Development of a Headspace Gas Chromatographic–Mass Spectrometric Method for Determining Methyl-Ketones and Secondary Alcohols in Blue Cheese

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Abstract

The aim of this work is to develop a rapid and direct gas chromatographic (GC) method for the analysis of methyl-ketones and secondary alcohols in blue cheese using headspace with constant heating temperature coupled to GC with a mass spectrometric detector. Repeatibility of the method is assessed; the coefficient of variation for individual methyl-ketones ranges from 4.5 to 9.6%, and the total mean value for methyl-ketones is 466.1 ± 5.1 µg/100 g with a coefficient of variation of 1.1%. The coefficient of variation found for individual secondary alcohols in this study ranges from 11.2 for 2-pentanol to 22.6 for 2-nonanol. The total mean concentration for secondary alcohols (2-propanol, 2-pentanol, 2-heptanol, and 2-nonanol) is 80.5 ± 5.0 µg/100 g with a coefficient of variation of 6.2% (2-pentanol and 2-propanol representing 62% of the total secondary alcohols studied). Recovery ranges between 80.3 to 88.6% for individual methyl-ketones and between 81.3 and 85.4% for secondary alcohols.

Introduction

Flavor is a key attribute in the acceptability of food. The mechanisms responsible for creating the taste sensation are not well understood. In general, many compounds may be involved in the generation of a particular flavor, and it is difficult to establish the importance that each individual compound has (1). Aroma perception is one of the foremost criteria of a cheese grader for product evaluation. To unfold the complex nature of a cheese flavor, earlier research was focused on flavor profiling (2,3) and the study of volatile formation during the ripening of cheese (4,5). Carbonyl compounds, which are strongly aromatic, make a significant contribution to the flavor of many cheese varieties, and methyl-ketones make a large contribution to the flavor of blue cheese. Although the nature of the molecules that determine the taste of some cheeses is well known, the difficulty is in defining the role of one molecular species or a class of molecules in the taste perception of the consumer. In blue cheeses, it has been shown that free fatty acids, methyl-ketones, and secondary alcohols have an important role in flavor-typifying these cheeses. 2-pentanone, 2-heptanone, and 2-nonanone are considered to be key flavor components of mold-ripened cheese (6,7). Gas chromatography (GC) coupled with a mass spectrometric (MS) detector has been most commonly used for the analysis of cheese volatile flavor compounds. Different analytical techniques have been applied for isolating the volatile components. These include simultaneous distillation–extraction (8–11), molecular distillation (2), solvent extraction (12–13), dynamic purge and trap (14–15), and headspace methods (16–18). The headspace methods do not require solvents or a special apparatus, and they are relatively short in time consumption.

This paper describes a rapid GC–MS direct static headspace method for the determination of methyl-ketones (2-propanone, 2-pentanone, 2-heptanone, and 2-nonanone) and secondary alcohols (2-propanol, 2-pentanol, 2-heptanol, and 2-nonanol) in blue cheese. The repeatability and recovery of the analytical procedure applied to cheese were also assessed.

Experimental

Standard solutions

Aqueous solutions of 2-propanone, 2-pentanone, 2-heptanone, 2-nonanone, 2-propanol, 2-pentanol, 2-heptanol, 2-nonanol, and propionic acid ethyl ester as an internal standard were prepared from high purity chemicals (> 98%) purchased from Sigma (St. Louis, MO) and Aldrich Chemie (Steinheim, Germany). The standards were individually prepared and stored in hermetically sealed headspace vials at –20°C until they were used.

Sample preparation

Three representative portions of commercially available Cabrales Cheese (soft blue cheese manufactured from a blend

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of cow's, ewe's, and goat's milk in Asturias, Spain) with a normal level of ripening were used for this study. The rind was completely removed, and each cheese portion was ground and mixed to prepare a homogeneous sample.

A 10-g sample and 15 µL standard solution containing 0.84 mg/mL of propionic acid ethyl ester as an internal standard in aqueous solution and 10 g of sodium sulphate anhydre to retain the water were combined and mixed with a spatula in a 20-mL headspace vial that was sealed hermetically with a polytetrafluoroethylene (PTFE) coated rubber septum and an aluminium cap until further analysis.

Instrumentation and operating conditions

Headspace analysis

A Hewlett-Packard (Palo Alto, CA) model HSS 19395 A headspace autosampler was used to monitor the static headspace quantitation of volatiles. Samples were equilibrated for 60 min at 80°C prior to analysis. The conditions of the HSS 19395 A were as follows: 5 s for pressurization, equilibration, and filling; 2-min injection. The 3-mL headspace loop temperature was set at 90°C.

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

GC–MS analysis

Analysis was performed on a Hewlett-Packard 5890 GC coupled with a 5972 MS detector. Manual tuning of the MS with perfluorotributylamine (PFTBA) was used to adjust the relative abundance for *m/z* 69, *m/z* 219, and *m/z* 502. The MS was run in the scan mode (*m/z* 33–250 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. GC operating conditions were as follows: silica capillary column, HP Innovax (Hewlett-Packard) cross-linked polyethylene glycol (60 m × 0.25 mm, 0.25-µm film thickness); flow rate, 36.5 cm/s at 33°C; split ratio, 7:1; temperature program, 33°C for 5 min, then to 38°C at 1°C/min, then to 210°C at 7°C/min, and held 10 min until the final time of the program; injection port temperature, 200°C; interface line to MS temperature, 280°C; electron energy voltage, 70 eV; and electron multiplier voltage, 1647 V.

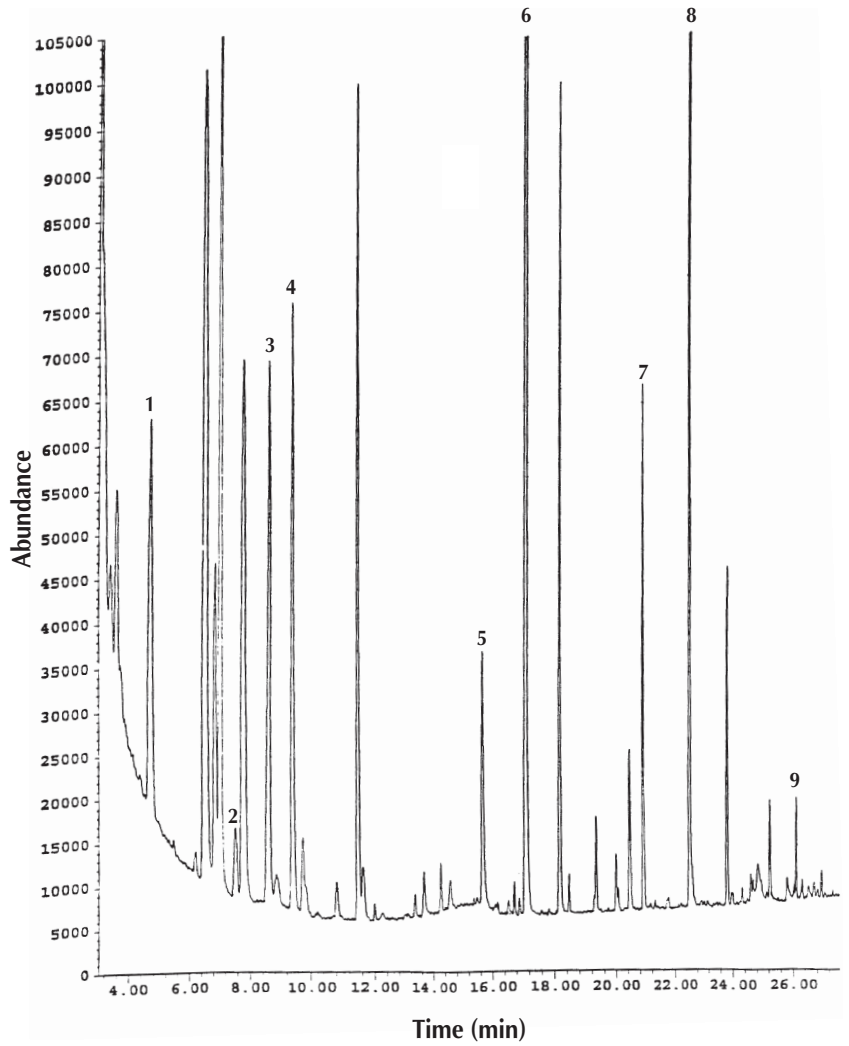

Figure 1. Capillary GC total ion chromatogram of the headspace volatiles methyl-ketones and secondary alcohols of a blue cheese sample. Peaks: 1, 2-propanone; 2, 2-propanol; 3, internal standard; 4, 2-pentanone; 5, 2-pentanol; 6, 2-heptanone; 7, 2-heptanol; 8, 2-nonanone; 9, 2-nonanol.

Results and Discussion

Headspace GC analysis

Headspace GC coupled with a MS detector is often used as one of the methods for the analysis of volatiles in samples that are impossible to directly syringe inject. The constant heating time (CHT) for the headspace sampler allows each sample to be heated for an equal time prior to analysis, avoiding the differences among samples caused by unequal equilibration periods (19). Therefore, it should be possible to achieve a high level of precision from sample to sample even when the analyte concentration varies with the heating time. For most analyses, equilibrium is established rapidly, and headspace analysis may be run in a CHT mode. In the present study in particular, methyl-ketones and secondary alcohols were practically stable at the incubation temperature and reached a good thermodynamic equilibrium during the incubation time.

The response factors were calculated in a mixture of 2-propanone, 2-pentanone, 2-heptanone, 2-nonanone, 2-propanol, 2-pentanol, 2-heptanol, and 2-nonanol with a ratio of 1:1 (w/w) with respect to the

internal standard, propionic acid ethyl ester. The response factors of low- and medium-molecular-weight volatile compounds are in the range of 0.83 to 1.09, corresponding to 2-propanona and 2-heptanona. However, for the volatile compounds with a high molecular weight (2-nonanone and 2-nonanol), the correction factors were 1.15 and 1.19, respectively, because of the fact that those compounds have differences in vapor pressure (20). Figure 1 shows a GC capillary total ion chromatogram of the headspace volatiles (methyl-ketones and secondary alcohols) of a sample of blue cheese. Propionic acid ethyl ester, which is practically not present in the sample, is well defined as the internal standard; although it elutes close to an unknown substance, it does not interfere with a correct quantitation.

Table I. Repeatability for Methyl-Ketones and Secondary Alcohols in Blue Cheese

	Concentration (µg/100 g)						
	1	2	3	4	5	X*	SD
2-propanone	48.1	49.3	43.1	47.6	51.5	47.9	3.1
2-pentanone	137.3	132.8	146.9	141.3	126.3	136.9	7.9
2-heptanone	216.5	229.7	221.3	205.8	208.7	216.4	9.7
2-nonanone	64.3	60.8	57.3	69.6	72.3	64.9	6.2
2-propanol	16.3	13.9	19.1	18.6	12.9	16.2	2.8
2-pentanol	32.6	36.3	39.9	29.3	31.3	33.9	3.8
2-heptanol	25.8	28.4	19.1	26.1	21.7	24.2	3.7
2-nonanol	5.3	7.1	4.3	7.9	6.3	6.2	1.4

* X, mean values.
† SD, standard deviation.

Repeatability of method

The repeatability of the analytical method for methyl-ketones (2-propanone, 2-pentanone, 2-heptanone, and 2-nonanone) and secondary alcohols (2-propanol, 2-pentanol, 2-heptanol, and 2-nonanol) was tested on a commercial blue cheese using propionic acid ethyl ester as an internal standard. Table I shows the individual and mean values and standard deviations for 5 replicate analyses of blue cheese for the determination of methyl-ketones and secondary alcohols. 2-pentanone and 2-heptanone were the most abundant ketones, representing 76% of the total methyl-ketones studied. The total mean value for methyl-ketones was 466.1 ± 5.1 µg/100 g, and the coefficient of variation for individual methyl-ketones ranges

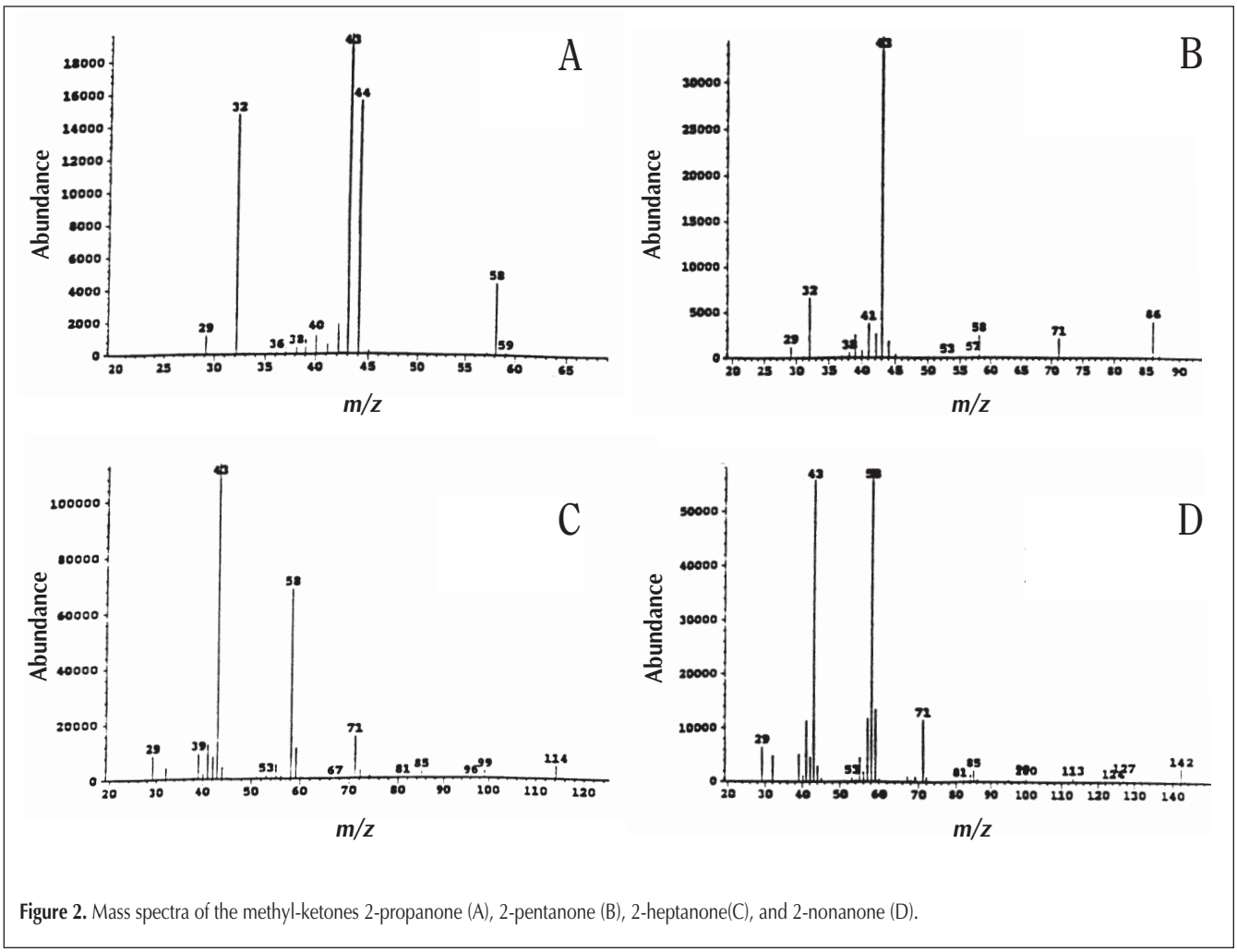

Figure 2. Mass spectra of the methyl-ketones 2-propanone (A), 2-pentanone (B), 2-heptanone(C), and 2-nonanone (D).

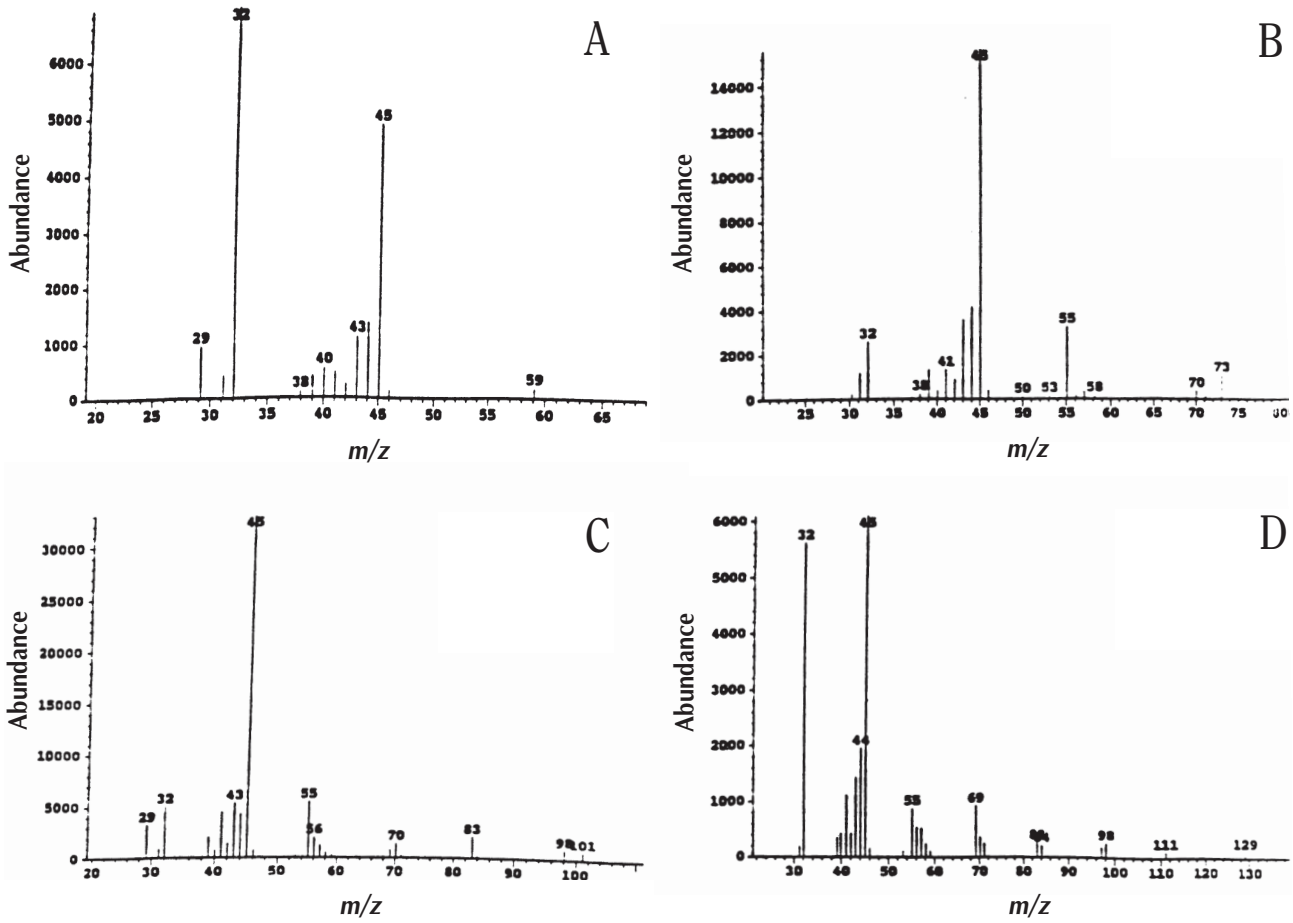

Figure 3. Mass spectra of the secondary alcohols 2-propanol (A), 2-pentanol (B), 2-heptanol (C), and 2-nonanol (D).

from 4.5 to 9.6%. Gallois and Langlois (21), in a study of volatile odorous compounds of French cheeses using a vacuum degassing and cold finger molecular distillation procedure, obtained a range of coefficients of variation from 2 to 33% for 2-pentanone, 2-heptanone, and 2-nonanone, higher than those reported in this work. The mass spectra for the 4 methyl-ketones are shown in Figure 2.

Odd-numbered secondary alcohols were found in blue cheeses mainly by the reduction of the corresponding aldehydes or ketones (22). Alcohols have less influence on cheese flavor than methyl-ketones; however, they may indirectly contribute to flavor because of their ability to form esters with fatty acids (15).

The total mean concentration for the 4 secondary alcohols was 80.5 ± 5.0 µg/100 g with 2-pentanol and 2-propanol representing 62% of the total secondary alcohols studied. The coefficients of variation found in this study for individual secondary alcohols ranged from 11.2 for 2-pentanol to 22.6 for 2-nonanol. The mass spectrum for the 4 secondary alcohols analyzed are shown in Figure 3.

Table II. Recovery of Methyl-Ketones and Secondary Alcohols in Blue Cheese

	Concentration (µg/100 g)					
	Initial account*			Recovery		
	X	SD†	Amount added	X	SD	%
2-propanone	47.9	3.1	22.3	65.8	2.9	80.3
2-pentanone	136.9	7.9	66.4	195.7	6.9	88.6
2-heptanone	216.4	9.7	108.3	309.1	5.9	85.6
2-nonanone	64.9	6.2	31.8	91.2	3.8	82.7
2-propanol	16.2	2.8	8.9	23.8	3.5	85.4
2-pentanol	33.9	4.2	16.8	48.1	4.0	84.5
2-heptanol	24.2	3.7	22.1	42.6	4.2	83.3
2-nonanol	6.2	1.4	3.2	8.8	2.7	81.3

* Mean value of 5.
† Mean value of 3.
‡ X, mean values.
§ SD, standard deviation.

Bosset and Gauch (23), in a study of 6 European cheeses not ripened by mold using a purge-and-trap system, reported dates of secondary alcohols for 2-pentanol and 2-nonanol with a range of coefficients of variation from 3 to 24%, which is in a similar order to those reported in this study.

Recovery of methyl-ketones and secondary alcohols

For recovery analysis, known amounts of methyl-ketones and secondary alcohols were added to a blue cheese sample in which individual methyl-ketones and secondary alcohols had been determined previously using propionic acid ethyl ester as an internal standard. Three different addition assays and replicate injections of each assay were made. Table II shows the initial methyl-ketones and secondary alcohols as determined by the proposed method and the percentage recovery values. Mean recovery calculated according to the formula given by Ulmann's Encyclopedia (24) was in the range of 80.3–88.6% for methyl-ketones (with a mean value of 84.3%) and 81.3–85.4% for secondary alcohols (with a mean value of 83.6%). Mean recovery values for methyl-ketones and secondary alcohols are quite similar, perhaps due to the approach in the vapor pressure for the volatility of those compounds.

De Haast et al. (25), applying a static headspace GC analysis, reported comparable recoveries for volatile organic compounds (2-pentanone, 2-heptanone, 2-nonanone, and 2-pentanol) from aqueous solutions of milk and coagulated milk, demonstrating that recoveries were situated in a similar order of magnitude for milk and coagulated milk. Ulberth (26), applying a standard addition method in milk with varying fat content, demonstrated that the fat did not significantly affect the results of headspace analysis for volatile organics compounds. This fact possibly suggests that eventual differences in the matrix in milk and fermented milk products do not affect headspace analysis (27).

Conclusion

The direct headspace GC method coupled with an MS detector at a constant heating temperature assayed here seems usable for the quantitative determination of methyl-ketones and secondary alcohols in blue cheese, and it will be a suitable method for the routine analysis of these carbonyl compounds. The application of the response factors evaluated in this study can also be used to determine with greater accuracy the concentration of the volatile organic compounds in blue cheese.

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