Volatile Trace Components in Gouda Cheese

Volatile components of Gouda cheese were isolated by low-temperature vacuum distillation after which they were divided into polar and nonpolar parts by solvent extraction. The nonpolar part was separated into fractions—containing higher levels of the minor components—by reversed phase adsorption chromatography on Amberlite XAD-2. The polar extract and some of the nonpolar extract fractions were analyzed by combined gas chromatography and mass spectrometry. The following trace components were detected for the first time in Gouda cheese: *p*-(1-propenyl)anisole (anethole), bis(methylthio)methane, and six alkylpyrazines.

The volatiles of a cheese flavor extract consist—apart from a large amount of fatty acids—also of small amounts of nonacidic components. In different types of cheese, a great number of neutral components have already been identified by combined gas chromatography and mass spectrometry (gc-ms); see, *e.g.*, the investigation of Blue cheese (Day and Anderson, 1965), Swiss cheese (Langler *et al.*, 1967), and Cheddar cheese (Liebich *et al.*, 1970b). Generally, there are qualitative and significant quantitative differences in the composition of the volatiles of different types of cheese (Liebich *et al.*, 1970a). Furthermore, it has been assumed that some trace components contribute significantly to the typical character of a cheese flavor. For the identification of trace components, pre-separations of the isolated compounds are necessary. To this end, an adsorbent has to be used on which no formation of artifacts or too strong an adsorption will take place in order to prevent loss of minor components. Naipawer *et al.* (1971) reported the use of Amberlite XAD-2—a cross-linked styrene-divinylbenzene copolymer—as an adsorbent to concentrate flavor components from aqueous solutions.

This paper reports the suitability of the reversed-phase adsorption Amberlite XAD-2 column for separating volatile flavor components in a nonaqueous medium and the identification of trace components in fractions of Gouda cheese volatiles.

EXPERIMENTAL SECTION

Materials. Nine- to twelve-month-old Gouda cheese of high quality was used. The Amberlite XAD-2 resin (20–50 mesh, ex Rohm and Haas Co.) was freed from its odor by wetting with methanol, washing five times with water, extraction with methanol in a Soxhlet apparatus for 16 hr, and vacuum drying in a rotary evaporator. The resin was then ground and sieved; the particles that passed through a DIN 40 screen (>100 mesh) were used for chromatography. The solvents were analytical grade.

Isolation of the Cheese Volatiles. Gouda cheese was ground and isopentane and water were added in the ratio cheese-water-isopentane 120:50:25 (w/v/v). The cheese slurry was placed in the round-bottomed flask of a bulb-to-bulb distillation apparatus and the volatile components were distilled off at 20° and trapped at −196° at 0.05 mm (6.66 Pa) for 18 hr. After extracting the distillate with isopentane, the extract was deacidified and concentrated by evaporating the solvent on a 12-cm Vigreux column. More polar substances were isolated by further extraction of the distillate with dichloromethane. After deacidification of this extract, it was concentrated at −20° under reduced pressure. The deacidifications of the isopentane and the dichloromethane extracts were carried out by shaking twice with a saturated sodium carbonate solution (100:2, v/v).

Column Chromatography. *Preparation of the Column.* The resin was stirred in a beaker filled with methanol and, after settling, the supernatant was discarded to remove too fine particles; this procedure was repeated three times. Then the slurry of the resin was poured into a glass column to provide a resin bed of 430 × 6 mm. Before the sample was brought onto the column, a 2-cm layer of Celite slurried in methanol was placed on top of the layer to prevent disturbance of the surface.

Model Compounds. The test mixture contained 0.2 mg/20 μl of pentane of each of the following compounds: hexanoic acid, 2-heptanol, 2-heptanone, ethyl hexanoate, and toluene. Elution was performed with methanol at a flow rate of 0.2 ml/min. Fractions of 0.5 ml were collected and each fraction was analyzed by gc on a 4 m × 4 mm 5% Carbowax 20M TPA column.

Cheese Flavor Extract. The deacidified isopentane concentrate of the Gouda cheese volatiles, obtained from 300 g of cheese, was brought onto the Amberlite XAD-2 column. Forty-five fractions of 1 ml were collected. Five chromatographic runs were performed using the same column and the corresponding fractions were combined for further analysis. To introduce the eluted compounds in a more volatile solvent, the methanol fractions were after dilution with two parts of water extracted with pentane. The odors of the fractions were evaluated by smelling, using a small glass rod. For further analysis, the fractions were concentrated by evaporating the pentane on a 12-cm Vigreux column.

Gas Chromatography and Odor Evaluation. A Varian Aerograph 2100 equipped with an effluent splitter and a flame ionization detector was used. Part of the dichloromethane concentrate was analyzed under the following conditions: glass column (length 2 m, 4 mm i.d.) packed with 10% Carbowax 20M on 60–80 mesh Chromosorb W, acid washed. Parts of the Amberlite column fractions were chromatographed on a glass column (length 4 m, 4 mm i.d.) packed with 5% Carbowax 20M TPA on 60–80 mesh Chromosorb W, acid washed; temperature program, 60–220° at 2°/min; injection block, 225°; detector, 225°. The effluent vapors were evaluated by sniffing at the outlet of the gas chromatograph.

Gas Chromatography–Mass Spectrometry. A combination of a Becker 1425 DPT gas chromatograph with flame ionization detector and an AEI MS 12 mass spectrometer was used. The concentrates and columns were similar to those mentioned above. The column temperature was programmed from 50 to 180° at a rate of 1°/min. The unknown compounds were identified by comparing the mass spectra with those of authentic compounds.

RESULTS AND DISCUSSION

Amberlite XAD-2 Column. Apart from some overlappings, all model compounds were separated from each other on the Amberlite XAD-2 column (Table I). We observed that aliphatic and aromatic hydrocarbons occur in a great variety in volatile cheese extracts, like in many flavor concentrates (Johnson *et al.*, 1969) which disturb the gc-ms analysis of trace components in a total flavor extract. The Amberlite XAD-2 resin is particularly suitable for removing these hydrocarbons. The neutral character of the resin prevents changing less stable com-

Table I. Chromatography of Model Substances on Amberlite XAD 2 (Column, 430 × 6 mm; Eluent, Methanol)

Fraction no.	Amount, ml	Compd detected by gc
1–18	9.0	Blank
19	0.5	Hexanoic acid, 2-heptanol
20–23	2.0	2-Heptanol
24,25	1.0	Blank
26,27	1.0	2-Heptanone
28–30	1.5	2-Heptanone, ethyl hexanoate
31–39	4.5	Ethyl hexanoate
40,41	1.0	Blank
42–68	14.0	Toluene
69–88	10.0	Blank

pounds, and because the compounds are separated in reversed order, loss of polar components by too strong an adsorption is also avoided. Separations obtained with the Amberlite column are highly reproducible. The same column can be used several times.

Cheese Volatiles. Of the fractions collected from the Amberlite column, fraction 14 possessed a strong odor, which suggested a sulfur-containing compound. During gc of this fraction, the same odor was recognized, corresponding to a very small peak situated between those of ethyl hexanoate and propyl hexanoate. On gc–ms analysis of fraction 14, a clear mass spectrum was obtained of this peak. Its molecular weight was 108 and in view of the isotope ratios it was likely that the compound contained two sulfur atoms (Figure 1). After comparison with reference mass spectra it was concluded that this compound could only be bis(methylthio)methane. This was confirmed by comparison of the retention time and the mass spectrum of synthesized bis(methylthio)methane which was prepared starting from formaldehyde and methanethiol (Böhme and Marx, 1941). Using a very small concentration of the synthesized product the typical odor of the sulfur compound perceived in the cheese flavor fractions could be reproduced.

Besides fatty acid esters and methyl ketones, a series of alkylpyrazines was detected in fractions 19 and 20. The following pyrazines were identified: tetramethylpyrazine, 2,5(or 2,6)-diethyl-3-methylpyrazine, and tentatively dimethylpyrazine, ethylmethylpyrazine, and 3-ethyl-2,6(or 2,5)-dimethylpyrazine. Owing to their small amounts, the question as to which isomer is present could not be elucidated.

Alkylpyrazines were already found in Emmental cheese (Sloot and Hofman, 1975) but the total content of pyrazines in Gouda cheese is likely to be lower than in Emmental cheese.

On gc–ms analysis of the deacidified dichloromethane extract, a new trace component was found. The mass spectrum of this compound was identical with that of 1-methoxy-4-propenylbenzene. As no information was obtained about the position of the double bond in the propenyl group, it was not clear whether the substance was anethole (*p*-(1-propenyl)anisole) or estragole (*p*-allylanisole). The gc retention times of these two compounds on a Carbowax 20M column, however, are quite different. By measuring the retention times of anethole, estragole, and the isolated compound relative to the retention time of acetophenone—one of the identified cheese flavor components—it appeared that the isolated compound had the same relative retention time as anethole.

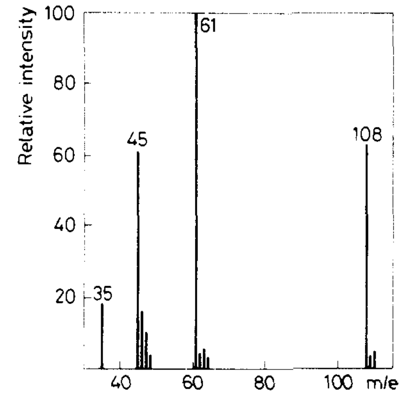

Figure 1. Mass spectrum of bis(methylthio)methane.

As far as we know bis(methylthio)methane and anethole have not been reported previously as components in any type of cheese. This is likely to be due to their very low concentration in cheese. Bis(methylthio)methane has been identified by Fiecchi *et al.* (1967) as an odorous principle of the white truffle *Tuber magnatum pico*. The compound can be considered as a dimethyl thioacetal of formaldehyde and methanethiol. The latter two compounds are known constituents of cheese. Like most types of sulfur-containing volatiles, bis(methylthio)methane has a very strong odor. Solutions of 0.003 mg/kg in vegetable oil and 0.0003 mg/kg in water could still be distinguished from a blank by a flavor panel. Since anethole and bis(methylthio)methane are considered to be important odor components of Gouda cheese patents have been filed concerning their use as flavoring agents.

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