FAM — INFORMATION

Département de l'intérieur
et de la santé publique

Laboratoire cantonal
vaudois
CH-1066 Epalinges

Dairy Research
Institute
CH-3097 Liebefeld-Bern

October 1990/209 W

Comparison of four extraction, concentration and injection techniques for volatile compounds analysis by GC-MS: an application to the study of the volatile flavour of Swiss Emmentaler cheese

B. Klein, R. Gauch and J.-O. Bosset

In: Flavour Science and Technology
Edited by Y. Bessière and A. F. Thomas
(Flavour 90 - 6th Weurman Symposium)
John Wiley & Sons, Chichester 1990

1 Introduction

Numerous techniques have been proposed for the extraction, concentration and GC-injection of volatile components of food products [1],[2], and especially of dairy products such as cheese. They can be divided into five main groups :

i) Headspace techniques, in which the analysed sample is a part of the gas phase containing volatiles released by food. In their classical static form, these techniques are limited to the most volatile components. The detection of flavor compounds depends strongly on their concentration and vapor pressure, as well as on the temperature and matrix of the food product. To obtain more concentrated extracts, dynamic headspace methods have been developped, in which the volatile components of the gas phase are continuously concentrated in a cold trap or adsorbed onto an inert support, and recovered either by thermal desorption or by elution with a suitable solvent.

ii) Steam distillation methods, which are combined with a continuous solvent extraction such as the Likens-Nickerson technique, or produce an aqueous extract which has to be concentrated by liquid/liquid partitionning or by cryo-concentration. These widely used and very popular techniques suffer from several problems, namely, low recoveries of very volatile components and/or masking of them by the chromatographic peak of the solvent, sample contamination by the solvent, and possible thermal degradation of labile compounds if the steam distillation is not carried out under reduced pressure.

iii) High-vacuum distillation methods, which are variants of the second group. They produce small volumes of concentrated aqueous extracts which are in turn extracted with organic solvents. These techniques prevent thermal degradations occuring by working at ambient or even sub-ambient temperature, but they usually require large amounts of sample and are very time-consuming.

iv) Direct extraction methods, in which an extract is obtained by liquid/liquid or liquid/solid partitionning. They are generally very rapid, but limited to samples with a very low fat content. Acetonitrile can be used to extract cheese flavors, due to its inability to dissolve triglycerides. However, the relatively high boiling point of this solvent (78°C) causes important losses of volatile components and masks several peaks in the chromatogram.

v) Supercritical fluid extraction methods, which avoid the problems of extract concentration, but are also limited in their application to samples with a low fat content, because of its simultaneous extraction with the volatile compounds. In the case of cheese samples, a SFE-SFC coupling seems at the present time to be the only possible way to make use of this promising technique [3].

2 Methods

2.1 SAMPLES

Analysed samples were obtained from a single ripe emmentaler swiss cheese loaf (approximately 1 year ripening), divided into two parts : the external zone consisting of the rind and 1 cm of associated cheese, and the central zone consisting of the remainder of the cheese. Both samples were finely grated, thoroughly mixed and stored at -20°C before use.

2.2 EXTRACTION, CONCENTRATION AND INJECTION TECHNIQUES

The four techniques compared in this work have already been published. They have been used as indicated in the original papers, with the following exceptions :

a) High-vacuum distillation ("Tower") [4], using 1.8 kg of grated cheese. The aqueous distillate was adjusted to pH 10.5, extracted with ether, concentrated on a Dufton column and injected in the split mode.

b) Steam distillation ("Rotavapor") [5], with 250 g of grated cheese dispersed in 250 ml of distilled water. The trap corresponding to pos. 9 of fig. 1 in the original work was empty and the resulting condensate was treated as described under a).

c) Dynamic headspace extraction ("Rektorik") [6], using the MWS-1 system from Rektorik, Geneva, CH, which traps the volatile compounds on graphite powder and desorbs them by ultra-fast microwave heating. In this technique, 25 g of cheese were suspended in 50 ml distilled water, and pH was adjusted to 7.5. The headspace volume aspirated through the graphite trap was 40 x 50 ml.

d) Purge & trap extraction followed by cryo-focussing ("Purge & trap") [7], using a Tekmar LSC-2000 purge & trap system and a Tekmar cryo-focussing unit. 3.5 g grated cheese were suspended in 3.5 ml distilled water, the pH was adjusted to 7.5, and the resulting slurry was poured in a 25 ml fritted disk sparger. Operating conditions were the following : purge gas He at 20 ml/min, trap Supelco # 2-0293 (1 cm 3% SP-2100 on Chromosorb + 23 cm Tenax TA), 0.75 min prepurge, 1 min preheat at 60°C, 15 min purge at 60°C, 11 min dry purge, 1min inject from -100°C to 200°C, 10 min bake at 210°C.

2.3 GAS CHROMATOGRAPHY AND MASS SPECTROMETRIC DETECTION

Gas chromatograph : Hewlett-Packard 5890. Column : J&W DB-WAX 60m x 0.32 mm i.d., df = 0.25 µm. Carrier gas flow : 0.71 ml He/min, inlet pressure 150 kPa. Injected volumes : 1 µl, split ratio 1:27 (for a, b and c). Injector temperature : 200°C (for a, b and c). Temperature program : 13 min at 45°C, heating to 220°C at 5 °C/min, and 10 min at 220°C. Detection : Hewlett-Packard mass-sensitive detector (MSD model 5970), working in the scan mode from 19 to 250 amu at 1.85 scan/s, ionization by EI at 70eV and 0.8 mA.

3 Results and discussion

Fig. 1 shows the chromatograms obtained under identical conditions with the four investigated methods, with ordinate expansion chosen to obtain the same baseline noise. One can see important differences that can be explained, among other factors, by differences in polarity, vapor pressure, solubility and partition coefficients of the volatile components in the various phases coexisting during the extraction and concentration steps.

158 volatile components have been identified : 31 hydrocarbons, 26 alcools, 21 aldehydes, 27 ketones, 5 ethers, 6 acids and 1 phenol, 22 esters, 4 lactones, 6 pyrazines, 2 sulfur compounds and 7 contaminants. Their occurence in cheese will be discussed in a

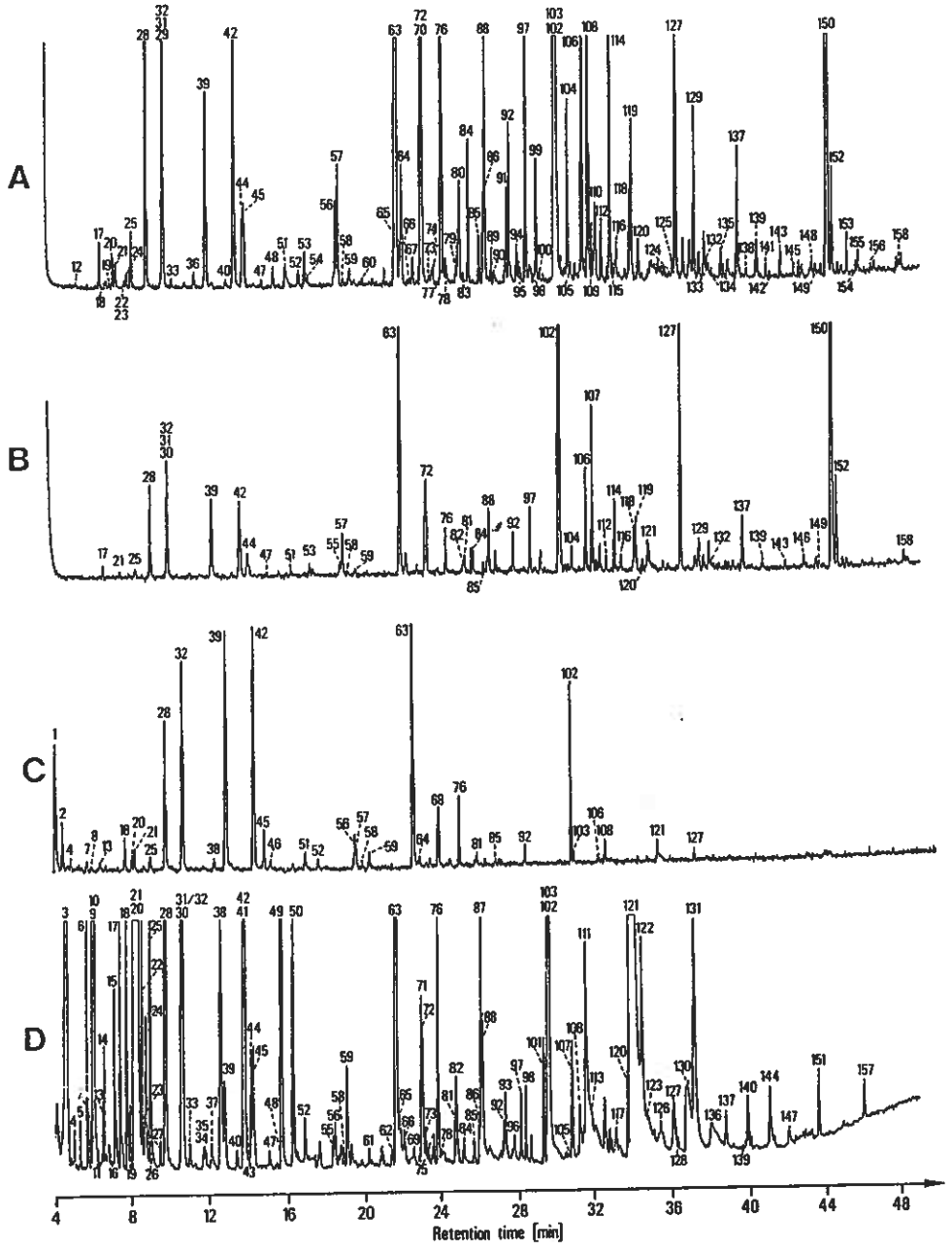

Fig. 1. Chromatograms obtained using four different extraction, concentration and injection methods for analysis of the volatile flavor of cheese. A: High-vacuum distillation. B: Steam distillation. C: Rektorik purge & trap extraction. D: Tekmar purge & trap extraction, followed by cryo- focussing.

forthcoming publication. The yield of the various extraction, concentration and injection techniques investigated is strongly dependent on the chemical class of the flavor compounds.

Fig. 2 shows the cumulated number of identified compounds as a function of the retention time. The Tekmar purge & trap system (d) is very efficient, especially for compounds with low (< 18 min) and middle (18 - 35 min) retention times. The tower extraction (a) gives the highest number of compounds, especially those with middle and long (> 35 min) retention times. The Rektorik MSW-1 system (c) is less efficient and is useful only for compounds with low to middle retention times. The Rotavapor method (b) also performs poorly, but it is not too selective with regard to the retention times of the extracted compounds.

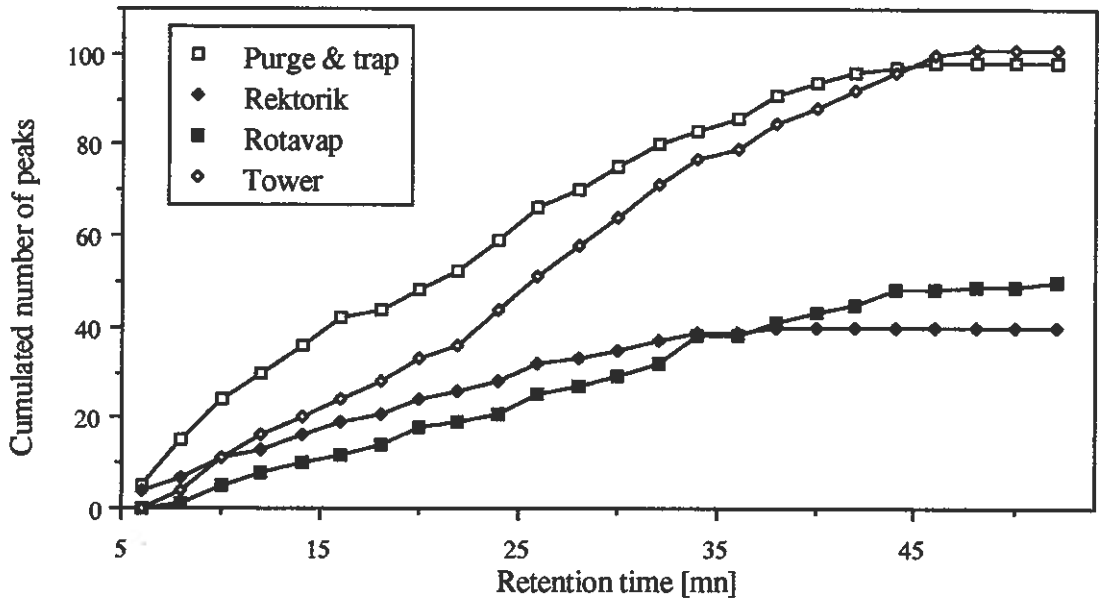

Fig. 2. Cumulated number of identified compounds vs. retention time

4 Conclusion

There exists no ideal method for the extraction, concentration and injection of volatile flavor components of cheese : all the investigated methods have advantages and drawbacks. In practice, the Tekmar purge & trap method is the most interesting, especially with regard to the small sample size (3.5 g), the analysis time (90 min, including sample preparation) and the large number of compounds obtained (98 peaks). Such a technique is suitable both for routine and for research work. The tower extraction technique results in the detection of slightly more compounds (102 peaks), but requires a very large sample size (1.8 kg) and is very time consuming (2-3 days). It can therefore be applied to research work only. The Rotavapor method is a good compromise with respect to sample size (250 g) and analysis time (3-4 hours), and can be used as a routine tool, especially if the compounds to be analysed are of relatively low polarity and low volatility. The Rektorik MSW-1 method works under very drastic desorption conditions (T = 400°C) and is limited to graphite and activated charcoal as adsorbents. Moreover, it includes no cryo-focussing step between the desorption and the injection. Its only advantage is that the traps can be easily loaded at distant location before analysis in the laboratory. It can be used as a routine tool, but detects few compounds, most of them with high volatilities.

5 References

1. C. Weurman, J. Agric. Food Chem. 17 370 (1969)
2. G.A. Reineccius and S. Anandaraman in Food Constituents and Food Residues, ed. by J.F. Lawrence, Marcel Dekker Inc., New York and Basel (1984), p. 195
3. W. Gmür, Ph. D. Thesis no 655, EPF - Lausanne (1986)
4. R. Liardon, J.-O. Bosset and B. Blanc, Lebensm.-Wiss. Technol. 15 143 (1982)
5. R. Imhof and J.-O. Bosset, Mitt. Gebiete Lebensm. Hyg. 88 409 (1989)
6. J.-O. Bosset and R. Gauch, J. Chromatogr. 456 417 (1988)
7. R.G. Westendorf, Am. Lab. (Fairfield) 17 72 (1985)