

Simple and Rapid Analysis for Quantitation of the Most Important Volatile Flavor Compounds in Yogurt by Headspace Gas Chromatography–Mass Spectrometry

L. Alonso*

Instituto de Productos Lácteos de Asturias (CSIC), Carretera de Infiesto s/n, 33300 Villaviciosa, Asturias, Spain

M.J. Fraga

Instituto del Frío (CSIC), Ciudad Universitaria 28040, Madrid, Spain

Abstract

The aim of this experiment is to develop a rapid, simplified, direct gas chromatographic (GC) method for the analysis of volatile flavor compounds in yogurt combining a headspace with constant heating temperature and GC with a mass spectrometric detect ion. Repeatability of the method is assessed. The relative standard deviation for individual flavor compounds range from 3.5% for acetaldehyde to 8.4% for acetone, with a total mean value of 52.4 ± 2.2 mg/kg for all of the studied aroma components. Recovery for individual flavor compounds range from 63.7% for acetone to 82.4% for acetic acid.

Introduction

Aroma perception is one of the foremost criteria for the evaluation of fermented milk because of its influence on consumer acceptance and preference. In order to understand the complex nature of a fermented milk sample, research has focused on the flavor and the study of volatile formation (1–4). The characteristics of yogurt are mainly the result of the metabolism of the culture organisms. The most important compounds from lactic starter cultures are known to be acetaldehyde, diacetyl, acetone, butanone, acetoin, and acetic acid. It is the balance between them that is thought to determine acceptability (5,6); the composition of the mixed strain starter may determine a pleasant flavor or a certain off-flavor (7). Gas chromatography (GC) is the method most commonly used for the analysis of volatile compounds in fermented milk. The analytical techniques that have been applied for isolating volatile compounds include simultaneous distillation–extraction (8–11), solvent extraction (12,13), dynamic purge trap (14,15), and headspace (HS) methods (16,17). HS methods do not require solvents or special appa-

ratus, and they are less time consuming.

This paper describes a rapid GC method coupled with a mass spectrometry (MS) direct static HS method used to assay volatile flavor compounds in yogurt. The repeatability and recovery of the analytical procedure applied to yogurt are also assessed.

Experimental

Chemicals

All reagents were analytical reagent grade. Aqueous solutions of acetaldehyde, acetone, butanone, diacetyl, acetoin, acetic acid, and propionic acid ethyl ester were prepared from high-purity chemicals (higher than 98%) purchased from Sigma Chemical Co. (St. Louis, MO) and Aldrich Chemie (Steinheim, Germany).

Yogurt sample

Commercial plain yogurt samples from the same manufacturing batch were purchased from a retail store and were used to test the repeatability and percent recovery of the method.

Sample preparation

In order to retain the water, 10 g of yogurt sample with a 15- μ L standard solution containing 0.54 mg/mL of propionic acid ethyl ester as the internal standard in aqueous solution and 10 g of anhydrous sodium sulfate were mixed with a spatula in a 20-mL HS vial that was sealed hermetically with a polytetrafluoroethylene-coated rubber septum and an aluminum cap. The sample was kept in the freezer until further analysis.

Instrumentation and operating conditions

HS analysis

A Hewlett-Packard (Palo Alto, CA) HS autosampler (Model HSS 19395 A) was used to monitor the static HS quantitation of volatiles. Samples were equilibrated for 60 min at 80°C prior to analysis. The settings of the HSS 19395 A were 5 s for pressur-

* Author to whom correspondence should be addressed: email lalonso@ipla.csic.es.

ization, equilibration, and filling and 2 min for injection. The HS loop (3 mL) temperature was set at 90°C.

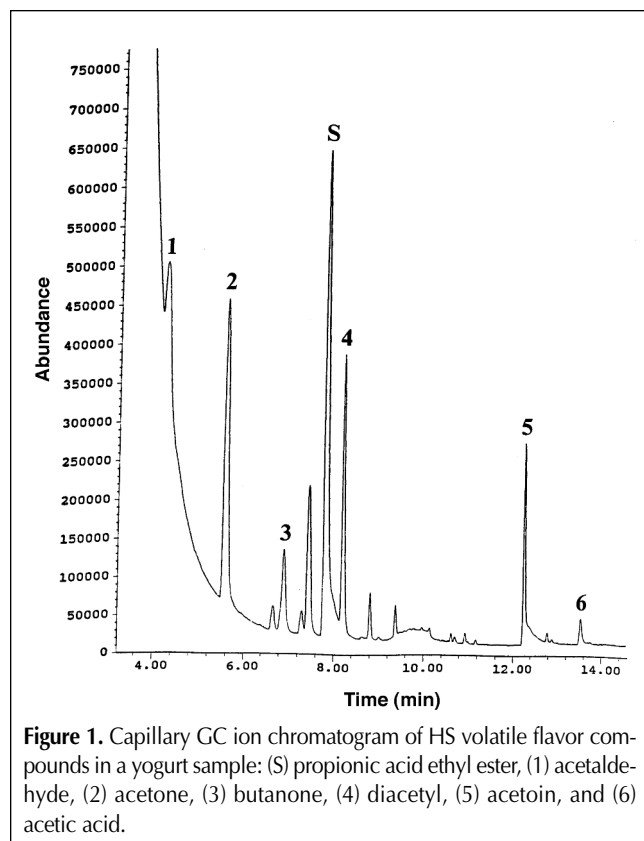
High-purity helium, filtered through moisture and oxygen traps (Hewlett-Packard), was used for vial pressurization, and an HSS sampler carrier gas at a flow rate of 17.5 mL/min was measured at the splitter outlet.

GC-MS analysis

Analyses were performed on a Hewlett-Packard 5890 GC coupled to a 5972 MS detector. Manual tuning of the MS with perfluorotributylamine was used to adjust relative abundance for m/z 69, 219, and 502. The MS was run in the scan mode (m/z range from 33 to 250 with a threshold of 100 and a sampling rate of 3 scans/s). Ultrapure helium was passed through moisture and oxygen traps and was used as the carrier gas. The following GC operating conditions were used: a silica capillary column HP Innovax cross-linked polyethylene glycol adipate (60-m \times 0.25-mm \times 0.25- μ m film thickness); a flow rate of 36.5 cm/s at 33°C; a split ratio of 7:1; the injection port set at 200°C and the interface line to the MS at 280°C; and the electron energy and electron multiplier voltage at 70 eV and 1647 V. The temperature program began at 33°C for 5 min; increased 1°C/min up to 38°C; and then increased 7°C/min up to 70°C until final time.

Results and Discussion

HS-GC coupled with MS is one of the methods for the analysis of volatiles in which direct injection is not feasible. The constant heating time (CHT) for the HS sampler allows each sample to be



heated for an equal amount of time prior to analysis, avoiding differences among samples resulting from unequal equilibration periods (18). For most analyses, equilibrium is established rapidly and HS analysis may be run in CHT mode. The response factors were calculated in a mixture of acetaldehyde, acetone, diacetyl, acetoin, and acetic acid at a ratio of 1:1 (w/w) with respect to the internal standard (propionic acid ethyl ester). The response factors for acetaldehyde, acetone, and butanone were 0.86, 0.80, and 0.89 respectively, and for diacetyl, acetoin, and acetic acid were 1.06, 1.11, and 1.16, respectively, because these compounds had differences in vapor pressure. Figure 1 shows a GC capillary total ion chromatogram of the HS volatile compounds of a yogurt sample. Propionic acid ethyl ester, which is practically absent from the sample, is well-defined as the internal standard and is therefore suitable for quantitative analysis in yogurt.

The repeatability of the analytical method for volatile flavor compounds was tested on a commercial yogurt using propionic acid ethyl ester as the internal standard. Table I shows the individual and mean values and the relative standard deviations (RSD) for acetaldehyde, acetone, butanone, diacetyl, acetoin, and acetic acid obtained for five replicate analyses of commercial yogurt. The mean value found for the repeatability of the method for the total volatile content was 52.4 ± 2.2 mg/kg, and the RSD for individual flavor compounds ranged from 3.5% to 8.4%. In a study for assaying volatile compounds by HS-GC in fermented milk, Monnet et al. (19) obtained a range from 2.5% for acetaldehyde to 7.7% for acetone. These results are comparable to our own study in which the RSD was generally low. The mass spectra for the volatile flavor compounds studied are shown in Figure 2.

For recovery analysis, known amounts of volatile flavor compounds (acetaldehyde, acetone, butanone, diacetyl, acetoin, and

Table I. Repeatability of Flavor Compounds in Yogurt

| Component | Concentration (mg/kg) | | | | | Mean | RSD (%) |
|--------------|-----------------------|-------|-------|-------|-------|-------|---------|
| | 1 | 2 | 3 | 4 | 5 | | |
| Acetaldehyde | 4.92 | 5.27 | 5.38 | 5.10 | 5.25 | 5.18 | 3.5 |
| Acetone | 23.94 | 21.71 | 20.32 | 22.32 | 25.18 | 22.69 | 8.4 |
| Butanone | 4.12 | 4.31 | 4.43 | 4.03 | 4.19 | 4.22 | 3.8 |
| Diacetyl | 11.70 | 10.86 | 11.36 | 10.98 | 11.90 | 11.36 | 4.0 |
| Acetoin | 7.46 | 6.91 | 7.58 | 7.31 | 7.13 | 7.28 | 3.7 |
| Acetic | 1.76 | 1.93 | 1.81 | 1.81 | 1.86 | 1.85 | 3.8 |

Table II. Recovery of Flavor Compounds in Yogurt

| Component | Concentration (mg/kg) | | | | | Mean | RSD (%) |
|--------------|-----------------------|---------|--------------|-----------|---------|------|---------|
| | Initial account* | | Amount added | Recovery* | | | |
| | Mean | RSD (%) | | Mean | RSD (%) | | |
| Acetaldehyde | 5.18 | 3.5 | 3.12 | 7.59 | 2.8 | 77.2 | |
| Acetone | 22.69 | 8.4 | 10.28 | 29.24 | 7.3 | 63.7 | |
| Butanone | 5.22 | 3.1 | 2.16 | 6.93 | 3.8 | 79.2 | |
| Diacetyl | 11.36 | 4.0 | 6.13 | 16.41 | 2.4 | 82.4 | |
| Acetoin | 7.28 | 3.7 | 3.94 | 10.37 | 2.2 | 78.4 | |
| Acetic | 1.85 | 3.8 | 1.16 | 2.85 | 2.8 | 86.2 | |

* Mean value of 5.

acetic acid) were added to a commercial yogurt sample in which individual flavor compounds had been determined using propionic acid ethyl ester as the internal standard. Five different addition assays were carried out. Table II shows the amounts of flavor compounds added to the yogurt sample and the recovery percentage values. In spite of the acetaldehyde peak being poorly resolved (Figure 1), the reproducibility of each recovery was high (RSD = 2.8%). These ranged from 63.7% for acetone to 86.2% for acetic acid with a mean value of 77.9% for all volatile compounds. De Haast et al. (20) using static HS-GC analysis reported slightly higher recoveries for volatile organic compounds from an aqueous solution of milk and fermented milk and demonstrated that the recoveries were of a similar order of magnitude. Imhof and Bosset (21) demonstrated that the fat did not significantly affect the quantitative results of HS analysis for volatile organic compounds. This fact suggests that any differences in the matrix in fermented products may not affect HS analysis.

The method assayed in this study is fast and simple and gives

good repeatability and acceptable recoveries. Therefore, it offers advantages for the analysis of volatile flavor compounds in yogurt and will be a suitable method for the routine analysis of volatile aroma compounds in fermented milk.

Acknowledgments

This work was supported by a grant from the Plan Nacional de I+D+I (Project AGL 2000-0727-C03-02).

References

1. H.T. Badings and R. Neeter. Recent advances in the study of aroma compound of milk and dairy products. *Neth. Milk Dairy J.* **34**: 9–30 (1980).

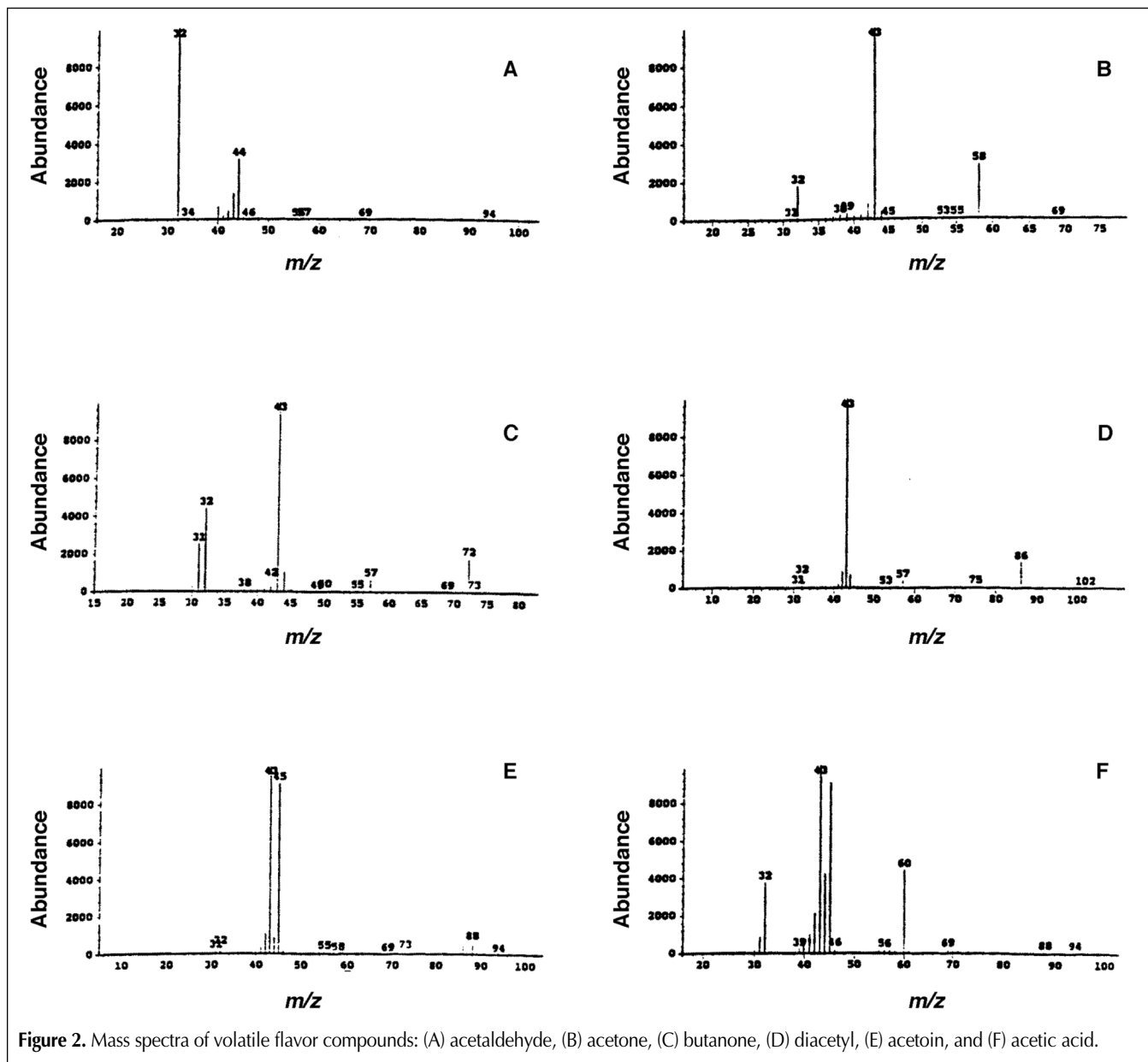


Figure 2. Mass spectra of volatile flavor compounds: (A) acetaldehyde, (B) acetone, (C) butanone, (D) diacetyl, (E) acetoin, and (F) acetic acid.

2. H.A. Veringa, E.H. Verburg, and J. Stadhouder. Determination of diacetyl in dairy products containing α -acetolactic acid. *Neth. Milk Dairy J.* **38**: 251–62 (1984).
3. J.C. Lin and I.J. Jeon. Headspace gas sampling/GC method for the quantitative analysis of volatile compounds in cheese. *J. Food Sci.* **50**: 843–44 (1985).
4. J. Hugenholtz and M.J.C. Starrenburg. Diacetyl production by different strains of *Lactococcus lactis* subsp. *Lactis* var. *diacetylactis* and *Leuconostoc* spp. *Appl. Microbiol. Biotechnol.* **38**: 17–25 (1992).
5. B. G'Osheva. The organoleptic and gas chromatographic characteristics of the flavour of Bulgarian yoghurt. *Khranitelna Promishlenost.* **33**: 36–38 (1984).
6. M. Kondratenko and B. G'Osheva. "Variation in the volatile components of yoghurt". In *XX International Dairy Congress*. Paris, France, 1978, pp. 297–98.
7. A.M. Gaafar. Volatile flavour compounds of yoghurt. *Int. J. Food Sci. and Technol.* **56**: 87–91 (1992).
8. T. Aishima and S. Nakai. Pattern recognition of GC profiles for classification of cheese variety. *J. Food Sci.* **52**: 939–49 (1987).
9. M. Careri, P. Manini, S. Spagnoli, G. Barbieri, and L. Bolzoni. Use of simultaneous distillation–extraction and dynamic headspace methods in the gas chromatographic analysis of Parmesan cheeses volatile components. *Chromatographia* **38**: 386–94 (1994).
10. M. De Frutos, J.Sanz, and I. Martinez–Castro. Characterization of artisanal cheeses by GC and GC/MS analysis of their medium volatility (SDE) fraction. *J. Agric. Food Chem.* **39**: 524–30 (1991).
11. I. Martinez–Castro, J. Sanz, L. Amigó, M. Ramos, and P. Martín–Alvarez. Volatile components of Manchego cheese. *J. Dairy Res.* **58**: 239–46 (1991).
12. H.C. Dunn and R.C. Lindsay. Comparison of methods for the analysis of higher boiling flavor compounds in Cheddar cheese aroma. *J. Dairy Sci.* **68**: 2853–58 (1985).
13. P. Vandeweghe and G.A. Reineccius. Comparison of flavor isolation techniques applied to Cheddar cheese. *J. Agric. Food Chem.* **38**: 1549–52 (1990).
14. W.T. Yang and D.D. Min. Dynamic headspace analysis of volatile compounds of Cheddar and Swiss cheese during ripening. *J. Food Sci.* **59**: 1309–12 (1994).
15. G. Arora, F. Cormier, and B. Lee. Analysis of odor-active volatiles in Cheddar cheese headspace by multidimensional GC/MS/sniffing. *J. Agric. Food Chem.* **43**: 748–52 (1995).
16. D.J. Manning and C. Moore. Headspace analysis of hard cheeses. *J. Dairy Res.* **46**: 539–45 (1979).
17. R. Marsili. Monitoring chemical changes in Cheddar cheese during aging by high-performance liquid chromatography and gas chromatography techniques. *J. Dairy Sci.* **68**: 3155–61 (1985).
18. L. Philip. "Headspace Analysis with Constant Sample Heating Time". Application note, Hewlett-Packard, 1998, pp. 228–58.
19. C. Monnet, P. Schmitt, and C. Divies. Method for assaying volatile compounds by headspace gas chromatography and application to growing starter cultures. *J. Dairy Sci.* **77**: 1809–15. (1994).
20. J. De Haast, P.M. Lategan, and J.C. Novello. Headspace analysis as a means of evaluating flavour producing volatiles in milk product. *South African J. Dairy Technol.* **10**: 155–58 (1978).
21. R. Imhof and J.O. Bosset. Quantitative GC–MS analysis of volatile flavour compounds in pasteurized milk and fermented milk products applying a standard addition method. *Food Sci. Technol.* **27**: 265–69 (1994).

Manuscript accepted March 23, 2001.