Unconventional Proteins as Aroma Precursors. Chemical Analysis of the Volatile Compounds in Heated Soy, Casein, and Fish Protein Model Systems

Ingmar H. Qvist* and Erik C. F. von Sydow

Model samples containing soy, casein, or fish protein were analyzed by gas chromatography and mass spectrometry. The headspace of samples, unheated and heated and with or without fat and starch added, was investigated. Over 150 compounds were identified representing aliphatic hydrocarbons, alcohols, aldehydes, ketones and furan derivatives, and sulfur-containing compounds. The absolute concentrations in the headspace gas were determined for about 80 compounds judged to be of at least potential interest from the aroma point of view. On heating, the concentrations of volatiles generally increase and new compounds are detected. Of particular importance for the aroma of all heated samples is the presence of branched chain aldehydes and sulfur compounds. Moreover, straight chain aldehydes and furans are important in the soy protein samples and hydrogen sulfide in the fish protein sample.

For economical and nutritional reasons it is sometimes desirable to replace a conventional protein such as meat with less expensive but still nutritious unconventional protein materials, such as soy, rapeseed, fish, and single cell proteins. In processed protein foods the protein part acts as one of several precursors for compounds responsible for different sensory properties, such as color, taste, and aroma.

Thus a change of the protein part is likely to cause changes in one or more of the sensory properties. There is very little to be found in the literature concerning aroma properties of protein concentrates or isolates from various sources. However, the sources themselves and products made using these raw materials are more often treated in the literature. This is particularly true with soybeans and food products made from soybean, such as soy sauce, miso, etc.

The chemistry of volatiles from soy is treated and reviewed by Manley and Fagerson (1970a,b), Maga (1973), and Cowan *et al.* (1973). Hrdlička and Čuda (1971) studied the change in sulfhydryl containing volatile compounds when heating soy protein with glucose. They found an increase of the total amount of such compounds with increasing temperature and/or increasing heating time.

Nakanishi and Itoh (1967), Ferretti *et al.* (1970), and Ferretti and Flanagan (1971) reviewed the chemistry of volatiles formed from casein by heating and they described their own results from experiments on heating casein with or without lactose. Heating without lactose for 60 min at 140° generates a number of aliphatic aldehydes, such as 2-methylpropanal, 3-methylbutanal, and 2-methylbutanal. When casein was heated with lactose at 75–80° for several days a number of alicyclic and heterocyclic compounds were formed.

Kato *et al.* (1972) heated casein at 250° for 1 hr and analyzed the volatiles. Of special interest was the detection of volatile sulfur compounds, such as hydrogen sulfide, methanethiol, and dimethyl disulfide.

Wick *et al.* (1967) analyzed the volatile constituents of a fish protein concentrate (unheated) and found the presence of some amines. Miller *et al.* (1973) measured dimethyl- and trimethylamine in fish protein concentrates. As far as we know, the headspace technique has not been used in any detailed analysis of volatiles from unconventional proteins. Neither has much emphasis been paid to the sulfur-containing volatile compounds.

Swedish Institute for Food Preservation Research (SIK), Fack, S-400 21 Göteborg 16, Sweden.

Maga and Lorenz (1972, 1973) reported on sensory evaluation of a number of protein sources and supplements. Most of the samples were not heat treated. Kalbrener *et al.* (1971) evaluated sensorically a number of commercial soybean flours, concentrates, and isolates. The results are not only quantitative in nature as the experimentors were concerned with a more descriptive evaluation including the usage of some odor and taste notes. With regard to rapeseed and single cell proteins, nothing can be found in the literature concerning aroma properties, either chemically or sensorically.

This investigation deals with the aroma properties of heat treated protein food models containing different amounts of unconventional proteins and it is directly linked to another investigation on canned beef (Persson and von Sydow, 1973, 1974a,b; Persson *et al.*, 1973a,b). The unconventional proteins investigated are: a soy protein isolate, sodium caseinate, a fish protein concentrate, and a rapeseed protein concentrate. They are analyzed alone in mixture with water and salt, both unheated and heated. Other models contain fat and starch also. Finally, the unconventional proteins are used together with beef protein in model samples containing water and salt also.

The analytical techniques employed were gas chromatography of the headspace volatiles and mass spectrometry for identification. Of special importance was the usage of a sulfur specific flame photometric detector, as sulfur-containing compounds are particularly important in connection with aroma generated by proteins.

Sensorically, an odor quality assessment technique was used. It was originally suggested by Harper *et al.* (1968a,b) but further developed and applied by us (von Sydow *et al.*, 1970; von Sydow and Karlsson, 1971; Persson *et al.*, 1973a). A somewhat similar technique has been used on soy sauce by Tanaka and Saito (1969). Models for correlation between instrumental and sensory data were applied according to Persson and von Sydow (1974b).

In this part of our investigation we present results from the gas chromatographic and mass spectrometric analyses of different samples containing soy protein, fish protein, and casein. Sensory data and data on rapeseed protein will be presented later as will also the results from experiments using mixtures of beef protein with the various unconventional proteins.

EXPERIMENTAL SECTION

Materials. The protein raw materials analyzed were soy protein isolate (Promine D from Central Soya, U. S.), fish protein concentrate (EFP 90 from Astra Nutrition AB, Sweden), and sodium caseinate (Sodinol V from A/S Lidano, Denmark). Analytical data of the proteins, as given

Table I. Analytical Data (Per Cent) of the Protein Raw Materials as Given by the Manufacturers

	Soy protein isolate Promine D	Sodium caseinate Sodinol V	Fish protein concentrate EFP 90
Protein	91.4	88	91
Fat	0.5		0.1
Ash	3.4	5	6
Water	4.7	5	3
pH	7.0	6.4–6.9	

by the manufacturers, are given in Table I.

For all experiments involving quantitative determinations one homogenous lot of each protein raw material was used. The fat used was one homogenous batch of minced pork back fat. The carbohydrate used was commercial potato flour (80% starch and 20% H_2O from Swedish Starch Producers Association). The salt was commercial table salt (from K. N. Z. Henzelo, Holland). The water used was distilled and filtered through activated charcoal.

For each protein a standard protein raw material was made by mixing the protein with water so that a sample containing 21% protein (as in the meat used by Persson and von Sydow, 1973) was obtained. These mixtures are called "protein–H_2O" in Table II. With these mixtures as basic material the formulations presented in Table II were prepared. Soy protein isolate and sodium caseinate were investigated in all three models, but fish protein concentrate was investigated only in models I and II. The cans used for experiments involving quantitative measurements were deep drawn from electrolytical tin plate (1.00/0.50 lb per bb), 73 × 28 mm in size holding 80 g of material.

Processing. The processing was carried out in a retort assembly designed for thermal death time (TDT) investigations (National Canners Association, 1968). The pressure and temperature of the vapor were controlled by a Honeywell Pneumatic controller (type 152 Brown Elektronik). This system gives a very high degree of control during the heating and cooling period and also a homogeneous temperature in the retorts. The processing was carried out at 121° until an F_c value of 28 was reached, which happened after 37–41 min. The temperatures in the retorts and in the can centers were measured with thermocouples manufactured by Ellab Instruments, Copenhagen, Denmark. The tip of the thermocouple was sheathed by a 50 mm long stainless steel needle, 1.0 mm thick (type TCK 8). In order to insert the needle into the can through the can wall, a packing gland manufactured by the same company (type TCG 24) was used. The temperature was registered on a Honeywell Temperature Recorder (type Elektronik 15-695-1658). The thermocouples were calibrated by comparing them with accurate (±0.15°) thermometers. To measure the F_c values a Telemetric Sterimeter (Telemetric Instruments, Sundbyberg, Sweden), which automatically integrates the time-temperature curve, was used. The accuracy after integration was ±3%. The processed cans were stored at −40° until analyzed within 2 months.

Concentrates of Volatiles. For mass spectrometric identification (see below) it was necessary to concentrate the components in the headspace gas. A slurry was prepared by mixing 1.2 kg of protein model sample and 1.2 l. of carbon-filtered distilled water. The protein model sample was obtained from a number of cans (see Materials) containing one of the formulations described in Table II. Volatiles from the protein model slurry were concentrated by low temperature distillation according to Forss *et al.* (1967) at 1 kPa and 20° after initial degassing with the slurry stirred at 0°. A mechanical stirrer was used. The distillation column temperature was 6° and the volatiles were condensed in a cold trap cooled by liquid nitrogen. Several batches were distilled until about 1 l. of concentrate was obtained. This concentrate was redistilled, giving about 100 ml of distillate. This technique is suitable for qualitative determinations rather than for quantitative ones.

Headspace Sampling Technique. The sampling technique described by von Sydow *et al.* (1970) was used with the following modifications: 75 g from one can was homogenized for 5 min at 0° with 75 ml of carbon-filtered distilled water in a 750-ml flask with a stainless steel lid, which also was used as the headspace sampling flask.

In this way transfer of the material from a homogenizer flask to a headspace flask was avoided, resulting in a better reproducibility. The flask was rotated for 45 min in an inclined position in a water bath held at 25 ± 0.1° to obtain equilibrium. A 500-ml sample of headspace gas was conveyed to the cold trap, which contained 70 mesh glass beads in the lower U-shaped part. For analysis of the distillates (25-ml samples) a 200-ml flask and a 150-ml headspace sample were used.

When analyzing the sulfur compounds with a Melpar Flame Photometric Detector a sampling system made entirely from glass and PTFE was used. After homogenization, the sample was transferred to a 750-ml headspace flask with a glass lid. The volatiles were transferred to the trap as described. All connections in the valve oven were made of PTFE tubing and the eight-port switching valve was substituted with an eight-port FEP/PTFE valve (Valco VSV-8-CI). One of the stainless steel cold traps was replaced by a glass trap, having otherwise the same configuration as the original one. This cold trap was used in the way described by von Sydow *et al.* (1970). The sample size was 400 ml and the valve oven was held at 60°. The other cold trap was replaced by a 2-ml sample loop made of PTFE tubing (2.2 mm o.d. × 1.2 mm i.d.). This was held at room temperature and was used for analyzing hydrogen sulfide and methanethiol which in some samples were present in such large amounts that the responses exceeded the working range of the FPD.

Gas Chromatography. The equipment consisted of a Perkin-Elmer Model 990 gas chromatograph with flame ionization detector and, when analyzing the sulfur compounds, a Perkin-Elmer Model 900 gas chromatograph with a Melpar sulfur specific Flame Photometric Detector (see below). The precolumn concentration equipment was connected to the chromatograph. When using the flame ionization detector the headspace gas was analyzed on an open tubular column, a 0.76 mm i.d. × 170 m long stainless steel tube coated with SF 96/Igepal CO 880 (95:5). The temperature was programmed from 20 to 140° at 2°/min after an initial isothermal period of 6 min. The helium carrier gas flow rate was 12 ml/min.

Table II. Formulations and Treatments of the Model Samples Analyzed

Model no.	Treatment	Formulation, %				
		Protein–H_2O	H_2O	NaCl	Starch	Fat
I	Unheated	79.3	20	0.7		
II	Heated (F_c = 28)	79.3	20	0.7		
III	Heated (F_c = 28)	61.3	20	0.7	5	13

For the absolute quantitative determinations of the components in the headspace gas the FID response factors were determined for the various compounds by experiment and from literature data (Dietz, 1967; Kaiser, 1962). The peaks were considered to have the same width. The peak heights were therefore used as a measure of the peak area. Using the absolute concentrations of thiophene, determined in the headspace gas according to the method described below, and the response factors, the absolute concentrations of the different compounds in the headspace gas were determined by measuring the peak heights (Persson and von Sydow, 1973).

When analyzing the sulfur compounds, the sulfur-specific Melpar Flame Photometric Detector (FPD) was used in conjunction with a Perkin-Elmer 900 gas chromatograph, to which the glass–PTFE sampling system (described above) was connected. The column used was a 3.0 mm i.d. × 6.3 m long glass column packed with Chromosorb G (acid-washed, DMCS treated, 80–100 mesh) coated with 5% Igepal CA 630 and percolated with 50 ml of 10% didecyl phthalate in acetone (Jansen *et al.*, 1971). The column end and the detector were connected with a PTFE tube (0.8 mm i.d. × 1.6 mm o.d.). For the 2- and the 400-ml samples the temperature was programmed from 20 to 120° at 10° and 4°/min, respectively, after an initial isothermal period of 10 min. The helium carrier gas flow was 45 ml/min. To calibrate the FPD with various sulfur compounds the procedure with permeation tubes calibrated gravimetrically was used (O'Keeffe and Ortman, 1966; Scaringelli *et al.*, 1970; Stevens *et al.*, 1971).

Mass Spectrometry. The samples were analyzed in a combined gas chromatograph–mass spectrometer, Perkin-Elmer 990 (FID)–LKB 9000 (TIC detector) with parallel detection in the gas chromatograph and the mass spectrometer. The headspace precolumn equipment described above was connected to the gas chromatograph. The gas chromatographic separation was made on the SF 96 open tubular column (see above). Mass spectra were recorded at 70 eV. The separator temperature was 200° and the ion source temperature was 270°. The compounds in the concentrate were identified by comparison with our own reference spectra or spectra given in the literature.

RESULTS AND DISCUSSION

Eight samples according to Tables I and II were investigated. EFP 90 with starch and fat added was not subject to investigation.

The headspace gas of a low temperature distillate of the various samples was analyzed in a combined gas chromatograph–mass spectrometer in order to obtain as much qualitative data as possible. In this way altogether over 150 compounds were identified, some of which are presented in Table III. Compounds not exemplified in Table III include 40 hydrocarbons, 4 aliphatic esters, 2 nitriles, 12 ketones, and 8 furan derivatives, most with only partially known structures. The hydrocarbons are generally uninteresting because of their high odor thresholds and the other compounds were present in such low concentrations that they cannot play a role in these samples. For the quantitative work straightforward analyses of the headspace gas of the eight samples were applied.

The chromatographic separations and quantifications were carried out using an SF 96 column with a FID for most of the non-sulfur-containing compounds and an Igepal CA 630–didecyl phthalate column with an S-FPD for the sulfur-containing ones. Typical examples of chromatograms obtained from analysis with FID and S-FPD are presented in Figures 1 and 2, respectively. Mass spectrometry was used again to ascertain the identity of the various eluted compounds. The five thiols (marked *f* in Table III) were not identified by mass spectrometry. Hydrogen sulfide was absorbed and masked by air and the four others were broken down in the analyzing system. The compounds marked *f* were identified using retention data obtained in our own experiments using known chemicals. The absolute concentrations were determined as described in the Experimental Section and by Persson and von Sydow (1973). The data are presented in Table III. Each value is a mean obtained from analysis of three or four replicates. The relative standard deviation in data varies between 5 and 25%. In some cases where the concentration is very high or very low or the compound extremely volatile, the variation can be larger. The concentrations of furan and 2-methyltetrahydrofuran are less accurate as these peaks are masked by pentane and benzene, respectively. Carbonyl sulfide was not determined quantitatively because of difficulties with the permeation tube technique.

Literature data for odor thresholds are included in Table III. The selection of compounds for Table III has been made from the odor point of view. Thus, the odor threshold data and the results from the investigation on canned beef (Persson and von Sydow, 1973, 1974a,b; Persson *et al.*, 1973a,b) were used when selecting the compounds. This does not mean that all compounds are of importance for the odor, only that they are of a potential interest.

From a chemical point of view the following observations can be made. The concentrations of almost all volatiles increase on heating, independent of protein source. Also a number of new compounds are detected in the heated samples as a result of thermally induced reactions. This is particularly true for sulfur compounds which are an interesting group of compounds from the sensory point of view. The concentrations of the alcohols do not seem to be affected very much by heat treatment or addition of starch and fat.

The concentrations of the straight-chain aldehydes in Promine D samples are greatly affected by heat, which does not seem to be the case for Sodinol V and EFP 90. For all three proteins the concentration of the branched chain aldehydes of low molecular weight increases markedly on heating, probably as a result of a Strecker degradation of the corresponding amino acids. This had earlier been pointed out by Nakanishi and Itoh (1967) in the case of casein.

The addition of starch and fat to Promine D and Sodinol V has only a small total effect on the concentrations of aldehydes. This can be the result of two opposite effects: the fat generates some aldehydes on heating (Watanabe and Sato, 1970) and the fat dissolves and retains some of the aldehydes generated through other mechanisms.

A large number of ketones were detected and determined. The concentration of the ketones generally increases on heating but the addition of fat reduces the increase, probably due to the solvent effect of the lipids.

The furans are more abundant in Promine D than in Sodinol V or EFP 90. However, independent of original concentrations, heat causes a large increase. The addition of fat diminishes the concentrations, probably due to a solvent effect.

In unheated samples there are very few sulfur compounds except in EFP 90. Heating results in drastic increases of concentrations and also results in the formation of sulfur compounds not detectable in the unheated samples. These findings are in accordance with Hrdlička and Čuda (1971) and Kato *et al.* (1972). The addition of fat has only a minor influence on the concentrations in general. The abundance of volatile sulfur compounds in EFP 90, unheated and heated, is probably caused by the high amount of S-containing amino acids in the protein, particularly methionine.

With the technique used in this investigation no pyrazines were detected although such compounds were found by Ferretti *et al.* (1970), Ferretti and Flanagan (1971), Kato *et al.* (1972), and Manley and Fagerson (1970a).

Table III. Absolute Concentrations (ppb, v/v) of Volatile Compounds in the Headspace Gas of Samples of Soy Protein (Promine D), Sodium Caseinate (Sodinol V), and Fish Protein (EFP 90)[a]

Compound	Peak no.	Promine D			Sodinol V			EFP 90		Odor thresholds, ppb in air (v/v)	References
		Unheated	Heated in H_2O[b]	Heated with fat, starch, and H_2O[b]	Unheated	Heated in H_2O[b]	Heated with fat, starch, and H_2O[b]	Unheated	Heated in H_2O[b]		
	See Fig 1										
Methanol	2	21	24	9						100,000	Leonardos *et al.* (1969)
Ethanol	8				24	26	10			10,000	Leonardos *et al.* (1969)
1-Butanol	26	0.8	3.2	1.1	1.2	0.3	0.8			303	Pliška and Reisenauer (1961)
1-Pentanol	40		1.4	2.2	0.1	0.1	0.1			255	Pliška and Reisenauer (1961)
1-Hexanol	51	4.1	9.2	0.7						1,030	Pliška (1962)
2-Propanol	6							83	20		
2-Methyl-2-propanol	14							Trace	1.0		
1-Penten-3-ol	27	3.5		1.5			0.7				
1-Octen-3-ol	59			Trace							
Ethanal	1	2500	4250	4400	540	4500	7700	6100	8250	210	Leonardos *et al.* (1969)
Propanal	5	Trace									
1-Butanal	10	17	125	108	3.7	4.2	10.5	3.3	14.6	9[d]	Guadagni *et al.* (1963)
1-Pentanal	22	68	780	720	3.6	4.1	24	0.2	3.1	12[d]	Guadagni *et al.* (1963)
1-Hexanal	38	260	1710	1390	11	15	39		0.6	4.5[d]	Guadagni *et al.* (1963)
1-Heptanal	50	2.2	41	21	2.4	5.1	4.9			3[d]	Guadagni *et al.* (1963)
1-Octanal	58	3.9	26	8.5	3.1	4.8	3.9		1.4	0.7[d]	Guadagni *et al.* (1963)
1-Nonanal	65	3.0	16	7.7	4.3	7.6	4.6		4.1	1[d]	Guadagni *et al.* (1963)
1-Decanal	69	0.1	0.3	0.2	1.6	3.0	0.7		2.2	0.1[d] 0.2	Guadagni *et al.* (1963) Teranishi (1967)
2-Methylpropanal	7	62	230	210	7.9	75	225	23	125	1[d]	Guadagni *et al.* (1972)
2-Methylbutanal	18	11	41	38	3.5	9.8	17	32	84		
3-Methylbutanal	16	5.6	36	52	5.8	14	36	23	70	0.2[d]	Guadagni *et al.* (1972)
a-Methylhexanal[c]	42	25	8.2	21							
2-Methylpropenal	9	68	21	13							
2-Methyl-2-butenal	29		4.9	3.5							
2-Methyl-2-pentenal	41	Trace	2.5	0.5				0.4			
2,4-Hexadienal	44		Trace								
2,4-Heptadienal	60			Trace							
2,4-Decadienal	70	0.3	0.2	0.1						0.07[d]	Guadagni *et al.* (1972)
Benzaldehyde	53	18	31	15	1.6	3.4	3.8	23	18	9,960	Pliška and Reisenauer (1961)
2-Propanone	3	360	4400	2200	31	410	1450	70	3800	100,000	Leonardos *et al.* (1969)
2-Butanone	11	64	190	140	11	37	210	14	33	10,000	Leonardos *et al.* (1969)
2-Pentanone	21	3.8	20	13	0.2	6.0	5.6	0.1	2.1		
2-Hexanone	35	4.6	21	9.9		0.4	0.3	0.1	0.5		
2-Heptanone	47	25	150	35	1.3	16	5.7	1.7	2.1	217	Weurman (1963)
2-Octanone	56	1.9	1.3	1.3	1.4	0.9	0.1	5.9	4.8		
2-Nonanone	63	0.7	2.9	0.4	0.9	4.2	0.4	0.6	1.3		
2-Decanone	68	0.1	0.3	0.3	0.1	0.2	0.1				
2-Undecanone	72				0.1	Trace	Trace			225	Pliška and Reisenauer (1961)
3-Pentanone	23								1.7		
3-Hexanone	34	0.4	1.0	0.5			0.1				
3-Heptanone	45	0.3	0.3				Trace		0.9		

3-Octanone	55	1.3	1.3	1.5							
5-Decanone	67	Trace	Trace	Trace							
3-Methyl-2-butanone	17	1.1	6.5	1.0					Trace		
3-Methyl-2-pentanone	31	0.4	1.3	0.4							
4-Methyl-2-pentanone	28	0.8	1.4	0.7	0.1	0.2	0.3		0.3		
2-Methyl-3-pentanone	30	0.7	1.7	0.5							
2-Methyl-5-decanone	71	0.5	0.5	0.3							
2,3-Butanedione	12					Trace	0.6				
Furan	4		~370	~170		~1800	~160	Trace	~120		
2-Methylfuran	13	14	310	110	9.8	165	110	12	52	27,000[e]	Evans *et al.* (1971)
2-Ethylfuran	24	130	4000	650	1.5	40	42	3.6	710	8,000[e]	Evans *et al.* (1971)
2-Propylfuran	37	1.5	57	2.7		0.6	0.2		1.9	6,000[e]	Evans *et al.* (1971)
2-Butylfuran	49	14	125	5.5		0.7				10,000[e]	Evans *et al.* (1971)
2-Pentylfuran	57	95	2700	110	7.7	15	3.2			2,000[e]	Evans *et al.* (1971)
2-Hexylfuran	64		0.2	0.1	0.1	0.4	0.1		1.6	6[d]	Buttery *et al.* (1971)
3-Methylfuran	15	9.1	59	16	0.5	3.2	4.8		0.2		
2,5-Dimethylfuran	25		31	11		0.8	1.4		Trace		
2-Methyl-5-ethylfuran	39	2.3	54	3.6		0.7	0.1	0.3	6.9		
2-Methyl-5-propylfuran	46	0.9	12	2.7		0.3					
2-Methyl-5-pentylfuran	62	0.1	1.4	0.1							
2-Methyltetrahydrofuran	19		~30	~30							
2-Propenylfuran	36	0.2	1.7				0.5		2.1		
	See Fig 2										
Carbonyl sulfide	S1	+	+	+	+	+	+	+	+		
Hydrogen sulfide[f]	S2	2.7	1690	1200	9.0	310	510	850	15,500	4.7	Leonardos *et al.* (1969)
Methanethiol[f]	S3		47	26	16	89	362	72	895	2.1	Leonardos *et al.* (1969)
Ethanethiol[f]	S4		0.8	0.7			0.9		3.7	1.0	Leonardos *et al.* (1969)
Dimethyl sulfide	S5	2.8	43	29	2.6	19	18	2.7	40	1.0	Leonardos *et al.* (1969)
Carbon disulfide	S6	0.4	13.2	17	1.2	19	19	2.7	12	210	Leonardos *et al.* (1969)
2-Propanethiol[f]	S7		1.1	1.1			3.2		34	0.45	Wilby (1969)
Ethylene sulfide	S9		3.3	5.6		6.3	15		3.8		
Methyl ethyl sulfide	S10		1.0	0.9		1.1	1.0	1.8	6.8		
Propylene sulfide	S12			Trace		Trace	Trace	0.3	0.6		
Methyl isopropyl sulfide	S13		0.7	1.1			2.3	1.2	7.1		
2-Methyl-1-propanethiol[f]	S14								2.8	1	Wilby (1969)
Diethyl sulfide	S15							5.3	1.7	5.9	Wilby (1969)
Methyl propyl sulfide	S17					1.2	1.6	Trace	2.1		
Dimethyl disulfide	S21		1.7	2.5	22	72	88	75	14	7.6	Wilby (1969)
Methyl pentyl sulfide	52, Fig 1		Trace								
2,3-Dithiohexan	48, Fig 1							Trace	Trace		
Thiophene	S16		130	24		26	2.7	0.5	6.9		
2-Methylthiophene	S22		3.9	0.8		2.3	0.9	Trace	11		
3-Methylthiophene	S23		1.4	Trace				0.8	1.4		
2-Ethylthiophene	S24		1.4	Trace					2.9		
2-Propylthiophene	54, Fig 1		3.7	0.3					Trace		
2-Butylthiophene	61, Fig 1		1.6	0.3							
2-Pentylthiophene	66, Fig 1	0.1	1.7	0.1							
3,5-Dimethyl-1,2,4-trithiolane[e,f]	S25		1.8	2.1	2.8	41	39	14	4		

[a] In water heated with and without starch and fat (*cf.* Table II). [b] At 121° to F_e = 28. [c] Tentative. [d] Parts per billion in water. [e] Parts per billion in cottonseed oil. [f] Identified by retention data only; all other compounds identified by mass spectrometry.

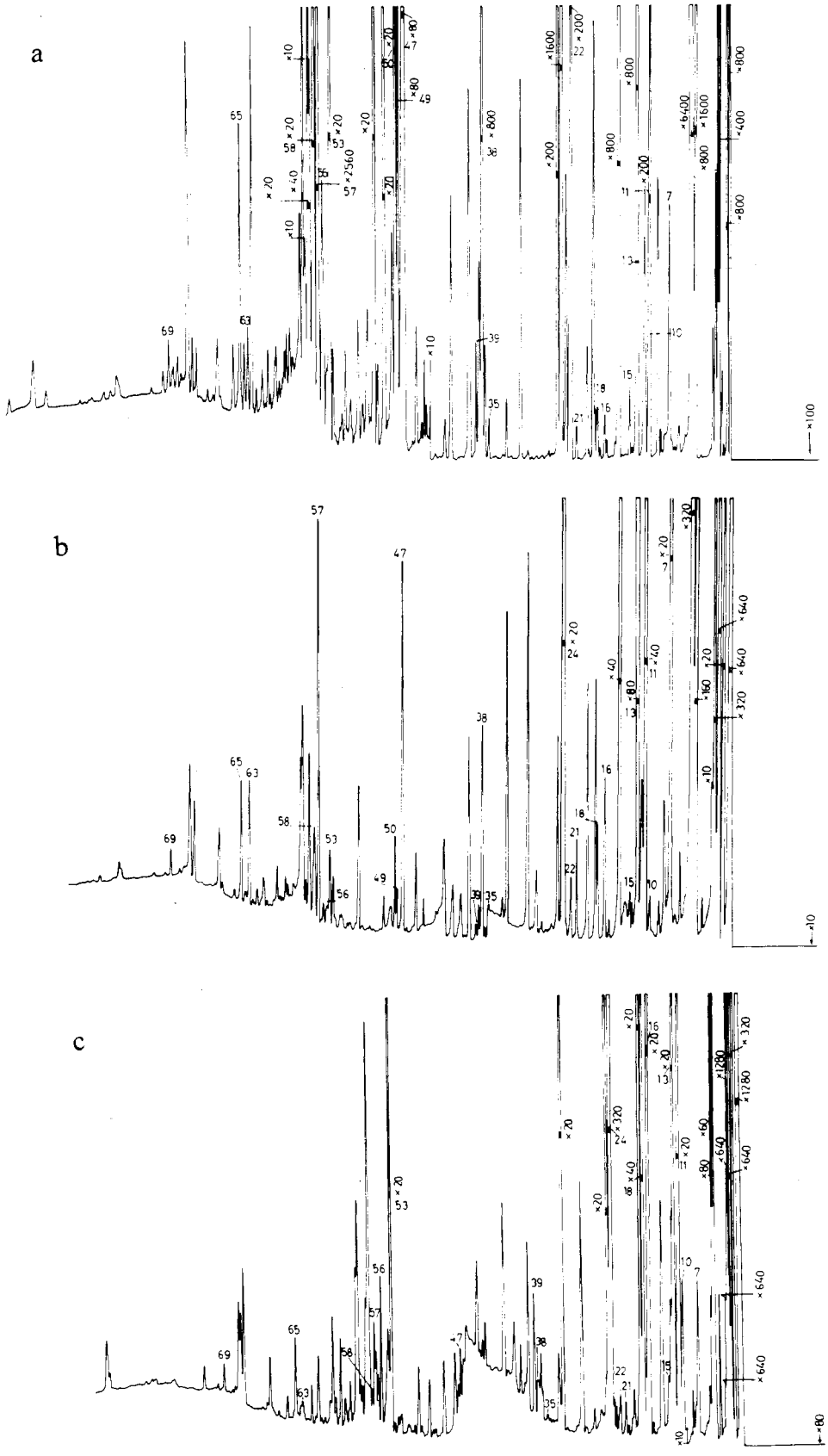

Figure 1. Gas chromatograms (FID) of 500-ml headspace samples of (a) Promine D, (b) Sodinol V, and (c) EFP 90; heated in water at 121° to F_c = 28; SF 96 column; peak numbers refer to Table III.

This discrepancy can be due to the fact that the heat processing conditions were very different or that the analytical procedure applied was also different.

A comparison of the volatiles in heated beef (Persson and von Sydow, 1973) and the heated unconventional proteins reveals a number of similarities and dissimilarities. Thus, aldehydes are important in all cases, but the concentrations of butanal, pentanal, and hexanal are much higher in the Promine D samples. The important branched chain aldehyde 2-methylpropanal is about four

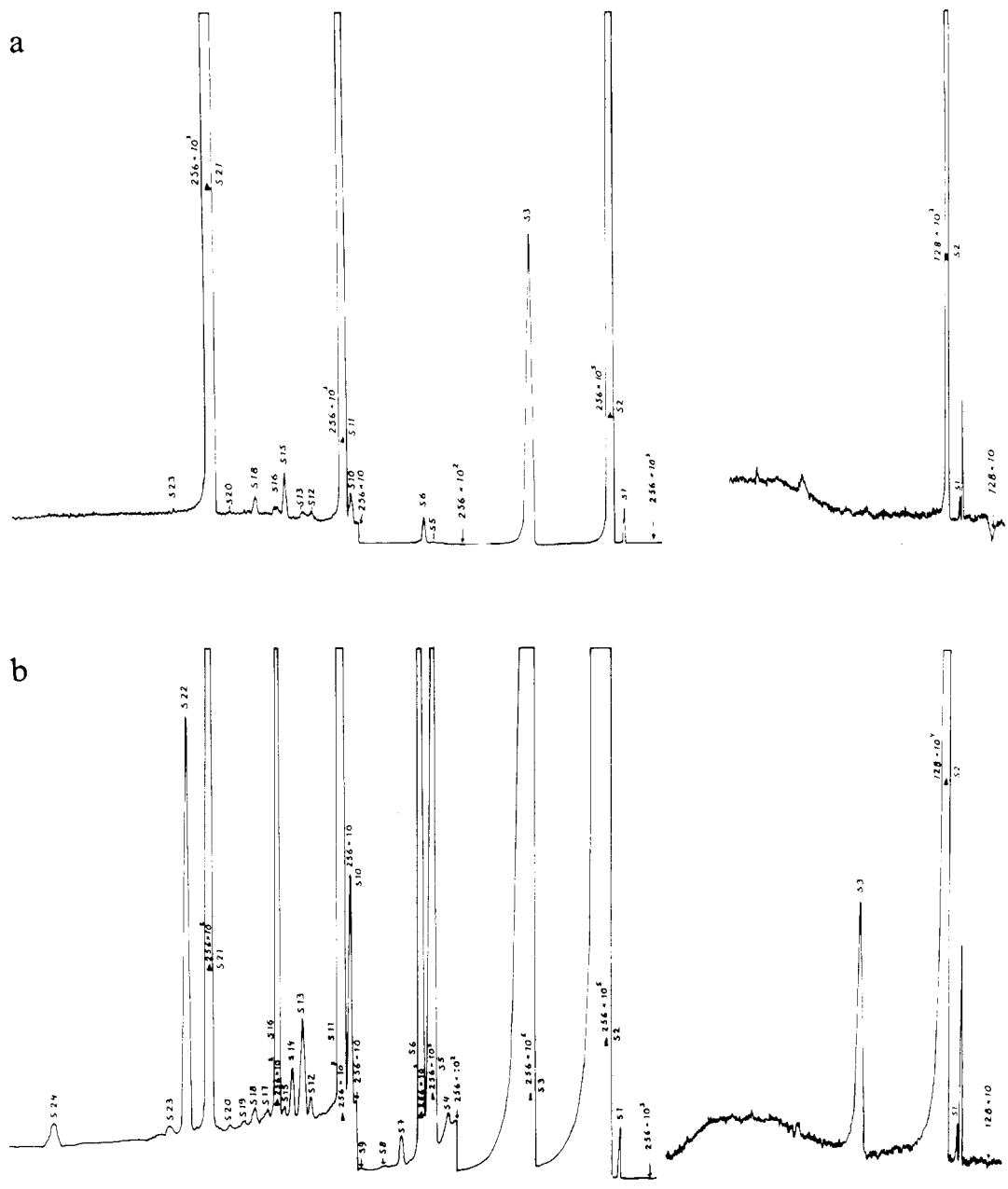

Figure 2. Gas chromatograms (S-FPD) of 2- and 400-ml headspace samples of EFP 90 in water: (a) unheated; (b) heated at 121° to F_c = 28; Igepal CA 630 column; peak numbers refer to Table III.

times more abundant in heated Promine D and Sodinol V (with fat added) than in the heated beef sample.

More furans were detected in the protein samples than in the beef samples and the concentrations of those present in both cases were generally higher in the protein samples. This is particularly true for Promine D.

Hydrogen sulfide, methanethiol, ethanethiol, dimethyl sulfide, and ethylene sulfide are present in larger concentrations in the heated beef samples than in the protein samples. One important exception is the very high concentration of H_2S in the heated sample of EFP 90. Dimethyl disulfide and thiophene are generally somewhat higher in concentration in the protein samples than in the beef samples.

From the odor point of view the following preliminary conclusions can be drawn based on odor threshold data and experience from samples of heat sterilized beef. The odor of all heated protein samples depends on the presence of low molecular weight branched chain aldehydes and sulfur compounds. Several straight-chain aldehydes and several furan derivatives are particularly important in Promine D. The high level of H_2S in heated EFP 90 is of importance for this sample. These matters will be dealt with in detail in further contributions.

ACKNOWLEDGMENTS

EFP 90 was kindly supplied by Astra Nutrition AB. Thanks are due to J. Andersson for helpful discussions and to R. M. Jansson and A. Moore for skillful technical assistance.

LITERATURE CITED

Buttery, R. G., Seifert, R. M., Guadagni, D. G., Ling, L. C., *J. Agr. Food Chem.*, **19**, 524 (1971).
Cowan, J. C., Rackis, J. J., Wolf, W. J., *J. Amer. Oil Chem. Soc.* **50**, 426A (1973).
Dietz, W. A., *J. Gas Chromatogr.* **5**, 68 (1967).
Evans, C. D., Moser, H. A., List, G. R., *J. Amer. Oil Chem. Soc.* **48**, 495 (1971).
Ferretti, A., Flanagan, V. P., *J. Agr. Food Chem.* **19**, 245 (1971).
Ferretti, A., Flanagan, V. P., Ruth, J. M., *J. Agr. Food Chem.* **18**, 13 (1970).

Forss, D. A., Jacobsen, V. M., Ramshaw, E. H., *J. Agr. Food Chem.* **15,** 1104 (1967).
Guadagni, D. G., Buttery, R. G., Okano, S., *J. Sci. Food Agr.* **14,** 761 (1963).
Guadagni, D. G., Buttery, R. G., Turnbaugh, J. G., *J. Sci. Food Agr.* **23,** 1435 (1972).
Harper, R., Bate-Smith, E. C., Land, D. G., Griffiths, N. M., *Perfum. Essent. Oil Rec.* **59,** 22 (1968a).
Harper, R., Land, D. G., Griffiths, N. M., Bate-Smith, E. C., *Brit. J. Psychol.* **59,** 231 (1968b).
Hrdlička, J., Čuda, P., *Sb. Vys. Sk. Chem. Tech. Potrav. Tech.* **E30,** 31 (1971).
Jansen, H. E., Strating, J., Westra, W. M., *J. Inst. Brew. London* **77,** 154 (1971).
Kaiser, R., in "Chromatographie in der Gasphase III," Bibliographisches Institut, Mannheim, 1962, p 116.
Kalbrener, J. E., Eldrige, A. C., Moser, H. A., Wolf, W. J., *Cereal Chem.* **48,** 595 (1971).
Kato, H., Hayse, F., Fujimaki, M., *Agr. Biol. Chem.* **36,** 951 (1972).
Leonardos, G., Kendall, D., Barnard, N., *J. Air Pollut. Contr. Ass.* **19,** 91 (1969).
Maga, J. A., *J. Agr. Food Chem.* **21,** 864 (1973).
Maga, J. A., Lorenz, K., *J. Milk Food Technol.* **35,** 131 (1972).
Maga, J. A., Lorenz, K., *J. Milk Food Technol.* **36,** 232 (1973).
Manley, C. H., Fagerson, I. S., *J. Agr. Food Chem.* **18,** 340 (1970a).
Manley, C. H., Fagerson, I. S., *J. Food Sci.* **35,** 286 (1970b).
Miller, A., III, Scanlan, R. A., Libbey, L. M., Petropakis, H., Anglemier, A. F., *J. Agr. Food Chem.* **21,** 451 (1973).
Nakanishi, T., Itoh, T., *Agr. Biol. Chem.* **31,** 1066 (1967).
National Canners Association, in "Laboratory Manual for Food Canners and Processors," 3rd ed, Avi Publishing Co., Westport, Conn., 1968, p 166.
O'Keeffe, A. E., Ortman, G. C., *Anal. Chem.* **38,** 760 (1966).
Persson, T., von Sydow, E., *J. Food Sci.* **38,** 377 (1973).
Persson, T., von Sydow, E., *J. Food Sci.* **39,** 406 (1974a).
Persson, T., von Sydow, E., *J. Food Sci.* **39,** 537 (1974b).
Persson, T., von Sydow, E., Åkesson, C., *J. Food Sci.* **38,** 386 (1973a).
Persson, T., von Sydow, E., Åkesson, C., *J. Food Sci.* **38,** 682 (1973b).
Pliška, V., *Sb. Vys. Sk. Chem. Tech. Potrav. Tech.* **6** (1), 37 (1962).
Pliška, V., Reisenauer, R., *Sb. Vys. Sk. Chem. Tech. Potrav. Tech.* **5** (3), 21 (1961).
Scaringelli, F. P., O'Keeffe, A. E., Rosenberg, E., Bell, J. P., *Anal. Chem.* **42,** 871 (1970).
Stevens, R. K., Mulik, J. D., O'Keeffe, A. E., Krost, K. J., *Anal. Chem.* **43,** 827 (1971).
Tanaka, T., Saito, N., *J. Ferment. Technol.* **47,** 772 (1969).
Teranishi, R., *Perfum. Essent. Oil Rec.* **58,** 172 (1967).
von Sydow, E., Andersson, J., Anjou, K., Karlsson, G., Land, D., Griffiths, N., *Lebensm.-Wiss. Technol.* **3,** 11 (1970).
von Sydow, E., Karlsson, G., *Lebensm.-Wiss. Technol.* **4,** 152 (1971).
Watanabe, K., Sato, Y., *Agr. Biol. Chem.* **34,** 1710 (1970).
Weurman, C., *Recent Advan. Food Sci.* **3,** 137 (1963).
Wick, E. L., Underriner, E., Paneras, E., *J. Food Sci.* **32,** 365 (1967).
Wilby, F. V., *J. Air Pollut. Contr. Ass.* **19,** 96 (1969).

Received for review February 22, 1974. Accepted June 17, 1974. Grants from the Swedish Board for Technical Development are gratefully acknowledged.

Fate and Disposition of Sucrose-*U*-^{14}C Acetate Isobutyrate in Humans, Rats, and Dogs

Ralph C. Reynolds,* Bernard D. Astill, Clarence J. Terhaar, and David W. Fassett

To determine the fate of sucrose acetate isobutyrate (SAIB) in mammals, SAIB-^{14}C, prepared from sucrose-*U*-^{14}C, was fed to humans, rats, and dogs and the elimination of radioactivity in breath, urine, and feces was studied. After a single oral dose of SAIB-^{14}C (1–1.2 mg/kg), humans eliminated within 30 days radioactivity in breath (41–66% of the dose), urine (15–21%), and feces (10%). Similarly, for two dogs fed 3 and 4.8 mg/kg, eliminations were 28 and 27% as $^{14}CO_2$, 7 and 5% in the urine, and 53 and 46% in the feces within 7 days, and for two rats fed 5.8 and 11.2 mg/kg, eliminations were 59 and 52% as $^{14}CO_2$, 11 and 13% in the urine, and 23 and 27% in the feces within 3 days. Urinary metabolites, detected solely by radiochromatography on paper, were apparently mostly partially esterified sucrose molecules with only traces of sucrose. They were similar in men and rats but possibly different in dogs. The results suggest that humans and rats handle SAIB similarly, the dog apparently differing both in the disposition of the dose and in the urinary excretory products.

Sucrose acetate isobutyrate (SAIB) is produced by the controlled esterification of sucrose with acetic and isobutyric anhydrides (Touey and Davis, 1960). The commercial material, more than 95% esterified with about 2 mol of acetate and 6 mol of isobutyrate per mol of sucrose, is an extremely viscous, colorless to light yellow liquid, insoluble in water, moderately soluble in aqueous ethanol, *n*-hexane, olive oil, and corn oil, and very soluble in ethanol and benzene. It has a very compact structure for its molecular weight (830–860). SAIB has potential use in soft drinks as a suspending agent for essential oils.

Octaesters of sucrose with short-chain fatty acids are well known and have some limited industrial uses, but little is known about their *in vivo* metabolism. Sucrose octaacetate, used to impart a bitter taste to animal feeds and rubbing alcohol to deter human consumption, had no adverse effect when fed to cows, ducks, chicks, and pigs and it could not be tasted after prolonged feeding in the flesh of fowl or animals or in cow's milk (Křiženecký, 1941a,b). Domingues *et al.* (1960) showed β-D-glucose pentaacetate to be completely absorbed and extensively utilized by the rat, undergoing some hydrolysis in the gut.

Feeding studies in rats (Krasavage *et al.*, 1973) showed SAIB to be without effect in 95-day feedings at up to 5% in the diet. Dogs had increased liver weights after feeding 0.6 and 2.0% in the diet (but not at 0.2%) and increased serum alkaline phosphatase activities and indocyanine green clearance times at the highest dose level. These effects were readily reversed when SAIB was withdrawn and were not seen in rats. We, therefore, investigated the fate

Health and Safety Laboratory, Eastman Kodak Company, Rochester, New York 14650.