Heating conditions can change the type and number of peptide fractions resolved by TLC analysis. The classification rule derived from the sequential TLC–HPLC analysis of the tryptic hydrolysates demonstrated a high degree of accuracy in discriminating and identifying proteins analyzed individually. This chromatographic analysis and statistical approach could be employed to detect nonmeat protein as an adulterant or additive in processed meat products. When a product is claimed to be all beef, the beef equation (eq 1) should give the highest score; otherwise, the product can be suspected of adulteration or indiscriminate use of nontraditional "meat" in meat products. Preliminary results indicate that a laboratory-formulated all-beef frankfurter can be discriminated from a standard frankfurter containing 35% pork protein.

Quantitation of the adulterant or protein additive is possible by establishing a calibration standard of protein mixtures. This would require a different statistical approach from what has been employed for the qualitative detection of adulterants or additives.

ACKNOWLEDGMENT

We thank Julio D. Pettinatti for his assistance in the use of the HPLC and Stanley A. Ackerman for his assistance in preparing the frankfurters.

LITERATURE CITED

Bailey, F. J. *J. Sci. Food Agric.* **1976**, *27*, 827–830.
Bailey, F. J.; Llewellyn, J. W.; Hitchcock, C. H. S.; Dean, A. C. *Chem. Ind. (London)* **1978**, *13*, 477–478.
Block, R. J.; Weiss, K. W. "Amino Acid Handbook"; Charles C. Thomas: Springfield, IL, 1956; pp 249–337.
Brenner, M.; Niederwieser, A.; Pataki, G. "Thin Layer Chromatography"; Stahl, E., Ed.; Springer-Verlag: New York, 1969; pp 730–786.
Eldridge, A. C. *J. Am. Oil Chem. Soc.* **1981**, *58*, 483–485.
Eldridge, A. C.; Wolf, W. J. *Proc.—World Soybean Res. Conf., 2nd, 1979* **1980**, 118–145.
FAO (Food and Agricultural Organization) "Amino Acid Content of Foods and Biological Data on Proteins"; Food and Agricultural Organization of the United Nations: Rome, 1970; pp 36–139.
Llewellyn, J. W.; Dean, A. C.; Sawyer, R.; Bailey, F. J.; Hitchcock, C. H. S. *J. Food Technol.* **1978**, *13*, 249–252.
Medina, M. B. Ph.D. Thesis, Rutgers University, 1978, pp 94–119.
Morton, R. K. *Methods Enzymol.* **1955**, *1*, 25–51.
Olsman, W. J. *J. Am. Oil Chem. Soc.* **1979**, *56*, 285–287.
Olsman, W. J.; Krol, B. "Methods for Detection and Determination of Vegetable Proteins in Meat Products", Report of the Study Group on Vegetable Proteins in Foodstuffs for Human Consumption in Particular in Meat Products EUR 6026 Commission of the European Communities; Luxemburg, 1978; pp 112–140.
Rao, C. R. "Linear Statistical Inference and Its Applications", 2nd ed.; Wiley: New York, 1973; pp 574–581.
Rivier, J. E. *J. Liq. Chromatogr.* **1978**, *1*, 343–366.
Stephens, R. E. *Anal. Biochem.* **1978**, *84*, 116–126.

Marjorie B. Medina*[1]
John G. Phillips[2]

[1]Eastern Regional Research Center
Agricultural Research Service
U.S. Department of Agriculture
Philadelphia, Pennsylvania 19118
[2]Consulting Statistician
Northeastern Region
Agricultural Research Service
U.S. Department of Agriculture
Philadelphia, Pennsylvania

Received for review December 21, 1981. Accepted June 28, 1982.

Effects of Some Food Additives and Fat Content in Meat on Lindane Transformation to Nonpolar Compounds during Cooking

The effects of antioxidant (butylated hydroxyanisole and ascorbic acid) and oxidant (H_2O_2) and acetic acid as well as the fat level on lindane (γ-BHC) transformation in meat during cooking were investigated. γ-BHC is transformed more readily in the presence of an antioxidant and in meat with a low fat level. Using capillary GL chromatography (EC detector), the nonpolar compounds 1,2-, 1,3-, and 1,4-dichlorobenzene, 1,2,3-, 1,2,4-, and 1,3,5-trichlorobenzene, γ-pentachlorocyclohexene, and hexachlorocyclohexene were detected as the major γ-BHC transformation products. The possible γ-BHC degradation pathway is briefly discussed.

The transformation of γ-BHC (lindane) in the environment proceeds by (1) dehydrogenation and HCCHE (hexachlorocyclohexene) formation, (2) dehydrochlorination and PCCHE (γ-pentachlorocyclohexene) formation, (3) isomerization to other BHC isomers (α, β, δ), and (4) transformation to HCB (hexachlorobenzene). Some authors (Engst et al., 1977, 1979; Haider, 1979; Kurihara et al., 1980) reported further degradation to chlorinated benzenes (CB) and phenols and dechlorination to nearly chlorine free compounds.

Only a few data on γ-BHC transformation in meat (Morgan et al., 1971; Ritchey et al., 1972; Mirna and Coretti, 1974; Mirna, 1976) during cooking can be found. The decrease in γ-BHC residues during meat processing is not yet a guarantee of toxicological safety; we must know the transformation products. In the present contribution, the influence of (1) some food additives—antioxidant [BHA (butylated hydroxyanisole) and ascorbic acid] in comparison to oxidant (H_2O_2)— and (2) fat content on γ-BHC degradation during meat cooking was investigated.

EXPERIMENTAL SECTION

Reagents. High-purity 1,2-dichlorobenzene (1,2-DCB), 1,3-dichlorobenzene (1,3-DCB), 1,4-dichlorobenzene (1,4,-DCB), 1,2,3-trichlorobenzene (1,2,3-TCB), 1,2,4-trichlorobenzene (1,2,4-TCB), 1,3,5-trichlorobenzene (1,3,5-TCB), 1,2,3,4-tetrachlorobenzene (1,2,3,4-TeCB), 1,2,3,5-tetrachlorobenzene (1,2,3,5-TeCB), 1,2,4,5-tetra-

0021-8561/82/1430-1253$01.25/0

chlorobenzene (1,2,4,5-TeCB), pentachlorobenzene (PCB), hexachlorobenzene (HCB), and Florisil, 60–100 mesh (Fluka, Buchs, Switzerland), α-, β-, γ-, and δ-BHC (Analabs Inc., NEN, Boston, MA), butylated hydroxyanisole (BHA) (Koch-Light Laboratories, Colbrook, Great Britain), and ascorbic acid, acetic acid, and hydrogen peroxide (Merck, Darmstadt, Federal Republic of Germany) were used as received. γ-Pentachlorocyclohexene (PCCHE) and hexachlorocyclohexene (HCCHE) were synthesized by standard methods. The solvents were distilled in a glass apparatus.

Sample Collection and History. After slaughter the sample of beef (M. Longissimus dorsi) was chilled to 3 °C and afterward stored. It was cut on the 14th day after slaughter; the pH was 5.8. Samples of beef containing either high (30.2%) or low (2.1%) levels of fat were taken. Fat content of the meat was determined by the Stoldt–Weibull method (Rauscher et al., 1972) with hydrochloric acid destruction. Meat was passed rapidly 3 times through a food chopper, being mixed thorougly after each grinding.

Gas–Liquid Chromatography. A Varian GC Model 3700 equipped with an electron capture detector (^{63}Ni) was used with capillary and packed columns. The columns used consisted of a 20-m glass capillary column with an i.d. of 0.22 mm coated with SE-30, as well as with OV-101 and a 2-m glass column with an i.d. of 2 mm packed with 1.5% OV-17 plus 1.95% QF-1 on 80–100-mesh Varaport 30 (supplied by Varian Associates, Palo Alto, CA).

Chromatographic conditions were as follows for the capillary column: temperatures were injector port 210 °C, detector 240 °C, initial oven temperature 60 °C held for 40 s (during the purge activation time) and then the temperature raised to 65 °C in 20 s and programmed from 65 to 180 °C at 5 °C/min; splitless injection, volume injected 2 and 3 μL; purge activation time 40 s; nitrogen flow was for the column 0.8 mL/min, injector purge 120 mL/min, and makeup 25 mL/min. The conditions for the packed column were as follows: temperatures were column 110 and 150 °C (isothermal), injector 210 °C, and detector 260 °C; nitrogen flow 30 and 40 mL/min, respectively.

The identification of all compounds was carried out by comparing their retention time with that of test substances. Two replications per meat extract were chromatographed on each column. Peak areas were measured by a Varian CDS 111 electronic integrator.

Procedure. γ-BHC was added at a concentration of 50 μg/g of meat. Ten-gram meat samples for processing were put in a individual glass vials and without degassing sealed and heated at 115 °C for 2 h. In some samples the additive acetic acid at 0.3%, ascorbic acid at 1%, BHA at 200 μg/g, or H_2O_2 at a 100 μg/g level was added. Some additional experiments were run in glass vials with cooking of 10 g of water with γ-BHC (50 μg/g of water) and 10 g of lean meat (2.1% fat) with α-BHC or PCCHE (50 μg/g of meat).

After being cooled, the vial was opened, 1 mL of 6 N sulfuric acid was added, and the content was extracted twice with 20 mL and finally with 10 mL of a solvent mixture of *n*-hexane and 2-propanol (1:1). The solvent extracts were decanted into the separatory funnel. For separation of 2-propanol, extraction with 100 mL of water (pH shifted below 2 with diluted sulfuric acid) was performed. The hexane layer was washed twice with 5% aqueous solution of K_2CO_3 to separate phenolic compounds and then with water and cleaned twice with concentrated sulfuric acid in a separatory funnel. The hexane extract was passed through a Florisil column 7 cm in height with an i.d. of 1 cm (activated overnight at 130 °C) with 0.5 cm of anhydrous Na_2SO_4 on the top and eluted with 20 mL of 6% diethyl ether in hexane. The eluate was concentrated to 5 or 2 mL at 50 °C in a nitrogen flow and analyzed by GL chromatography. At least two replications of the experiment were run with a reproducibility of ±10%.

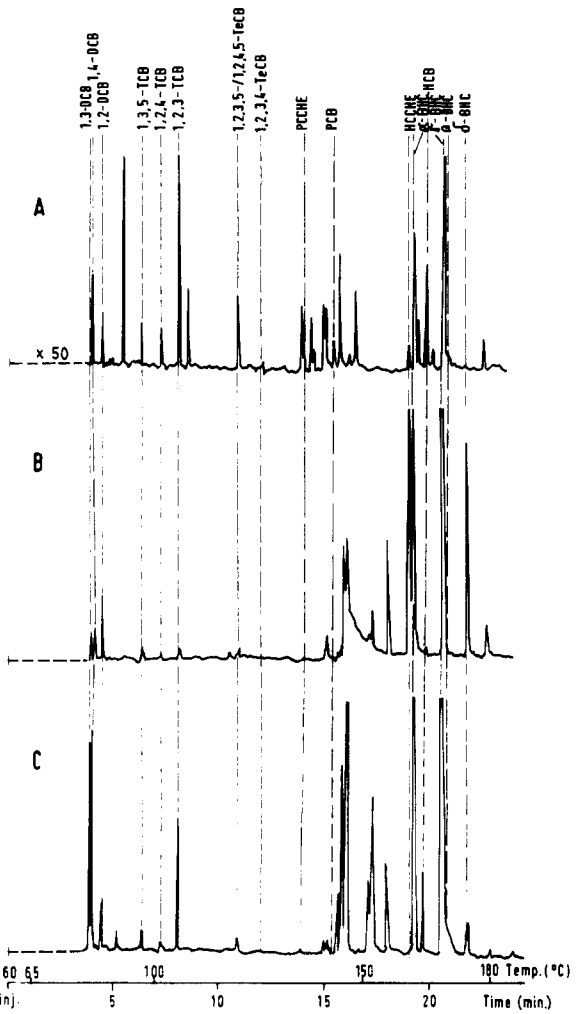

Figure 1. Chromatograms on the SE-30 capillary column of the low-fat (2.1%) meat extracts. (A) Uncooked meat (enhanced response) and meat processing with γ-BHC (B) in antioxidative BHA and (C) in oxidative H_2O_2 milieu.

Recoveries of organochlorine compounds added to cooked meat were studied after spiking at 0.05 and 0.5 μg/g levels except for γ-BHC, which was studied at 1–10 μg/g levels. Recoveries were in the range of 75–94% (for γ-BHC 90%) except for DCB isomers (64–72%) where the high volatility could be responsible for the losses.

RESULTS AND DISCUSSION

Some γ-BHC transformation products determined from the chromatograms obtained with capillary and packed columns are given in Table I. Figure 1 illustrates the chromatographic patterns of the nonpolar extracts of the uncooked meat and cooked with γ-BHC in oxidative and antioxidative milieu, and Figure 2 illustrates the chromatographic patterns of the γ-BHC transformation products after cooking meat of different fat levels. Some peaks in the chromatograms could not be identified.

In the products from γ-BHC degradation (Table I), DCB and TCB predominate, with negligible amounts of TeCB, PCB, and HCB. In some treatments PCCHE and HCCHE appear.

The additives in meat during cooking considerably decrease the γ-BHC level, except in acetic acid medium, which has nearly no influence on γ-BHC transformation at the concentration used. The formation of DCB pre-

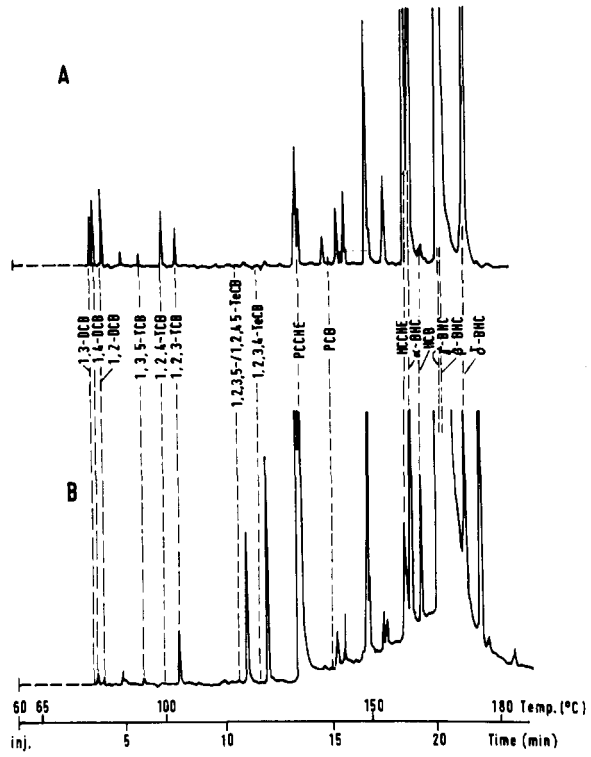

Figure 2. Chromatograms on the SE-30 capillary column of the transformation products of γ-BHC after cooking in the meat of different fat levels. (A) Lean meat (2.1% fat); (B) fatty meat (30.2% fat).

dominates in the oxidative milieu. The fat content plays an important role on the stability of γ-BHC in meat during cooking; it seems that γ-BHC due to its lipophilic character is protected in a fatty medium from reaction. In the extract made 4 h postcontact of meat with γ-BHC at room temperature, the PCCHE was detected. PCCHE was detected neither in raw meat nor in the γ-BHC standard. The opinion that the biotransformation of γ-BHC (Engst et al., 1977, 1979) takes place could be accepted.

For elucidation of the transformation of γ-BHC during the cooking of meat, a sample of lean meat is cooked with PCCHE to demonstrate that DCB and TCB as a transformation products of γ-BHC could be produced during one of the pathways via PCCHE. After meat processing with PCCHE, DCB and TCB were formed with elimination of PCCHE. The ratio to DCB degradation of the γ-BHC was approximately 1, 2, and 2 and of the PCCHE 1.5, 15, and 5 for the 1,3-, 1,4-, and 1,2-DCB isomers, respectively.

γ-BHC isomerization to other BHC isomers is difficult to prove because in purified γ-BHC other isomers also exist. There is, however, an indication of possible γ-BHC → α-BHC isomerization: in meat cooked with γ-BHC a small increase of α-BHC occurs, although cooking meat with α-BHC caused a decrease of that isomer.

The γ-BHC transformation proceeds in the presence of meat. No DCB was detected after cooking γ-BHC in water at the same temperature as the meat was cooked. The TCB and PCCHE were obtained only in trace levels, but some authors (Stein et al., 1977) reported that at 180 °C this degradation is more significant.

Our investigation shows that some additives and the fat level in meat during cooking influence on the transformation of γ-BHC. It is very likely that γ-BHC degradation goes through a PCCHE intermediate, which is further degraded. However, from the appearance of HCCHE as one γ-BHC degradation intermediate, we could not ignore

Table I. Levels of Some Nonpolar Compounds[a] Originating from γ-BHC Transformation in Meat during Cooking (Micrograms per Gram of Wet Meat Basis)

treatment	meat type[b]	preparation[c]	additives	DCB			TCB			TeCB							
				1,3-	1,4-	1,2-	1,3,5-	1,2,4-	1,2,3-	1,2,3,5- and/or 1,2,4,5-	1,2,3,4-	PCB	HCB	PCCHE	HCCHE	α-BHC	γ-BHC
1	LF	R[d]		nd[f]	0.01	tr[g]	tr	tr	tr	tr	nd	tr	tr	tr	tr	tr	tr
2	HF	R[e]		nd	0.01	0.01	0.01	tr	0.01	tr	nd	tr	tr	0.05	tr	0.09	36.20
3	LF	R		nd	0.02	0.01	0.01	tr	0.02	tr	nd	tr	0.01	0.06	tr	0.08	35.00
4	LF	C		0.40	0.72	0.70	0.03	0.10	0.06	tr	nd	tr	0.01	tr	0.09	0.13	1.40
5	HF	C		nd	0.01	tr	tr	tr	0.02	tr	nd	tr	0.02	0.04	0.02	0.07	9.70
6	LF	C	BHA	0.08	0.15	0.10	tr	tr	tr	tr	nd	tr	tr	tr	0.03	tr	0.01
7	LF	C	ascorbic acid	0.03	0.06	0.04	tr	tr	0.01	tr	tr	tr	tr	tr	0.01	tr	0.01
8	LF	C	H_2O_2	2.52	5.25	2.20	0.11	0.06	0.06	0.01	tr	tr	tr	tr	nd	0.03	0.13
9	LF	C	acetic acid	0.50	0.90	0.80	tr	0.01	0.01	tr	tr	tr	tr	tr	nd	0.04	1.80

[a] For compound abbreviations, see the text. [b] HF = high fat (30.2%); LH = low fat (2.1%). [c] C = cooked; R = raw. [d] Meat with no addition of γ-BHC. [e] Four hours postcontact with γ-BHC (50 μg/g of meat); values not corrected on recovery. [f] nd = not detectable. [g] tr = <0.01 μg/g.

other degradation pathways. Reduction of the γ-BHC level in cooked meat is not yet detoxification; from the toxicological aspect, CB is of similar toxicity to γ-BHC.

LITERATURE CITED

Engst, R.; Macholz, R. M.; Kujawa M. *Residue Rev.* **1977**, *68*, 59.
Engst, R.; Macholz, R. M.; Kujawa, M. *Residue Rev.* **1979**, *72*, 71.
Haider, K. *Z. Naturforsch., C: Biosci.* **1979**, *34C*, 1066.
Kurihara, N.; Yamakawa, K.; Fujita, T.; Nakajima, M. *Nippon Noyaku Gakkaishi* **1980**, *5*, 93.
Mirna, A. 1976, Report IAEA-R-1002-F.
Mirna, A.; Coretti, K. *Fleischwirtschaft* **1974**, *54*, 81.
Morgan, K. J.; Zabik, M. E.; Funk, K. *Poult. Sci.* **1971**, *51*, 470.
Rauscher, K.; Engst, R.; Fremuth, U. "Untersuchung von Lebensmittel"; VEB Fachbuchverlag: Leipzig, 1972.
Ritchey, S. J.; Young, R. W.; Essary, E. O. *J. Agric. Food Chem.* **1972**, *20*, 291.
Stein, K.; Portig, J.; Koransky, W. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1977**, *298*, 115.

Jurij Jan*
Stojan Malneršič
Public Health Institute of Slovenia,
Trubarjeva 2, 61000 Ljubljana, Yugoslavia

Received for review March 25, 1981. Revised manuscript received December 28, 1981. Accepted June 14, 1982. This work was financially supported by the Research Council for Slovenia. Presented at the World Congress on Foodborne Infections and Intoxications, West Berlin, 1980.

Identification of *N*-Methylsaccharin as a Peak at the Retention Time of Methylated (2,4,5-Trichlorophenoxy)acetic Acid in Human Urinary Exposure Samples

Human exposure to (2,4,5-trichlorophenoxy)acetic acid (2,4,5-T) may be detected by analysis of a 24–48 h postexposure urine sample. Acid hydrolysis of the urine followed by extraction with benzene and methylation of the extract with diazomethane will yield a gas chromatographic peak when a 6 ft long by 4 mm i.d. 4% SE-30/6% OV-210 column is used that matches that of a similarly treated standard of 2,4,5-T. Evidence is shown that *N*-methylsaccharin has an almost identical retention time and can be confused with 2,4,5-T methyl ester. Saccharin in diet drinks provides adequate amounts of urine saccharin for detection. GC/MS confirmation analysis supports the identification of *N*-methylsaccharin at the same retention time. Alternate columns can be used to detect 2,4,5-T exposure by using the same hydrolysis and extraction procedure. These would include a 6 ft long × 4 mm i.d. 1.5% OV-17/1.95% OV-210 and a 15 ft long by 4 mm i.d. SE-30/OV-210 column, both of which resolve the 2,4,5-T and saccharin peaks. Human exposure to 2,4,5-T is frequently determined by use of a modified Bevenue procedure (Bevenue et al., 1968) as described by Rivers et al. (1970). The procedure requires the benzene extraction of an acid-hydrolyzed urine or blood sample, followed by methylation of the extract with diazomethane. Determination is by electron-capture GC. Of the several options for column selection in the GC determination, it is frequently a choice of the analyst to use 4% SE-30/6% OV-210 as the liquid phase because of its general applicability to pesticide chemistry (Watts, 1980). This paper will describe work wherein an analyst can easily report a false positive for 2,4,5-T when the compound may actually be *N*-methylsaccharin [2-methyl-1,2-benzisothiazol-3(2*H*)-one 1,1-dioxide]. Diet drinks commonly yield enough saccharin in the urine so that a strong indication of 2,4,5-T could easily be suggested if a 6 ft long SE-30/OV-210 column is used for GC separation of peaks. Obviously, other column selections should be made, and the present work describes some successful alternatives.

EXPERIMENTAL SECTION

Apparatus. A Tracor Microtek 220 gas–liquid chromatograph equipped with a tritium electron-capture detector was fitted with a borosilicate glass column $^1/_4$ in. o.d. × 4 mm i.d. × 6 ft long. The column was packed with 4% SE-30/6% OV-210 on Gas-Chrom Q (80–100 mesh) and conditioned as described in the manual of analytical methods for the analysis of pesticide residues in human and environmental samples (Watts, 1980). Conditions for operating the chromatograph include the following: nitrogen flow, 100 mL/min.; column temperature, 160 °C; inlet temperature, 235 °C; detector temperature, 210 °C. The chart speed was $^1/_4$ in./min. The mass spectra were recorded on two separate instruments. Samples from subjects D.G. and D.M. were run on a Hewlett-Packard 5985, while the sample from subject M.C. was run on a Finnegan 4023 GC/MS/data system.

Reagents. Solvents were obtained from Burdick and Jackson, Muskegon, MI. Saccharin was purchased from Pfaltz and Bauer, Stanford, CT. Diazomethane was prepared from *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine purchased from Aldrich Chemical Co., Milwaukee, WI. Sulfuric acid was reagent grade, and a 0.1 N solution was extracted with Burdick and Jackson benzene prior to use. 2,4,5-T was from the EPA repository at Research Triangle Park, NC. Standard solutions were prepared in benzene with suitable dilutions. The diet cola consumed was a commercial product.

Sample Handling. Two subjects, M.C. and D.G., submitted urine samples after having had an alleged exposure to 2,4,5-T. The urine samples were collected several days postexposure and examined by the Bevenue–Rivers method. In both instances when the sample was analyzed by using the 6 ft SE-30/OV-210 column, a significant peak resulted within 0.5 mm or less of a similarly treated standard of 2,4,5-T. Because of the importance of identifying the 2,4,5-T, each sample was subjected to electron-impact GC/MS analysis. In Figure 1 are shown the spectra of the compounds isolated from two urine samples at the principal retention time of the methyl ester of

0021-8561/82/1430-1256$01.25/0