid, 16: 2,4-decadienal, 17: benzyl alcohol, 18: 2-phenylethanol.

TABLE II
Minor Volatile Compounds Identified from Bread Crumb

Volatile Compound	Retention Time	Ion m/z	Volatile Compound	Retention Time	Ion m/z
Alcohols			Acids		
1-Pentanol	21.18	42	Pentanoic acid	33.69	60
1-Octen-3-ol	26.93	57	Hexanoic acid	35.85	60
1-Heptanol	27.12	56	Heptanoic acid	37.90	60
2-Ethyl-1-Ethanol	27.99	57	Octanoic acid	39.82	60
1-Octanol	29.62	56	Benzenes		
1-Nonanol	31.94	55	Toluene	10.96	91
Furfuril alcohol	32.16	81	Ethylbenzene	15.16	91
3-Nonen-1-ol	32.51	55	1,2-Dimethylbenzene	15.51	91
3-Decen-1-ol	34.85	68	1,4-Dimethylbenzene	15.80	91
Phenol	39.05	94	1,2-Ethylmethylbenzene	19.30	105
Aldehydes			1,4-Ethylmethylbenzene	19.44	105
Pentanal	8.67	44	Styrene	20.95	104
Octanal	22.11	43	Thrimethylbenzene	21.61	105
2-Heptenal	23.36	41	1,2-Dichlorobenzene	26.72	146
Nonanal	25.35	57	Others		
2-Octenal	26.43	55	Decane	9.06	57
Esters			Limone	17.91	68
Isobutyl acetate	9.97	43	2-Pentylfuran	19.85	81
Buthyl acetate	12.87	43	Dimethyltrisulfide	24.94	125
Ethyl lactate	24.11	45	2-Butoxiethanol	25.84	57
Ethyl Octanoate	26.63	88	α -Terpineol	32.82	59
2-Phenylethanol acetate	35.60	104	Azulene	33.94	128
γ -Nonalactone	39.68	85	Stragole	35.65	148
Ketones			Linalol	36.22	71
2-Octanone	22.02	43	4-Vinil-2-metoxiphenol	42.61	150
1-Octen-3-one	22.36	55			
6-Methyl-5-hepten-2-one	23.82	43			

at 60 min, whereas for the other compounds, such as 2-phenylethanol, peak areas increased until 120 min. From these results, an extraction time of 60 min was chosen to get a period of time which coincides with chromatographic run times.

Tables I and II show the volatile compounds extracted from bread crumb analyzed under the conditions above. Besides the 18 studied analytes (Table I), more than 45 additional compounds were extracted with good resolution (Table II). To improve analytical determination, the ion in greatest abundance was used to integrate each compound.

Linear Range, Limits of Detection, and Precision

Once the conditions for the SPME method to analyze the main volatile compounds from bread crumb were established, quality parameters such as linearity, limits of detection (LOD), and precision were calculated. Two compounds were used as internal standards (4-methyl-2-pentanol and 2-ethylbutyric acid). Both showed properties suitable to act as internal standard: retention times different from those of the compounds studied, good peak resolution, and they were not reported previously as aroma compounds in bread. The ranges of linearity for each compound are given in Table I. In all cases, the correlation coefficients were acceptable ($r > 0.99$).

The LOD for each compound was calculated with the Detarchi computer program (Sarabia and Ortiz 1994) from calibration graphs and taking into account the standard deviations and α and β errors (false positive or false negative).

The recovery of the SPME method was determined for a single French baguette and Italian ciabatta crumb. The test was performed by measuring volatile compounds in the bread spiked with known amounts of a standard mixture of all studied compounds (matched matrix standard method). Then we compared the concentration added with the concentration calculated by interpolation of the chromatographic response in the calibration graphs. Thus, we calculated the recovery, which was defined as the percentage ratio between concentration of analyte calculated and concentration of analyte added. This value was determined at two

different concentration levels in the calibration range specified in Table I. The recoveries were $\approx 100\%$ for all studied compounds, although ethanol, benzyl alcohol, and acetic acid showed higher recoveries (Table III). This could be explained because these compounds present different areas in comparison with internal standard, and a minor error could cause a major deviation. If the final method determines compounds with different behavior, it is very difficult to always obtain recoveries close to 100%. On the other hand, relative standard deviations were generally $< 20\%$. Similar values have been reported previously when CAR/PDMS fiber was used to analyze other foods (Yang and Peppard 1994; Mestres et al 1999; Brunton et al 2000).

Finally, the optimized SPME method was applied for determination of volatile compounds in different commercial precooked frozen baguettes ($n = 5$) and ciabattas ($n = 3$). The results are shown in Table III. These results are similar to those obtained by other authors (Luning et al 1991; Gassenmeier and Schieberle 1995; Schieberle 1996), who quantified some of the acids, alcohols, and aldehydes studied in French baguette.

CONCLUSIONS

Solid-phase microextraction (SPME) technique has been successfully applied to analyze the volatile compounds from bread crumb. In comparison with the other solvent extraction methods, the sample preparation process is simpler, does not involve the use of organic solvents, is less time-consuming, and can be used as a routine method to analyze bread crumb volatiles. Furthermore, this method allows quantification with a good precision and accuracy of the main volatile compounds responsible for the aroma profile of bread crumb. The volatile concentrations obtained in some commercial bread crumb analyzed agreed with values previously reported by other authors.

ACKNOWLEDGMENTS

Supported by Europastry, S. A., St. Cugat del Vallès, Barcelona, Spain.

TABLE III
Recovery Percentages^a and Relative Standard Deviations (in parentheses) and Concentrations of Major Volatile Compounds Found in Commercial Precooked Frozen Baguettes^b and Ciabattas^c

Volatile Compound	Baguette			Ciabatta		
	Recovery		Concentration ($\mu\text{g/g}$)	Recovery		Concentration ($\mu\text{g/g}$)
Low Level	High Level	Low Level		High Level		
Alcohols						
Ethanol	156 (19)	153 (23)	200–1900	170 (4)	144 (6)	200–1100
Isobutyl alcohol	83 (9)	109 (21)	0.45–1.50	94 (20)	72 (9)	0.20–3.00
Isoamyl alcohol	107 (9)	120 (16)	0.45–2.25	125 (5)	82 (5)	0.45–3.60
1-Hexanol	96 (13)	100 (5)	0.04–0.10	105 (9)	113 (9)	0.03–0.10
Benzyl alcohol	142 (15)	106 (25)	0.03–0.11	145 (5)	153 (5)	nd–0.05
2-Phenylethanol	109 (36)	110 (13)	1.50–2.40	126 (13)	121 (7)	0.40–2.25
Aldehydes						
Hexanal	89 (24)	112 (27)	0.05–0.10	87 (22)	83 (4)	0.04–0.22
Furfural	111 (28)	116 (16)	0.03–0.06	145 (19)	168 (2)	0.08–0.24
Benzaldehyde	101 (17)	112 (5)	0.03–0.05	108 (15)	135 (1)	0.04–0.06
2-Nonenal	106 (10)	81 (24)	0.03–0.08	80 (12)	118 (14)	0.01–0.06
2,4-Decadienal	141 (15)	67 (28)	0.02–0.03	120 (12)	156 (7)	nd–0.04
Acids						
Acetic acid	164 (13)	121 (8)	200–400	160 (12)	153 (3)	250–4000
Propionic acid	106 (16)	123 (10)	2.0–3.1	126 (10)	114 (3)	1.2–20.7
Isobutyric acid	100 (8)	105 (10)	2.4–2.8	99 (24)	95 (11)	2.0–4.4
Butyric acid	107 (9)	109 (3)	0.4–5.2	112 (10)	126 (7)	0.4–0.8
Isovaleric acid	107 (2)	102 (5)	1.4–2.6	112 (20)	103 (6)	2.4–5.7
Ketones						
2,3-Butanedione	112 (20)	132 (19)	0.03–0.09	130 (6)	98 (5)	0.02–0.07
3-Hydroxy-2-butanone	86 (9)	101 (12)	4.6–31.2	90 (13)	81 (2)	nd–30.4

^a Recovery percentage mean of three duplicates ($n = 3$) for a single sample.

^b Five different commercial precooked frozen baguettes.

^c Three different commercial precooked frozen ciabattas.

LITERATURE CITED

- Arthur, C. L., and Pawliszyn, J. 1990. Solid-phase microextraction with thermal desorption using fused silica fibers. *Anal. Chem.* 62:2145-2148.
- Brunton, N. P., Cronin, D. A., Monahan, F. J., and Durcan, R. 2000. A comparison of solid-phase microextraction (SPME) fibres for measurement of hexanal and pentanal in cooked turkey. *Food Chem.* 68:339-345.
- Caul, M. 1972. La flaveur des aliments et le consommateur. *Bull. Anc. Eleves Ec. Meun. ENSMIC* 251:242-246.
- Frasse, P., Lambert, S., Richard-Molard, D., and Chiron, H. 1993. The influence of fermentation on volatile compounds in French bread dough. *Lebensm. Wiss. Technol.* 26:126-132.
- Gassenmeier, K., and Schieberle, P. 1995. Potent aromatic compounds in the crumb of wheat bread (French-type)—Influence of pre-ferments and studies on the formation of key odorants during dough processing. *Z. Lebensm. Unters Forsch* 201:241-248.
- Hau, M. Y. M., Gray, D. A., and Taylor, A. J. 1994. Binding of volatiles to starch. Pages 109-117 in: *Flavor-Food Interactions*. R. J. McGorrin, and J. V. Leland, eds. ACS: Washington, DC.
- Luning, P. A., Roozen, J. P., Moëst, R. A. F. J., and Posthumus, M. A. 1991. Volatile composition of white bread using enzyme active soya flour as improver. *Food Chem.* 41:81-91.
- Maga, J. A. 1974. Bread flavor. *CRC Crit. Rev. Food Technol.* 5:55-142.
- Mestres, M., Sala, C., Martí, M. P., Busto, O., and Guasch, J. 1999. Headspace solid phase microextraction of sulphides and disulphides using carboxen-polydimethylsiloxane fibers in the analysis of wine aroma. *J. Chromatogr. A* 835:137-144.
- Pawliszyn, J. 1997. *Solid-Phase Microextraction: Theory and Practice*. Wiley-VCH: New York.
- Roberts, D. D., Pollien, P., and Milo, C. 2000. Solid-phase microextraction method development for headspace analysis of volatile flavor compounds. *J. Agric. Food Chem.* 48:2430-2437.
- Sarabia, L., and Ortiz, M. C. 1994. DETARCHI: A program for detection limits with specific assurance probabilities and characteristic curves of detection. *Trends Anal. Chem.* 13:1-6.
- Schieberle, P. 1996. Intense aroma compounds—Useful tools to monitor the influence of processing and storage on bread aroma. *Adv. Food Sci.* 18:237-244.
- Seitz, L. M., Chung, O. K., and Rengarajan, R. 1998. Volatiles in selected commercial breads. *Cereal Chem.* 75:847-853.
- Yang, X., and Peppard, T. 1994. Solid-phase microextraction for flavour analysis. *J. Agric. Food Chem.* 42:1925-1930.

[Received June 18, 2002. Accepted November 4, 2002.]