Comparison of Flavor Isolation Techniques Applied to Cheddar Cheese

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Three methods for the isolation of Cheddar cheese flavor (distillation, dialysis, and solvent extraction) were compared. The solvent extraction (acetonitrile) method gave the least concentrated isolate. It was, however, the fastest and cheapest and gave the most characteristic flavor isolate of the three techniques. Dialysis yielded the most concentrated flavor isolate. The gas chromatographic effluent was sniffed to determined odor character of the volatiles isolated. Many odor-active compounds, some of them in concentrations below the detection threshold of the analytical instrument, contribute to Cheddar cheese flavor. While one component was characterized as being cheesy in character, we feel that Cheddar cheese derives its characteristic flavor from a balance of many components. Four chemicals, 2-propanol, 1,3-butanediol, δ-undecalactone, and γ-decalactone, were tentatively identified in Cheddar cheese for the first time.

INTRODUCTION

After more than 50 years of research, both the origin and the composition of Cheddar cheese flavor remain puzzling questions (Aston and Dulley, 1982). This lack of understanding of Cheddar cheese flavor is a detriment to the cheese manufacturer. However, it may be possible to develop predictive models that will help the cheese manufacturer in this task. For example, a model correlating analytical data (peak areas of a gas chromatographic profile of a Cheddar flavor concentrate) and sensory data may point out the chemicals that are the best predictors of sensory attributes (overall quality or specific defects). This model may help to determine the chemicals that contribute the most to Cheddar cheese flavor. A different model could aim at predicting the gas chromatographic profile (therefore, sensory quality) of a Cheddar cheese when it reaches 6 or 9 months of age from its profile after 3 weeks of ripening. If this model could be established, the manufacturer would be able to reduce production costs by aging only the cheese that will develop a good flavor. Overall quality could also be increased by selling the cheese when it reaches its optimum flavor.

To develop these models, we must first develop techniques that allow the chemist to obtain representative gas chromatographic profiles of Cheddar cheese flavor. The ideal technique would be not only reproducible and sensitive but also simple, inexpensive, and able to process a large number of samples in a short amount of time.

Many methods of obtaining gas chromatographic profiles of Cheddar cheese already exist: steam distillation (Aishima and Nakai, 1987), molecular distillation (Scarpellino and Kosikowski, 1961; Libbey et al., 1963; Day and Libbey, 1964; Morris et al., 1966; McGugan and Howsam, 1962; Manning and Robinson, 1973), headspace sampling (Manning and Moore, 1979; Price and Manning, 1983; Marsilli, 1985; Dunn and Lindsay, 1985; Lin and Jeon, 1985; Dickeman, 1988), solvent extraction (Wong and Park, 1968; Lamparski and Klimes, 1981), dialysis (Benkler and Reineccius, 1980), and direct injection of Cheddar cheese oil obtained by centrifugation (Liebich et al., 1970).

We chose to evaluate the potential of two techniques judged to be fast and easy enough to fulfill our objectives: solvent extraction and membrane dialysis. Steam distillation was also used since this technique can produce a very concentrated sample and thus serve as a reference method. The second objective of this work was to produce the aromagram of the three Cheddar cheese flavor isolates obtained and to evaluate the possible uses of aromagrams in the investigation of Cheddar cheese flavor.

MATERIALS AND METHODS

Sample Preparation. A 5-lb block of vacuum-packaged, medium Cheddar cheese was obtained from Land O Lakes, Inc. (Minneapolis, MN). This cheese was informally tasted and determined to be free of off-flavors. A half-centimeter slice from each edge was removed to minimize the possibility of package off-flavors migrating into the cheese sample used for this study. The remaining block was cut into 16 pieces (150–200 g each). Each piece was placed in a 1-qt glass Mason jar and sealed with a screw-capped lid. All jars were stored in the dark at 6 °C until analysis. Maximum storage time was 3 weeks.

The Cheddar cheese flavor essences were prepared by using one of the three techniques described below.

Distillation–Extraction. A slurry of cheese was prepared by grinding a 150-g sample of cheese with 650 mL of distilled water for approximately 1 min with an Osterizer blender. The blender was set at the lowest speed (grate). The homogeneous slurry obtained was then poured into a 2-L flask. The blender jar was rinsed twice with 50 mL of water to assure complete transfer of the slurry. The 2-L flask containing the slurry was then connected to a rotary evaporator (Buchler flash evaporator), and the distillation was started. The slurry was maintained at 50 °C by a water bath. A vacuum of 700 mmHg was pulled by using a water aspirator. This vacuum was regulated precisely by using a Weiss vacuum gage. The condenser temperature was maintained at 0 °C by circulating ice water. The distillation was stopped when 650 mL of distillate was obtained. The distillate was extracted six times with 25 mL of methylene chloride (Burdick and Johnson, capillary GC–GC–MS solvent, GC[2]) by using a separatory funnel. The combined methylene chloride extracts then were dried with anhydrous magnesium sulfate (Mallinkrodt), filtered by using Whatman No. 3 filter paper, and finally concentrated to 0.5 g under nitrogen gas. This distillate–extract was stored in a screw-capped vial at −10 °C until GC analysis.

Dialysis. This technique has been developed by Benkler and Reineccius (1979, 1980) and modified by Chang and Reineccius (1985). The dialysis unit consisted of two heavy-walled stainless steel half-cylinders (diameter 102 mm and depth 10 mm), each with six holes for the bolts and an access hole. The

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membrane was placed between the two half-cylinders, and the entire unit was held securely in place by two metal brackets that were screwed tightly together. A perfluorinated membrane (Nafion 117, Du Pont) was used. The access hole on each half-unit was plugged with a Teflon plug during dialysis. One of the two half-units possesses a ridge for a Teflon O-ring. This Teflon O-ring assured a tight contact between the two half-units and prevented any leaks.

A fresh membrane was used for each dialysis. Prior to use, a membrane was cut into a circle having an approximate diameter of 130 mm (the outside diameter of the dialysis unit). The membrane was soaked in boiling water for 40 min and then left in the water until it cooled to room temperature (20–25 °C) (during the soaking procedure the membrane expands). After 30 min in boiling water, the membrane is considered to be totally hydrated and has reached its maximum size. This preparation procedure is necessary to assure uniformity of membranes from dialysis to dialysis. This is important because chemical activity and pore size of the membrane is a function of the degree of membrane hydration. One half-cylinder was filled with water-saturated, diethyl ether (Mallinkrodt, analytical reagent). The wet membrane was laid on this half-cylinder, and the second half-cylinder was then placed on top of the membrane. The unit then was screwed together with the six bolts. Next, the unit was placed between the two metal brackets that were screwed tightly together.

The empty side of the dialysis unit was filled with a diethyl ether extract of the cheese. This cheese extract was prepared by grinding 20 g of cheese with 100 mL of diethyl ether in a mortar and pestle. The ether extract was removed, and the cheese solids remaining in the mortar were discarded. The diethyl ether extract was transferred from the mortar to the dialysis unit with a 30-mL glass syringe. The syringe needle was fitted with a 15-cm Teflon tube. This system allowed easy transfer of the diethyl ether extract from the mortar to the dialysis unit and reduced the risk of making a hole in the membrane during this operation.

Dialysis was allowed to proceed at room temperature for 72 h. The level of solvent in each side of the unit was checked daily. No evaporation was observed due to the use of the Teflon plugs. After 72 h, the contents of the diethyl ether side were collected with the previously described syringe. The contents of the other side (which was the side originally containing the cheese extract) were discarded. The dialysate then was dried with anhydrous magnesium sulfate, filtered, and concentrated to 0.5 g under a flow of nitrogen. This dialysate was then stored in a capped glass vial at -10 °C until dialysis by GC.

Solvent Extraction. This technique was developed by Wong and Park (1968). A 15-g sample of cheese was ground with 15 g of Celite 545 (Fisher Scientific Co.) by using a mortar and pestle. The homogeneous, yellow, free-flowing powder obtained was then poured into a 50-mL glass syringe (Perfektum, Popper & Sons Inc., New Hyde Park, NY) plugged with a disk of Whatman No. 3 filter paper. Another disk of filter paper was laid on top of the syringe. The contents then were packed by using the plunger of the syringe. The plunger was depressed by hand until the volume of the Celite–cheese-packed column was 36 mL (as read on the graduation of the syringe). Twenty milliliters of acetonitrile (Mallinkrodt, Chrom AR, HPLC grade) was then poured on top of the column. The first 0.5 g of eluent was collected into a glass vial equipped with a screw cap. The vial was stored at -10 °C until analysis by GC.

Evaluation of the Aroma of the Three Flavor Concentrates. This evaluation was informal. Six persons from our laboratory were used as a sensory panel. They first were asked to describe each of the five samples without any knowledge of their identity. A blotter was dipped in each flavor essence, and the samples were then evaluated by smelling the blotter after evaporation of the solvent. In the second part of the evaluation, the judges were asked to rank the samples, from the most similar to the least similar to the piece of cheese used for this study.

Gas Chromatographic Analysis of the Three Flavor Concentrates. For the analysis of the Cheddar cheese flavor essence, a Hewlett-Packard Model 5890 gas chromatograph equipped with a flame ionization detector (FID) and connected to a Hewlett-Packard Chem Station was used. Separation was accomplished on a 15 m × 0.21 mm (i.d.) capillary column (DB 225, J & W Scientific, Rancho Cordoba, CA). Helium was used as the carrier gas at a flow rate of 50 mL/min. The head pressure was set at 20 psi. The column flow was 3 mL/min. The injection port and detector temperatures were 275 °C. The column temperature was programmed from 40 to 250 °C at 5 °C/min with a 2-min postinjection hold and a 30-min final hold at 250 °C. A 1-μL sample was injected each time. The injection was splitless with a valve delay of 45 s.

Sniffing of the Gas Chromatographic Effluent. A vacant detector block heated to 275 °C and terminated with a bent copper tube was used for sniffing the gas chromatographic (GC) effluent. The GC column was passed through the heated detector block and the copper tube. Descriptions of the odors sniffed were recorded with a tape recorder; starting time and duration of this odor were recorded by using a hand-held button that controlled an external input to the integrator. Once the sniffing run was recorded, the end of the column was removed from the sniffing detector port and connected to the FID detector and another sample injection was done to record the GC profile. Chromatographic conditions were the same as those described previously except for the head pressure, which was set at 15 psi. The three concentrates were sniffed two to four times by a panel of five judges. All sniffers were members of our laboratory and familiar with the apparatus and task.

Mass Spectrometry. A Hewlett-Packard 5890 gas chromatograph coupled with a Hewlett-Packard 5970 mass spectrometer, direct inlet injection, and connected to a Hewlett-Packard Chem Station was used. The ionization mode was electron impact, and the ionization voltage was 70 eV. Chromatographic conditions were similar to those used for the establishment of the aromagrams.

RESULTS AND DISCUSSION

Evaluation of the Three Techniques. The time needed to run a sample by the distillation–extraction technique discourages the use of this method for routine analysis. An average of 5 h should be allowed for the preparation of a single sample. Distillation of the cheese slurries typically requires 2 h. Cleaning of the distillation apparatus between samples, done by distillation of 1 L of water, requires another 2 h. Extraction and concentration can be achieved in approximately 1 h. Therefore, a technician would be able to prepare a maximum of two to three samples a day. This method is not practical when many samples must be prepared in a short amount of time. In addition, due to the number of steps in this technique, artifact formation can be a problem (Jeon et al., 1976). Lawrence (1963) showed that methyl ketones were formed as artifacts during steam distillation of Cheddar cheese and recommended the use of other techniques of flavor isolation. However, the samples obtained were very concentrated, and this method would be useful for the identification of components. A typical chromatogram obtained via this isolation technique is shown in Figure 1. The aroma of the concentrate was described as cheesy but possessing a buttery background.

The aroma of the concentrate obtained by dialysis was described as cheesy with butyric and ketone notes. Our results agree with those of Benkler and Reineccius (1980), who described the aroma of a concentrate obtained with a similar technique as possessing more buttery notes than the distillate–extract obtained from the same sample of cheese. A rather unexpected result was the high yield of concentration obtained with this technique. For example, although 20 g of cheese was used in the preparation of this concentrate versus 150 g for the distillate–extract, some chemicals are present in similar concentrations in these two isolates (Figure 1). The main disadvantages of this method are its cost and the time required in sample preparation. The approximate cost of one dialysis unit

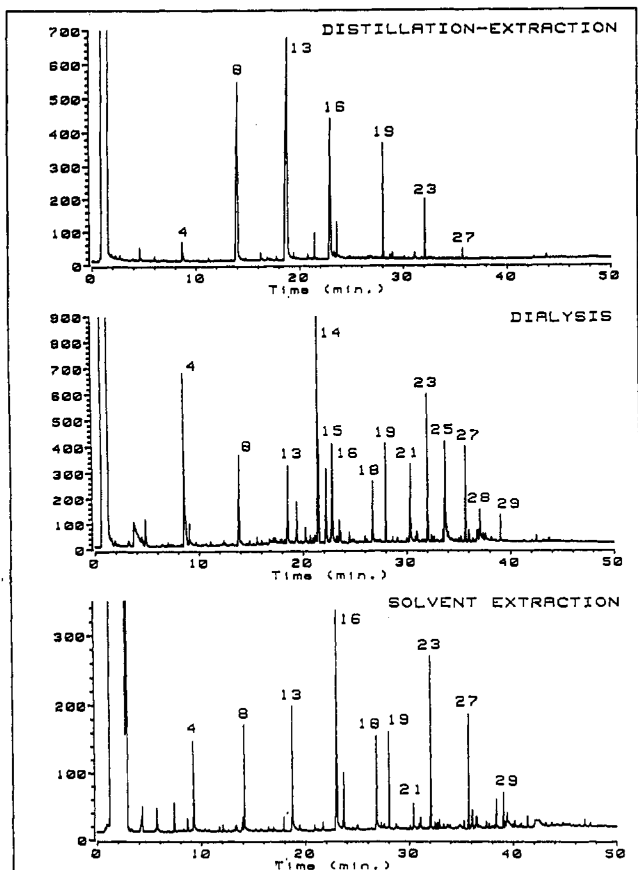

Figure 1. Gas chromatographic profiles (flame ionization detector) of Cheddar cheese flavor isolates prepared by distillation–extraction (top), dialysis (middle), and solvent extraction (bottom).

is $100. The membrane used cost $750/m^2$, which brings the membrane cost to between $10 and $15 per sample. A dialysis time of 3 days was used in this study. This delay was chosen to match an industrial setting where a technician would start the dialysis on Friday and concentrate and inject the sample on Monday. Since no attention is needed during this 3-day period, this method in conjunction with the use of many dialysis units may be attractive in some applications.

The solvent extraction technique gave a less concentrated flavor essence than the two other methods (Figure 1). This occurrence can be attributed to the absence of a concentration step in this method. Because of the rather high boiling point of acetonitrile (bp_{760} = 81.6 °C), an additional extraction with a more volatile solvent would be necessary before further concentration would be feasible. Wong and Park (1968) have suggested the extraction of the acetonitrile extract with petroleum ether. This method without an additional concentration step is rapid relative to the other isolation techniques investigated. A sample can be prepared in approximately 2 h. Furthermore, many samples can be run at the same time. A technician can easily prepare eight samples in a day, which is more than can be run on one gas chromatograph during the same period of time. Since nearly no materials are needed, the cost of this method is minimal. The aroma of this concentrate was described as cheesy, but more importantly, all judges agreed that this concentrate was the one possessing an aroma closest to the original cheese sample. For these two reasons, rapidity of sample preparation and preservation of the sample aroma, this method appears to be the most suitable for projects requiring analysis of many samples in a small amount of time. The fact that its cost is the lowest of the three methods discussed here is another advantage.

In comparing the three methods of flavor isolation (Figure 1), the resulting chromatographic profiles are different. Most compounds are present in each of the three concentrates; however, their ratios differ from one to another. This supports the observation that the aromas of the three extracts, although described as cheesy, were found to be different.

Odor-Active Compounds in Cheddar Cheese Flavor Concentrates. The most widely accepted theory on Cheddar cheese flavor is the component balance theory whereby cheese flavor is supposedly composed of a number of different compounds that must be present in the correct proportions (Mulder, 1952). Close to 200 compounds have been identified in Cheddar cheese (van Straaten, 1980; Lamparski and Klimes, 1981). Sniffing of the GC effluent can help in establishing the odor character and number of compounds contributing to Cheddar flavor.

Very few workers have reported sniffing the effluent of their GC during Cheddar cheese analysis. Manning and Robinson (1973) sniffed the chromatographic effluent of the headspace above a vacuum distillate of Cheddar cheese and assigned odor qualities to some of the compounds they identified. Lamparski and Klimes (1981) sniffed their gas chromatograph effluent to verify the odor contribution of the chemicals they identified using GC/MS. Since they were unable to determine any real flavor impact compounds, they supported the component balance theory.

In this current study, about 86 components were found to be odor active. The vast majority of these compounds possessed odors characteristic of fatty acids, ketones, and saturated and unsaturated aldehydes. Only one component was considered to have a cheeselike aroma. This component had a weak aroma and eluted from the GC just after butyric acid. We were unable to identify it. Since there was only one weak component which had a cheeselike aroma, we also have to support the component balance theory for Cheddar cheese flavor.

Compounds Identified. Table I lists the compounds identified, or tentatively identified, in each of the flavor concentrates. As can be seen from this table, fatty acids and lactones are the major compounds present in these three Cheddar cheese flavor essences. Alcohols, carbonyls, and aromatic compounds are among the other chemicals tentatively assigned in these flavor concentrates.

Four compounds, 2-propanol, 1,3-butanediol, γ-decalactone, and δ-undecalactone, were tentatively identified for the first time.

SUMMARY AND CONCLUSIONS

Each of the flavor isolation procedures yielded isolates that had a cheesy character but were somewhat different from each other. The solvent extraction technique produced an isolate with the most characteristic Cheddar aroma, while the other two methods produced isolates with a more buttery note. Their analytical profiles presented some similarities. Most peaks were observed in each profile; however, the relative concentrations of the chemicals isolated varied. Dialysis gave a sample with a high concentration of flavor compounds. The solvent extraction method did not generate a sample as concentrated but was the fastest and cheapest technique. Furthermore, this method gave an aroma concentrate most similar to the original Cheddar cheese sample.

Sniffing the effluent of the gas chromatograph allowed the evaluation of individual aroma components. While one

Table I. Compounds Identified, or Tentatively Identified, in Cheddar Cheese Flavor Isolates with Absolute Areas Obtained by Using the MS Detector

peak	proposed identification	IT[a]	absolute area ($\times 10^6$)			RT[e]
			DE[b]	Di[c]	SE[d]	
1[f]	2-propanol	1	11.37			3.75
2	heptanone	2	14.22			5.12
3	hexanol	2	16.27			5.64
4	butyric acid	2	0.79	50.75	11.74	7.34
5[f]	1,3-butanediol	1		1.60	8.86	8.11
6	methylbutyric acid	1		3.59		8.50
7	aldehyde	1	24.82	2.44		10.16
8	hexanoic acid	2	93.79	27.33	9.74	12.55
9	2-phenylacetaldehyde	1			14.04	12.72
10	undecanone	2	12.27			15.26
11	phenol	1	5.34			15.52
12	2-phenylethanol	1	8.81			16.03
13	octanoic acid	2	661.89	37.37	24.58	17.29
14[g]	BHT	1		81.60		20.38
15	benzoic acid	1		30.68	2.51	20.79
16	decanoic acid	2	638.97	72.20	76.09	21.68
17	γ-octalactone	1	63.11			23.36
18	dodecanoic acid	2	94.48	45.97	52.49	25.57
29	δ-decalactone	2	138.77	27.52	11.54	26.64
20[f]	δ-undecalactone	1	7.97			28.65
21	tetradecanoic acid	2		84.15	53.15	29.17
22[f]	γ-decalactone	1	18.84			29.66
23	δ-dodecalactone	2	81.92	12.72	23.51	30.66
24[g]	diisobutylphthalate	1	22.31			31.55
25	hexadecanoic acid	2		113.39	31.14	32.52
26[g]	dibutylphthalate	1	3.85			33.70
27	δ-tetradecalactone	2	16.65	38.08	18.25	24.28
28	octadecanoic acid	2		1.41		35.63
29	δ-hexadecalactone	2	5.94	6.25	5.87	37.63

[a] IT, identification type: 1, mass spectra; 2, mass spectra and GC retention time. [b] DE, distillation–extraction. [c] Di, dialysis. [d] SE, solvent extraction. [e] RT, retention time. [f] Compounds tentatively identified for the first time. [g] Artifacts.

component yielded a cheesy note, we agree with the majority of the literature which suggests that Cheddar cheese flavor is the result of the contribution of many compounds, which, in the correct ratio, produce a good flavor.

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LITERATURE CITED

Aishima, T.; Nakai, S. Pattern recognition of GC profiles for classification of cheese variety. *J. Food Sci.* **1987**, *4*, 939–942.

Aston, J. W.; Dulley, J. R. Cheddar cheese flavour. *Aust. J. Dairy Technol.* **1982**, 59–64.

Benkler, K. F.; Reineccius, G. A. Separation of flavor compounds from lipids in a model system by means of membrane dialysis. *J. Food Sci.* **1979**, *44*, 1525–1529.

Benkler, K. F.; Reineccius, G. A. Flavor isolation from fatty food via solvent extraction and membrane dialysis. *J. Food Sci.* **1980**, *45*, 1084–1085.

Chang, Y. I.; Reineccius, G. A. Isolation of flavor compounds in model systems by countercurrent continuous dialysis. *J. Agric. Food Chem.* **1985**, *6*, 1168–1173.

Day, E. A.; Libbey, L. M. Cheddar cheese flavor: Gas chromatographic and mass spectral analysis of the neutral components of the aroma fraction. *J. Food Sci.* **1964**, *29*, 583–589.

Dickeman, J. K. Sensory, Textural and chemical analysis of Cheddar cheese during aging. M.S. Dissertation, The University of Missouri—Columbia, 1988.

Dunn, H. C.; Lindsay, R. C. Comparison of methods for the analysis of higher boiling flavor compounds in Cheddar cheese. *J. Dairy Sci.* **1985**, *68*, 2853–2858.

Jeon, I. J.; Reineccius, G. A.; Thomas, E. L. Artifacts in flavor isolates produced by steam vacuum distillation and solvent extraction of distillate. *J. Agric. Food Chem.* **1976**, *24*, 433–434.

Lamparski, D.; Klimes, I. Cheddar cheese flavour—Its formation in the light of new analytical results. In *Flavour '81*; Schreier, P., Ed.; de Gruyter: Berlin, 1981.

Lawrence, R. C. Formation of methyl ketones as artifacts during steam distillation of Cheddar cheese and butteroil. *J. Dairy Res.* **1963**, *30*, 161–170.

Libbey, L. M.; Bills, D. D.; Day, E. A. A technique for the study of lipid soluble food flavor volatiles. *J. Food Sci.* **1963**, *28*, 329–334.

Liebich, H. M.; Douglas, D. R.; Bayer, E.; Zlatkis, A. Comparative characterization of different types of cheese by gas-liquid chromatography. *J. Chromatogr. Sci.* **1970**, *8*, 351–354.

Lin, J. C.; Jeon, I. J. Headspace gas sampling/GC method for the quantitative analysis of volatile compounds in cheese. *J. Food Sci.* **1985**, *50*, 843–844, 846.

Manning, D. J.; Moore, C. Headspace analysis of hard cheese. *J. Dairy Res.* **1979**, *46*, 539–545.

Manning, D. J.; Robinson, H. M. The analysis of volatile substance associated with Cheddar cheese aroma. *J. Dairy Res.* **1973**, *40*, 63–75.

Marsilli, R. Monitoring chemical changes in Cheddar cheese during aging by high performance liquid chromatography and gas chromatography technique. *J. Dairy Sci.* **1985**, *68*, 3155–3161.

McGugan, W. A.; Howsam, S. G. Analysis of neutral volatiles in Cheddar cheese. *J. Dairy Sci.* **1962**, *45*, 495–500.

Morris, H. A.; Angelini, P.; McAdoo, D. J.; Merrit, C., Jr. Identification of volatile components of Cheddar cheese. *J. Dairy Sci.* **1966**, *49*, 710–716.

Mulder, H. Taste and flavour forming substances in cheese. *Ned. Melk Zuiveltijdschr.* **1952**, *6*, 157–168.

Price, J. C.; Manning, D. J. A new technique for the headspace analysis of hard cheese. *J. Dairy Res.* **1983**, *50*, 381–385.

Scarpellino, R.; Kosikowski, F. V. A method of concentrating ripened cheese volatiles for gas chromatography. *J. Dairy Sci.* **1961**, *44*, 10–15.

Van Straaten, S. *List of volatile Compounds in Food*, 4th ed., Suppl. 7; Van Straaten, S., Ed.; TNO: Zeist, 1980.

Wong, N. P.; Park, O. W. Simple technique for extracting flavor compounds from fatty foods. *J. Dairy Sci.* **1968**, *11*, 1768–1769.

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