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Dynamic Headspace Analysis of Volatile Constituents of Swiss Cheese Whey Protein Concentrate

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Abstract

Volatile flavor compounds in the headspace of swiss cheese whey protein concentrate (WPC) were analyzed by dynamic headspace analyzer, gas chromatography, and mass spectrometer. Sixty one compounds were detected from the headspace of dry WPC and 23 compounds from the headspace of an aqueous solution of WPC. The major components were propanol, hexanal, 2-butanone, 2-pentanone, 2,3-butanedion, 2-propanol, acetic acid, dimethyl disulfide and benzothiazole. An external dynamic headspace sampler, devised for this study, effectively collected volatiles from the headspace of dry WPC and aqueous WPC solutions.

Key words: volatile compounds, dynamic headspace analysis (DHA), whey protein concentrate (WPC)

INTRODUCTION

Although fresh whey protein concentrate (WPC) generally exhibits a bland flavor, it develops a typically stale off-flavor during storage due to complex, inter-related reactions such as lipid oxidation, Maillard browning reaction, and decomposition of riboflavin (1-3). The off-flavors developed in whey protein concentrate (WPC) during storage greatly limit its use in many food applications (4). In order for industry to manufacture WPCs with improved flavor stability as well as functionality, it is necessary to understand the mechanism of off-flavor formation available on flavors and off-flavors of WPC, largely due to concentrations (3,5-9).

There are many methods for sampling of volatile organic compounds, such as steam distillation, molecular distillation, solvent extraction, membrane dialysis, and dynamic or static headspace analysis (9-17). Headspace analysis is a simple, rapid and reliable method for analyzing volatile flavor compounds (3,18-24). This method provides a more accurate flavor profile than other method, since the volatiles in the headspace are the same proportion and concentration as in the food system. However, sampling a few milliliters of equilibrated vapor from the headspace as by statistic headspace method, gives satisfactory results only for those volatile compounds with a sufficiently high vapor pressure and concentration (25). The dynamic headspace analysis (DHA) has advantages over static headspace analysis. DHA, which utilizes an adsorbent trapping system and cryogenic focussing, provides improved sensitivity and reproducibility (12,26). In DHA, the headspace volatiles are continuously purged and chemically adsorbed onto a polymer trap such as a Tenax. Trapped volatile compounds are, then thermally desorbed and transported to the gas chromatographic column and cryogenically focussed with liquid nitrogen at the capillary interface. The cryofocussed compounds are subsequently volatilized by rapidly injected onto the column with a minimum injection volume.

Mills (9) analyzed the headspace volatile compounds of lactic acid WPC solution by DHA and identified 10 compounds that included diacetyl, pentanal and hexanal. his DHA results are somewhat from those of previous studies by Ferretti & Flangan (5,6,27), who identified 24 volatile compounds from a steam distillate of commercial spray-dried whey (28). Compounds identified in this latter study included 7 alkylpyrazines, 3 furans 2 pyrroles, α-methyl-γ-butyrolactone, isobutyramide, N-methyl-2-pyrolidinone, 3-hydroxy-2-butanone, benzaldehyde, phenol, benzyl alcohol, maltol, dimethylsulfone, and propionic, butyric and benzoic acids. Alkylpyrazines were the major volatile components of spray-dried whey protein. Comparison of the results from these two studies indicates that DHA is simpler, quicker, and produces fewer artifacts than other analytical methods.

The present study describes the DHA technique with

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a modified external sampler device to analyze volatile compounds in the headspace of both dry WPC and aqueous WPC solutions.

MATERIALS AND METHODS

Materials

Fresh Swiss cheese whey was obtained from Holmes Cheese Company, Millersburg, OH. The whey was pasteurized at 72°C for 15sec and fractionated with a model PM 50 ultrafiltration (UF) unit (Romicon, Woburn, MA, USA) equipped with a 25 ft^2, 60 mil, 50,000 dalton molecular weight cut-off hollow fiber membrane at 42 ~ 43°C. The whey retentate was concentrated 20 : 1 v/v, diafiltered with 5 volumes of demineralized water, and spray dried in a laboratory spray dryer (APC Crepaco, size No.1) with an exhaust air temperature of 80 ~ 82°C. The WPC was collected in a Ziploc^R heavy duty freezer bag, sealed and stored at -20°C until analysis.

Dynamic headspace sampling

Two grams of WPC were placed in a 30 mL serum bottle and sealed air tight with a Teflon^R-faced rubber septum (Supelco, Inc., Bellefonte, PA, USA; part No. 3-3200) and aluminum cap. An external dynamic headspace sampler was designed as shown in Fig. 1 to recover volatiles from the headspace of the sample in the serum bottle. Special needles were purchased from Tekmar (Part No. 145186553) and cut to length as needed. An on-line filter with a replaceable 0.4 μm pore size frit (Upchurch, Oak Harbor, WA, USA; Part No. A-316 and A-103) was inserted between the sample bottle and the auxiliary heated transfer line to prevent fine sample particles from being carried into the headspace analyzer. The serum bottle was connected to the Tekmar Purge and Trap Concentrator (Model LSD 2000; Cincinnati, OH, USA). Volatile compounds were swept from the headspace for 10 min with ultrapure helium at a flow rate of 40 mL/min. The volatiles were chemically adsorbed by the Tenax TA (a polymer of 2,6-diphenyl- ρ -phenylene oxide) trap (Tekmar Part No. 12-0083-003). In addition to the initial conditioning which is similar to those reported (29,30) the Tenax trap was baked 10 min at 250°C immediately before each analysis to remove any residual compounds from the previous trial.

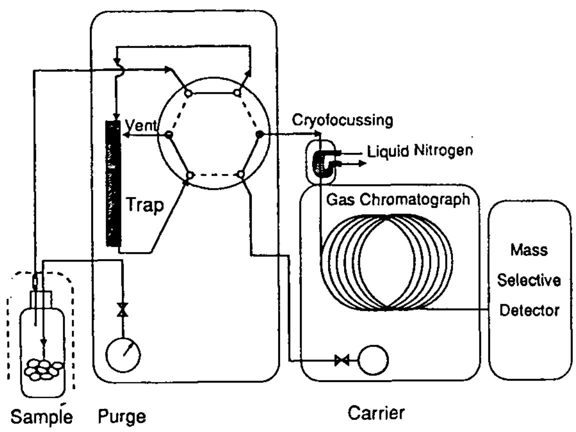

Fig. 1. Analytical system for dynamic headspace analysis (DHA) with an external sampler device.

The reproducibility of the headspace volatile compound recovery was calculated by the % relative standard deviation (% RSD) as described by Sensel & Griffiths (31) and Ha et al. (23).

Gas chromatography - mass spectrometry (GC-MS)

A 5890 Series II Gas Chromatography (GC) and a 5971A Mass Selective Detector (MSD) (Hewlett-Packard, Palo Alto, CA, USA), equipped with a 30 m × 0.25 mm, 0.25 μm film thickness DB-Wax fused silica capillary column (J & W Scientific Inc., Rancho Cordova, CA, USA), were used to analyze headspace volatiles. Ultrahigh purity helium was used as carrier gas at a flow rate of 1 mL/min. The column temperature was programmed from 32°C to 160°C at a rate of 2°C/min without an initial holding period, and then increased to 220°C at a rate of 10°C/min. The ion source was maintained at 180°C. Mass spectral data was collected within the mass range of m/z 33 ~ 350 with an electron energy of 70 eV. Other details of MSD conditions have been described by Ha et al. (23).

The retention index values for volatile compounds were determined according to the methods of Van den Dool & Kratz (32) by using a series of normal alkanes (C_7 ~ C_{15}). RI values greater than 1500 were extrapolated by linear regression of log values of RI and retention times of standard normal alkanes. Volatile compounds were identified on the basis of RI and mass spectral data as reported by Ha et al. (23).

RESULTS AND DISCUSSION

Dynamic headspace sampling with a modified external sampling device

Volatile flavor compounds in the headspace of Swiss cheese WPC were determined by DHA, using an external dynamic headspace sampling device (Fig. 1) instead of the original Teckmar sampling device. This homemade device with its on-line-filter allowed us to sample the headspace of dry WPC, since the filter prevented fine powder particles from being swept into the DHA equipment, where they might from heat-induced volatile compounds as artifacts.

DHA generally does not require high sampling temperatures and, for this reason, is less likely to form artifact volatile compounds than by steam distillation and other analytical techniques that require high temperatures for volatilization or concentration of volatile compounds. However, sampling temperature was found to affect the recovery of headspace volatiles (Fig. 2). The intensity of individual peaks and the number of peaks recovered from the headspace both increased as the headspace purging temperature was increased from 40°C to 60°C and 80°C. Total ion chromatograms (TICs) of volatile compounds recovered at the three purging temperatures were quantitatively different, but qualitatively similar, indicating that, as expected, more volatiles were recovered from the headspace of WPC at higher sample purge temperatures. Whey proteins reportedly bind flavor compounds (8,33,34). Therefore, the elevated headspace purging temperatures should allow the proteins to release more volatile compounds from the WPC than at lower purging temperatures. The Tenax trap reportedly has a lower affinity for water vapor than most other chemical traps (Tekmar data sheet B111981). However, it retains sufficient moisture to impair recovery and quantitation of volatile compounds from various food systems (23,35-37). Researchers have developed an 'off-line dry purge procedure' to overcome the moisture related problems in DHA (35,36).

Fig. 2 (C) and 2 (D) provide a comparison of total ion chromatograms (TICs) from two gas chromatographic runs of dry WPC and an aqueous solution of WPC, respectively. Sixty one compounds were detected from dry WPC, as compared to 23 compounds from an aqueous solution of WPC (Table 2). The aqueous WPC solution was not heated because this would have resulted in a greater amount of moisture adsorption and impaired volatile compound adsorption on the Tenex trap. As expected, this assumption was confirmed by the recovery of a larger number of volatile compounds from the headspace of dry WPC than from the headspace of an aqueous WPC solution.

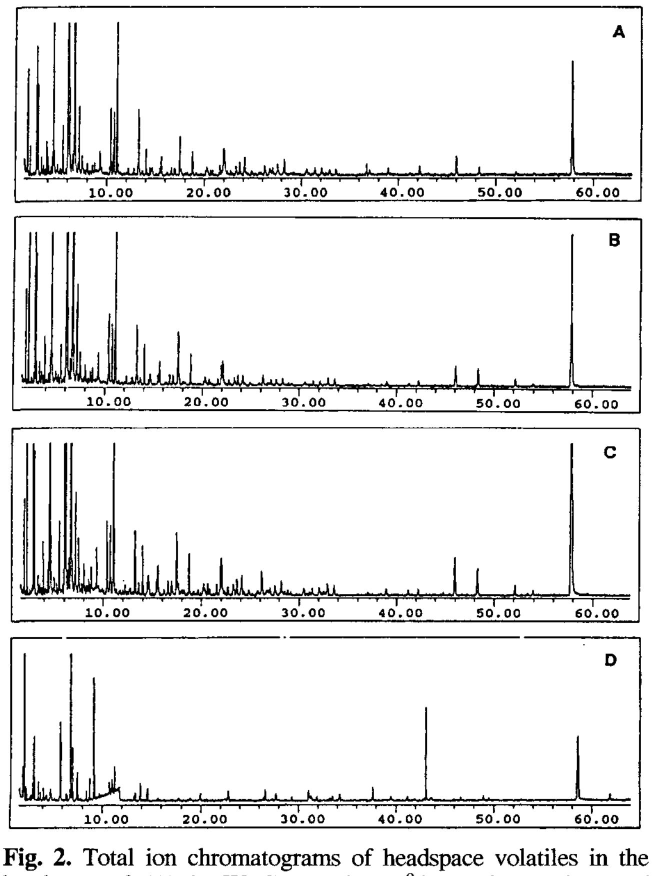

Fig. 2. Total ion chromatograms of headspace volatiles in the headspace of: (A) dry WPC purged at 40°C; (B) dry WPC purged at 60°C; (C) dry WPC purged at 80°C; (D) an aqueous solution of WPC purged at 25°C.

Reported recovery values for headspace volatile compounds by the Tenax TA purge and trap system ranged from 93.7% to 112% with a reproducibility of recovery range of 1.7% to 2.8% RSD (29). However, the reproducibility of recovery of headspace volatiles of soaked soybeans ranged from 17.0 to 33.1% RSD, due largely to the high moisture concentration in the headspace (23). In this study, the reproducibility of four key compounds in dry WPC analyzed by DHA, modified with the external sampling device, ranged from 0.5% for 2-propanol to 31.4% for benzothiazole (Table 1).

Headspace volatile compounds of Swiss cheese WPC

A total of 71 volatile compounds were identified from the headspace of both dry and solution forms of Swiss cheese WPC (Table 2) on the basis of RI and mass spectral data of reference compounds. These compounds are grouped by functionality.

Many of the compounds isolated from WPC were common to both dry and aqueous solution matrices. Hexanal, 2-pentanone, 2,3-butanedione, benzaldehyde, and octane have been also reported in the headspace of lactic acid WPC solution (9,38). Diacetyl, propanal and hexanal were reported as major compounds in the headspace of lactic acid WPC solution. In this study, 2-propanol, 1-octanol, propanal, 2,3-butanedione, benzothiazole, were detected as major flavor compounds. Aldehydes and ketones are lipid oxidation related products and may possibly contribute to the fermented flavor of WPC. However, no compounds have been identified that would be associated with "dairy" or "milky" flavors in WPC. Mills (9) suggested that "dairy" or "milky" flavors may either be present at low concen-

Table 1. Reproducibility of recovery of selected volatile compounds from dry WPC

Compound	RI	% RSD[1)]
2-Propanol	935	5.0
Ethanol, butoxy-	1402	0.5
Acetic acid	1455	17.2
Benzothiazole	1964	31.4

[1)]Data from a single GC analysis from each three replicate samples of dry WPC.

Table 2. Volatile compounds recovered from dry WPC and aqueous WPC solution[1)]

Compound	RI[2)]	Peak area (×10^6) Dry	Solution
Alcohols			
2-Propanol	935	196	-
Ethanol, butoxy-	1402	5	3
1-Hexanol, 2-ethyl-	1493	4	-
1-Octanol	1564	12	4
2-Furanmethanol	1644	7	-
Phenol	1988	-	4
Aldehydes			
Propanal	788	37	-
Butanal	873	3	-
Hexanal	1086	8	23
Heptanal	1188	3	6
Nonanal	1390	8	-
Benzaldehyde	1502	5	-
Acids and esters			
Acetic acid, methyl ester	819	-	7
Acetic acid, ethyl ester	887	1	-
Acetic acid	1455	5	4
Ketones			
2-Butanone	900	7	3
2-Pentanone	971	-	24
2,3-Butanedion (diacetyl)	976	15	-
Ethanone, 1-phenyl-[3)]	1630	2	-
Aliphatic hydrocarbons			
Cyclohexane	<700	2	-
Heptane	700	-	1
Octane	800	-	1
Nonane, 4-methyl-	956	5	-
Decane	1000	9	-
Undecane	1100	6	-
Dodecane	1200	-	-
Tridecane	1300	3	-
Aromatic hydrocarbons			
Benzene, methyl-	1037	-	7
Benzene, ethyl-	1126	33	3
Benzene, 1,4-dimethyl-(p-xylene)	1133	35	4
Benzene, 1,3-dimethyl-(m-xylene)	1140	71	5
Benzene, C3-alkyl-[3)]	1173	3	-
Benzene, 1,2-dimethyl-(o-xylene)	1182	26	2
Limonene	1195	38	-
Benzene, propyl-	1206	8	-
Benzene, C3-alkyl-[3)]	1220	5	-
Benzene, C3-alkyl-[3)]	1223	10	-
Benzene, 1,3,5-trimethyl-	1240	5	-
Benzene, C3-alkyl-[3)]	1256	3	-
Benzene, C3-alkyl-[3)]	1273	14	-
Benzene, C4-alkyl-[3)]	1294	5	-
Benzene, butyl-	1304	1	-
Benzene, C3-alkyl-[3)]	1325	16	-
Benzene, C4-alkyl-[3)]	1345	4	-
Benzene, C3-alkyl-[3)]	1351	5	-
Benzene, C4-alkyl-[3)]	1358	3	-
Benzene, C4-alkyl-[3)]	1359	4	-
Benzene derivative[3)]	1422	3	-
Benzene, dichloro-	1432	2	-
Benzene, C5-alkyl-[3)]	1458	4	-
Benzene, 1,2,3-trichloro-	1619	-	<1
Naphthalene	1698	15	-
Benzene, C6-alkyl-[3)]	1718	4	-
Naphthalene, 1-methyl-[3)]	1844	7	-
Naphthalene, 2-methyl-[3)]	1884	3	-
Sulfur compounds			
Disulfide, dimethyl-	1074	3	5
Methane, sulfonylbis-	1910	11	27
Benzothiazole	1964	85	-
Miscellaneous			
Methane, trichlorofluoro-[3)]	<700	34	-
Ethane,1,1,2-trichloro-1,2,2,-trifluoro-[3)]	<700	-	141
Unknown	807	-	7
Methane, dichloro-	927	9	-
Ethane, trichloro-[3)]	991	2	-
Cyclotetrasiloxane, octamethyl-[3)]	1012	9	-
Methane, trichloro- (chloroform)	1022	1032	10
1,3,5-Cycloheptatriene[3)]	1038	34	-
Unknown	1079	5	-
Unknown	1198	5	3
1,3,5,7-Cyclooctatetraene[3)]	1254	26	-
Unknown	1316	3	-
Formamide, N,N-dimethyl-[3)]	1321	15	-

[1)]Identified by retention index (RI) values and mass spectral data. Dry WPC was purged at 80°C and aqueous solution of WPC was purged at 25°C.
[2)]Experimental RI values.
[3)]Tentatively identified by mass spectral data alone.

trations, have a very low volatility or have a low flavor threshold. 2,3-Butanedione, which had an intense buttery odor (38), was found in the headspace of a dry WPC.

Three sulfur-containing compounds, benzothiazole, sulfonyl bis-methane (dimethyl sulfone), and dimethyl disulfide, were identified in this study. As shown in Fig. 2, benzothiazole, which was eluted approximately at 58.5 min, showed larger peak area when sample was purged at a higher temperature, due possibly to its high boiling point of 231°C. Benzothiazole and dimethyl disulfide have been also recovered in the headspace of Swiss cheese by Lee et al. (39) who found that the intensity of these sulfur-containing compounds increased during storage.

Eight aliphatic hydrocarbons were identified (Table 2). Saturated aliphatic hydrocarbons have no known contribution to flavor in foods (36,40,41). Saturated aliphatic hydrocarbons might result from decarboxylation or splitting of carbon chains of higher fatty acids (42), or reaction of alkyl free radicals, which are the product of thermally decomposed hydroperoxides with hydrogen free radicals (43). Hydrogen gas might result from hydrogen free radical ($H^{\bullet} + {}^{\bullet}H \rightarrow H_2$) during the thermal oxidation of a fat (44,45).

Halogenated compounds, such as dichloromethane and trichloromethane, were detected. Sources of halogenated compounds are not clear. Pesticide contamination was suspected as a probable source of halogenated compounds by

Vejaphan et al. (36). Detergents or tap water used during the manufacturing of cheese and whey protein concentrate may be possible sources. Quantitative analysis of trichloromethane showed that the concentration was below 100 ppt level, thus far below the concentration of halogenated compounds present in drinking water.

A series of alkyl bezenes were identified among the volatiles in the headspace of Swiss cheese WPC. These compounds have been found in tea (46), corn (47), nuts (48), egg (49,50) and boiled crayfish meat (34). However, The origin of alkyl benzenes is uncertain. Some may have entered the sample from the environment (46) or have been formed by thermal degradation of sugar (51). However, a sugar browning reaction model system did not produce significant amounts of alkyl benzenes (52). Watanabe & Sato (42) reported formation of various alkyl benzenes from beef fats during heating. Benzene, toluene, styrene, and biphenyl may have originated as decomposition products of Tenex in the purge and trap concentrator (Tekmar data sheet B111981). MacLeod & Ames (29) compared the artifact background on thermal desorption of preconditioned Tenex GC and Tenex TA and proposed an efficient preconditioning procedure to minimize background on thermal desorption. The possibility of artifact background on this study was eliminated by running dry blanks immediately before each sample analysis.

CONCLUSION

The DHA technique described with a modified external sampler device is simple, mild, effective and provides reliable results for the composition of the volatiles in the headspace of WPC. This technique should be useful for further investigations into the reactions and mechanisms for formation of off-flavors in WPC as a function of temperature, water activity, oxygen, light, metal ions, antioxidants and other key compositional and processing variables.

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