Analysis of Odor-Active Volatiles in Cheddar Cheese Headspace by Multidimensional GC/MS/Sniffing

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Volatile compounds were isolated from Cheddar cheese headspace by purge and trap extraction, enriched by cryofocusing, and analyzed by multidimensional GC equipped with odor port and by GC/MS. In total, 5 aldehydes, 6 ketones, 8 alcohols, 3 esters, 11 hydrocarbons, 3 halides, and 3 sulfur compounds were positively identified. Ethanol, 2,3-butanedione, and 3-hydroxy-2-butanone were the major components isolated in cheese headspace. The paper describes the aroma characteristics of some of these volatiles and their impact on the overall Cheddar flavor. Esters, aldehydes, methyl ketones, and sulfur compounds had direct impact on Cheddar aroma by virtue of their characteristic aroma. On the other hand, alcohols and hydrocarbons formed the major portion of the GC profile but contributed little to the aroma.

Keywords: *Headspace analysis; purge and trap; GC/MS; odor-port evaluation; Cheddar cheese aroma; flavor volatiles*

INTRODUCTION

Aroma perception is one of the foremost criteria of a cheese grader for product evaluation and of a consumer for its acceptance and preference. To unfold the complex nature of Cheddar cheese flavor, early research was focused on flavor profiling (Manning and Robinson, 1973; Sandine and Elliker, 1970) and the study of volatile formation during ripening (Adda, 1984; Law, 1984). GC/MS analysis of volatile and nonvolatile cheese extracts revealed the presence of over 180 such compounds in the cheese matrix, though none of these compounds, by itself, represented the characteristic aroma of Cheddar. The widely accepted component balance theory states that the flavor of Cheddar is the outcome of a synergistic odor effect of the right blend of some of these compounds in a balanced proportion. Sulfur compounds, methyl ketones, aldehydes, esters, alcohols, lactones, and free fatty acids that are generated from numerous sequential and simultaneous biochemical reactions involving milk lipids and proteins form the principal components of Cheddar aroma (Moskowitz and Noelck, 1987).

Previous research on flavor profiling of solvent extracts (Biede and Hammond, 1979; Wong and Park, 1968) and steam distillates (Banks *et al.*, 1992; Lin and Jeon, 1985) of cheese samples provided an extensive list of compounds in Cheddar flavor. Further, attempts were made to find ways to obtain a representative sample of cheese aroma with a reduced number of compounds and without sacrificing the characteristic Cheddar aroma. McGugan (1975) partitioned the extract into volatile and nonvolatile fractions and observed that while the volatile fraction had an important bearing on aroma, the nonvolatile fraction imparted background taste. Manning and Robinson (1973) isolated a cheese distillate fraction containing low molecular weight volatile compounds which had a typical Cheddar flavor. Banks *et al.* (1992) analyzed steam distillates of many commercial Cheddar cheese samples and found good correlation between their sensory attributes and the principal volatiles detected. Horwood (1989) described a headspace sampling technique to obtain a more representative sample of cheese aroma as perceived by consumer's olfactory senses and detected 28 low molecular weight volatile compounds in Cheddar aroma. Recently, Yang and Min (1993) and Wood *et al.* (1994) attempted to develop a more reproducible and quantitative method to analyze headspace volatiles of Cheddar cheese.

In spite of extensive research on Cheddar cheese flavor, formulating or even regulating the production of Cheddar aroma remains a difficult task due to lack of knowledge of flavor compounds and their contribution to the overall aroma. The first step in the control of flavor biogeneration lies in the identification and aroma intensity of individual aromatic compounds. In the past various workers have designed techniques for olfactory analysis of gas effluent. These techniques are mainly based on threshold levels of odor-active compounds (Roth and Thomas, 1963), relative odor potency of compounds in an extract (Acree *et al.*, 1984), and direct measurement of odor description and intensity (Miranda-Lopez *et al.*, 1992). In the present investigation, volatiles from progressively heated cheese samples were purged into a Tenax trap by a dynamic headspace technique, concentrated by cryofocusing, heat-desorbed onto the gas chromatographic column, and analyzed by multidimensional GC/MS. The olfactory analysis of the separated individual compounds was performed by directing half of the gas effluent of GC column to the odor port. This study on the analysis of headspace volatiles will provide a better understanding on the formation of cheese aroma. The paper also describes the odor profile of those volatiles and their relative significance to the characteristic aroma of Cheddar.

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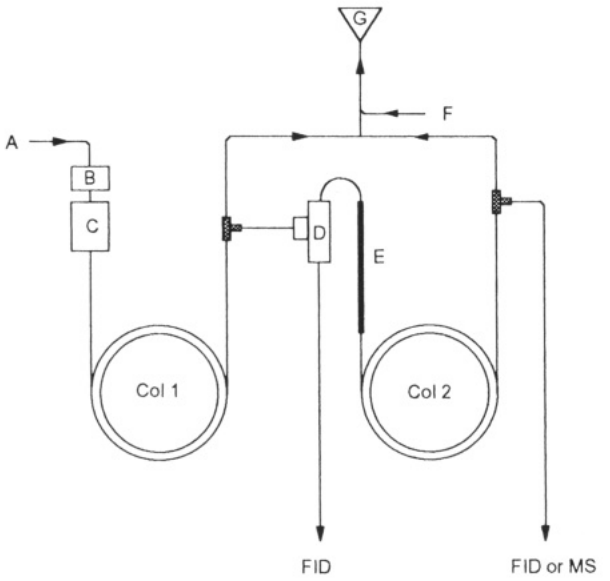

Figure 1. Schematic of multidimensional GC/MS/sniffing connections: (A) concentrated volatiles from desorber; (B) cryofocusing unit; (C) GC injector; (D) heart-cutting switch; (E) cryofocusing column; (F) humidified air; (G) odor port.

MATERIALS AND METHODS

Purge and Trap Extraction. Cheese samples were drawn from a 25 lb Cheddar cheese block matured for one year. Five grams of cheese sample and 3.5 g of sodium sulfate were ground with the mortar and pestle, transferred into a glass test tube, and stored at −20 °C. Frozen samples in the test tubes were ground to powdered form and prepurged with 40 mL/min helium gas at 30 °C for 15 min. The test tubes were heated to 70 °C over a period of 15 min and maintained at 70 °C for 5 min. The volatile compounds emitted under these conditions were swept onto a stainless steel trap (4.5 mm i.d. × 105 mm long) containing Tenax TA 60/80 mesh (Supelco Inc., Toronto, ON). The traps were stored overnight in a desiccator at 4 °C and, prior to desorption, purged with helium gas (50−60 mL) to remove residual moisture. Desorption and enrichment of trapped volatiles was carried out with a Tekmar Model GT-5010 (Tekmar Co., Cincinnati, OH) desorber under the following conditions. The traps were heated rapidly to 110 °C for 8 min, and the volatiles were enriched onto a glass-lined stainless steel tube in the desorber by cryofocusing at −150 °C with liquid nitrogen. The volatiles were transferred onto the second cryofocusing unit by heating (110 °C for 3 min) and refocused with liquid nitrogen at −150 °C (part B of Figure 1). The sample was injected into the column by heating the cryofocusing unit at 110 °C. Tenax traps after each run were reconditioned overnight at 225 °C in a stream of helium (80 mL/min) and stored in a desiccator at 4 °C. Blank GC runs were conducted with the reconditioned traps before their use in flavor analysis.

Gas Chromatography. Separation of volatiles was performed using a multidimensional GC Model 8700 (Perkin-Elmer Canada Inc., Montreal, PQ) equipped with a nonpolar (DB-1) followed by a polar (DB-Wax) megabore chromatographic column (0.75 mm i.d. × 60 m long each; Figure 1). Helium carrier gas was allowed to flow to the GC injector through the Tekmar desorber at 20 psi. Following the injection of the sample, the oven temperature was kept at 40 °C for 7 min and then heated to 130 °C at a rate of 3 °C/min. The column was held at 130 °C until no more components eluted. The effluent at the exit of each column was split at a 50:50 ratio so that half of the sample was directed to an odor port while the other half was directed to either a flame ionization detector or to a mass spectrometer. The odor port was supplied with a stream of humidified air at 100 mL/min (Figure 1). Three chromatographic runs were made with three individual replicate samples of the same cheese block in separate test tubes. The odor evaluation of each GC run was carried out in two successive sniffing sessions of around 20 min each with two different panelists. Panelists were asked to assess the odor characteristic and intensity (on an ordinal scale of 0−5) of each peak detected (Cormier *et al.*, 1991).

Gas Chromatography/Mass Spectrometry. The compounds separated by the two GC columns were identified by coupling the exit of effluent splitter to a quadrupole mass spectrometer model 9000 (Perkin-Elmer). Mass spectrometric analysis was carried out with the following operating conditions: ionization voltage, 70 eV; source temperature, 200 °C; multiplier voltage, 1050 V; scan range, 30−300 *m/z*; scanning speed, 5 scans/ms. Compounds were identified by comparing their retention times with pure standard compounds (Omega Chemicals, Quebec, PQ) under identical operating conditions of both GC columns and/or by matching their mass spectra with on-line NIST library of standard compounds.

RESULTS AND DISCUSSION

Preliminary trials on flavor analysis showed the presence of excess moisture in the trapped volatiles which resulted in poor resolution by GC and very high mass spectral background. This problem was resolved by desiccating the filled traps overnight in a desiccator at 4 °C, prepurging the sample-filled Tenax trap with helium for 5 min before injection, and using a nonpolar column, *i.e.* DB-1 in the first stage of GC separation. In addition, the chromatograms showed the presence of high levels of very low molecular weight compounds which originated from thermal degradation of labile volatiles during sample purging and desorption. This caused a high GC background profile and continuous odor background during sniffing trials. The problem was overcome by initial purging of the cheese sample at 30 °C, followed by slow heating of the sample to 70 °C. Higher temperatures up to 100 °C did not show the trapping of additional aroma compounds. Relatively lower desorption temperatures (110 °C) and isothermal GC separation at 40 °C for 7 min further minimized thermal degradation which was evident from GC chromatogram as well as odor detection.

By standardizing sample size, conditions of extraction and enrichment, and GC separation, it was possible to make comparison between GC, MS, and odor profile results of Cheddar volatiles. Certain trace compounds (below MS detection limits) but with detectable aroma profiles were identified by carrying out chromatographic separation of the volatiles from several identical traps, followed by heart-cutting of the desired peak (Bicchi and Joulain, 1990). The desired peak from different runs was stored in a cryofocusing column (part E of Figure 1), and the accumulated fraction was then analyzed on a second GC column (DB-Wax). Table 1 lists the peak numbers, retention times, compounds identified, and their odor evaluation. Most of the components detected by GC (Figure 2) were also identified. In total, 41 positive identifications were made by comparing their retention times with known standards and/or by GC/MS. The major components of 1 year old Cheddar aroma were eluted at 13.80 (peak 11), 17.37 (peak 17), and 32.49 (peak 40) min and were identified as ethanol, 2,3-butanedione, and 3-hydroxy-2-butanone, respectively. These results were different from the headspace profiles of 9 week old Cheddar cheese, which indicated the predominance of 2-butanone and 2-butanol (Yang and Min, 1993). Some of the lower concentration and longer retained components were not identified because of higher MS background and/or structural degradation of those compounds at the higher temperature of separation.

Table 1. Volatile Flavor Compounds Identified in the Headspace of Cheddar Cheese

peak[a]	compound	retention time (min)	odor description[b]
1	butane	7.68	
2	2-methylbutane	9.41	
3	pentane	9.75	
4	1,1,2-trichloro-1,2,2-trifluoroethane[c]	10.16	
5	propanal	10.91	
6	pentane	11.19	
7	ni[d]	11.52	
8	dimethyl sulfide	12.08	pomegranate
9	carbon disulfide[c]	12.50	
10	2-propanone	2.75	wood pulp, hay
11	ethanol	13.80	dry, dust
12	ethanol	14.36	dry
13	2-propanol	15.13	fruity
14	dichloromethane[c]	15.67	
15	2-butanone	16.58	butterscotch
16	ethyl acetate	17.01	fruity, pineapple
17	2,3-butanedione	17.37	cheesy, caramel
18	hexane	17.75	
19	1-propanol	18.40	sweet (candy)
20	2-methyl-1-propanol	18.81	plastic, bad
21	pentanal	19.32	chemical
22	trichloromethane	20.04	hay
23	2-pentanone	20.61	orange peel
24	ni	21.01	
25	2-butanol	21.50	
26	ni	22.00	
27	ni	22.66	
28	1-butanol	23.25	
29	2-methylpropanal	23.78	floral
30	ni	24.33	
31	dimethyl disulfide	26.00	
32	toluene	26.80	nutty, bitter almond
33	ni	27.50	
34	2,4-dimethylpentane[c]	27.91	
35	ethyl butanoate	29.02	pleasant, green fruit
36	2-methylbutanal	29.54	slightly caramel, nutty
37	hexanal	30.10	slightly fruity (balsam)
38	1-pentanol	30.57	fruity
39	ni	31.58	
40	3-hydroxy-2-butanone	32.49	sour milk
41	ni	35.25	
42	ni	36.50	
43	ni	36.83	
44	ni	37.16	
45	2-heptanone	39.75	
46	heptanal	41.00	soapy
47	ni	42.16	
48	2,4-dimethylheptane[c]	43.53	
49	acetic acid	44.69	
50	ethyl hexanoate	45.44	young cheese
51	ni	46.08	
52	ni	46.36	sulfur
53	ni	47.77	rotten mushroom
54	dodecane[d]	48.29	dirt
55	ni	48.75	salty beef broth
56	undecane	49.33	
57	2,5-dimethylheptane[c]	50.13	
58	ni	51.25	tobacco
59	ni	52.27	sulfurous
60	ni	52.99	

[a] Numbering of peaks as shown in Figure 2. [b] Compounds need not be responsible for the observed odor. [c] ni, not identified. [d] Compounds identified from GC/MS only. All other compounds were identified by GC retention time of corresponding standard compounds and by mass spectra (GC/MS).

The aroma description and intensity for the isolated components are listed in Table 1 and Figure 2. Although evaluators differed on terminology, they were able to recognize the different aroma-bearing components of the sample. Data included in this publication list only those aromas that were reported in at least two of three evaluations and were of intensity equal to or higher than 0.5 on the scale of 0 (none) to 5 (extreme). The average values of odor intensity detected under the conditions of the experiment are shown in Figure 2. Odor-port assembly permitted assignment of the intensity and description quality of different odors to the respective peaks. In all, 27 odor-active compounds were detected during sniffing. Although two successive columns provided sufficient resolution among peaks, in many cases, odor descriptions associated with some of the separated components were not typical of the characteristic aroma normally associated with pure compounds (peaks 11, 32, and 54). This could be attributed to coelution of trace components and/or carryover from previous eluting components. The problem was partly overcome by directing only a selected portion of the GC profile obtained from the first column to the second GC column with the help of a heart-cutting device and cryofocusing unit (parts D and E of Figure 1).

Cheddar aroma was primarily composed of low molecular weight alcohols, aldehydes, ketones, hydrocarbons, esters, and sulfur and halogen compounds, which are typical of the flavor profile of Cheddar (Moskowitz and Noelck, 1987). The headspace profile did not show the presence of hydrogen sulfide and methional, which has been closely related to good Cheddar flavor (Manning and Price, 1977). Absence of sulfur compounds in the cheese headspace chromatogram also was reported by Horwood (1989) and was attributed to poor retention of these compounds by the trap adsorbent material (Porapak Q). However, by using Tenax as adsorbing material, we could detect the presence of carbon sulfide, dimethyl sulfide, and dimethyl disulfide which are considered to be originated from methional (Parliment *et al.*, 1982). Another headspace study based on direct cryofocusing of volatiles also failed to detect hydrogen sulfide and methional and proposed them to be lost during cheese maturation or during sample preparation (Wood *et al.*, 1994).

In total, eight different alcohols were detected in the gas chromatogram of cheese volatiles. Ethanol was the major alcohol component formed as a common end product in the breakdown of glucose. The other alcohols from C_3 to C_8 are produced mainly by the reduction of their corresponding aldehydes and/or ketones. Alcohols have little influence on cheese flavor; however, they may indirectly contribute to flavor because of their ability to form esters with fatty acids. The aliphatic hydrocarbons are not important for aroma, though they may act as precursors of a number of other aroma compounds. The bitter almond note observed in peak 32 may be due to another coeluted compound rather than toluene (Table 1). The presence of three halogen compounds was also detected, but none of them seems to have any influence on cheese aroma intensity.

Sensory evaluation profiles showed that some major compounds detected by gas chromatography contributed little to the overall aroma and *vice versa* (Figure 2). Lipid-derived components, such as carbonyl compounds (aldehydes and ketones) and esters, which represented only a small fraction of the GC profile, were major contributors to the total aroma. This is further strengthened from the fact that these components are very volatile in nature at ambient temperatures. Since ethanol is the most abundant alcohol in Cheddar cheese, all fatty acid esters were ethyl derivatives. While most of the esters separated in this experiment had a buttery to fruity aroma, thioesters formed by the reaction of

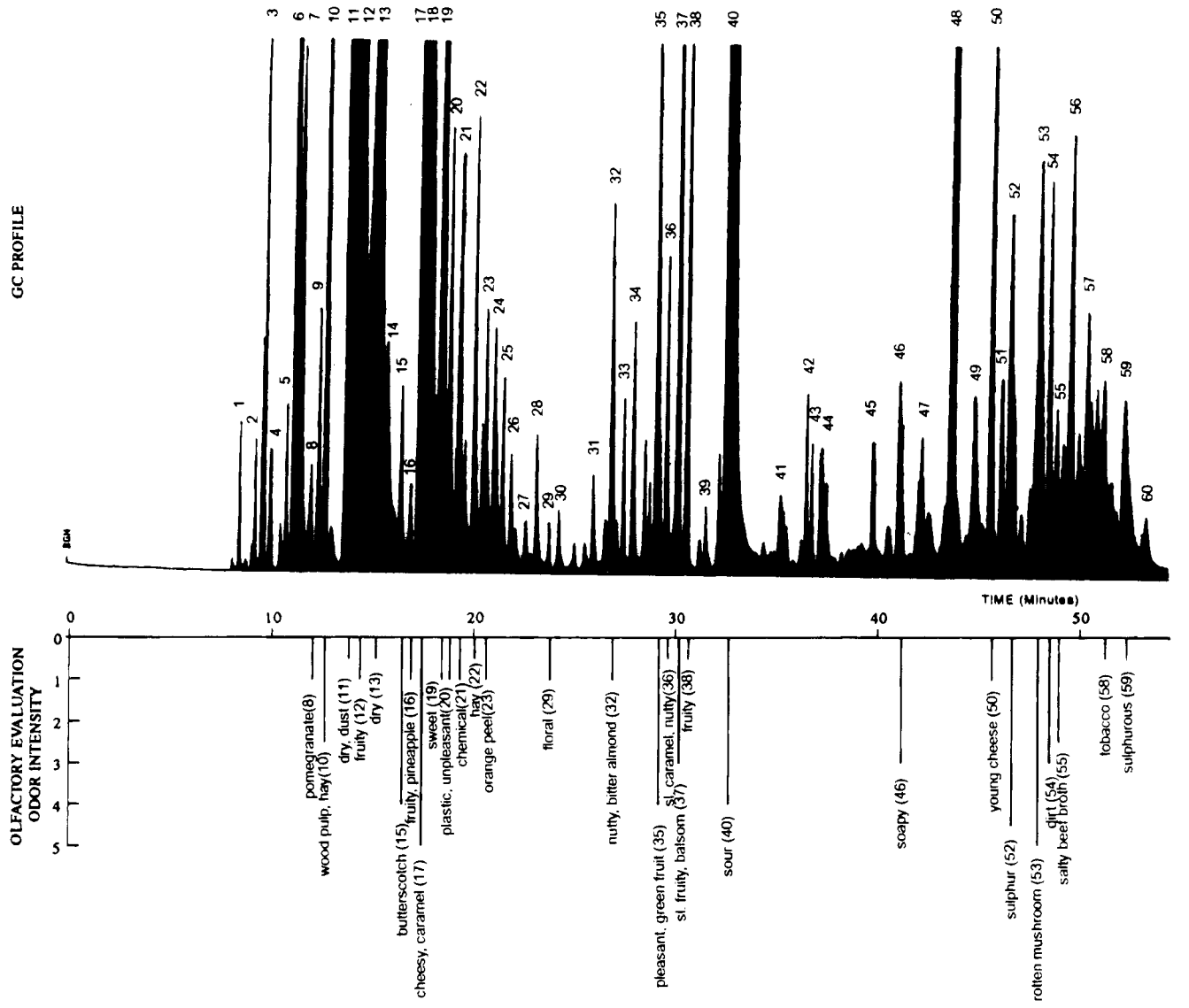

Figure 2. Gas chromatogram and mean value of odor description and intensity at the odor port of headspace volatiles in Cheddar cheese. Numbering of GC profile peaks and odor peaks is as shown in Table 1.

esters of short-chain fatty acids with methional are reported to impart the characteristic cheesy aroma to Cheddar cheese (Law, 1984). In addition to volatile fraction of headspace, solvent extracts of high boiling point fatty compounds are also considered a major contributor of cheese flavor (Biede and Hammond, 1979). However, specific extraction techniques are necessary for their detection. The major influence of lipid-derived components on Cheddar flavor has led researchers to find cheese starter strains with superior esterase activities (Arora *et al.*, 1990) or use esterase enzymes directly for desirable flavor notes during cheese maturation (Kosikowski and Iwasaki, 1975; Lee and Lee, 1990; Moskowitz and Noelck, 1987).

CONCLUSIONS

The experimental design used in this study was an attempt to identify odor-active components from a complex mixture of volatiles present in Cheddar cheese. Combining dynamic headspace sampling, enrichment of volatiles by cryofocusing, and heart-cutting in multidimensional GC provided flexibility in manipulating the concentrations of various components in complex cheese volatile mixtures. At the same time, simultaneous sensory analysis and mass spectrometry detection permitted the assignment of aroma description to odor-active components in the complex mixture of volatiles. However, certain variables in headspace analysis techniques need to be standardized to make the system quantitative. The cheese aroma obtained by this design showed the presence of 60 compounds, of which 27 were odor active. Lipid-derived aldehydes, methyl ketones, and esters were the principal aroma-bearing components present in the volatiles isolate from Cheddar cheese. However, to understand the respective contribution of each aroma-bearing compound to the overall cheese aroma, quantitative studies are needed to relate the concentration of an individual component with respect to its threshold level and also to observe any synergistic effect at the odor port.

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Registry No. Supplied by the Author: 2-Methylbutane, 78-78-4; dichloromethane, 75-09-2; 1,1,1-trichloroethane, 71-55-6; 2,4-dimethylpentane, 108-08-7; 2,2,4-trimethylpentane, 540-84-1; 2,2,3,4-tetramethylpentane, 1186-53-4; 1,1,2-trichloro-1,2,2-trifluoroethane; 2-propanol, 67-63-0; 2-butanol, 78-92-2; 2-butanol (*R*)-, 14898-79-4; 2,3-butanediol, 513-85-9; 2-hexanol, 626-93-7; 2-heptanol 543-49-7; acetonitrile, 75-05-8; 2-heptanone, 110-43-0; 2-butanoic acid, 107-92-6; ethyl butanoate, 105-54-5; ethyl hexanoate, 123-66-0; 2,2,5-trimethyl-3,4-hexanedione, 20633-0308; paramethadione, 115-67-3; ethyl formate, 109-94-4; ethyl acetate, 141-78-6; 1,1-ethanediol diacetate, 542-10-9; 1,2-ethanediol diacetate, 111-55-7; methyl propionate, 554-12-1; carbon disulfide, 75-15-0; dimethyl sulfide, 75-18-3.

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