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Analysis of Acrolein from Heated Cooking Oils and Beef Fat

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Acrolein formed from heated cooking oils and from beef fat was quantified as the morpholine derivative. Headspace volatiles formed from cooking oils or beef fat heated at various temperatures were purged into an aqueous morpholine solution with either a nitrogen or an air stream. 3-Morpholinopropanal produced from acrolein and morpholine was extracted with dichloromethane and subsequently analyzed by a gas chromatograph equipped with a thermionic detector and a fused silica capillary column. Five cooking oils and beef fat were separately heated at 300 °C for 2 h, and the quantities of acrolein formed were determined. The amount of acrolein formed from 120-g samples ranged from 30 mg (soybean oil) to 72 mg (olive oil).

Acrolein is the simplest α,β-unsaturated aldehyde. It has been known as a lachrymator, and the vapor causes eye, nose, and throat irritation. Acrolein is used for many purposes including that of a biocide for aquatic weed control and that of an intermediate in the synthesis of many organic chemicals. Thus, it is often present in commerical products as a trace impurity. Acrolein has been found at very low levels in ambient air in urban and suburban areas (Brodzinsky and Singh, 1982), in emission from plants manufacturing acrylic acid (Serth et al., 1978), and in exhaust gas from a cornstarch manufacturing works (Hoshika et al., 1981).

Acrolein has been found in various foods such as sugarcane molasses (Hrdlicka and Janicek, 1968), souring salted pork (Cantoni et al., 1969), cooked horse mackerel (Shinomura et al., 1971), and white bread (Mulders and Dhont, 1972). Kishi (1975) detected acrolein at levels between 2.5 and 30 mg/m^3 in the air 15 cm above the surface of a heated oil. Acrolein was proposed to form from the dehydration of glycerol when animal or vegetable fats were heated to high temperatures (Izard and Libermann, 1978). In the present study, the amounts of acrolein formed from various heated cooking oils and from beef fat were determined as the morpholine derivative, 3-morpholinopropanal.

EXPERIMENTAL SECTION

Materials. Morpholine, acrolein, and tributylamine were purchased from Aldrich Chemical Co., Milwaukee, WI. The extraction solvent, dichloromethane, was obtained from J. T. Baker Chemical Co., Philipsburg, NJ. Corn oil was from Sigma Chemical Co., St. Louis, MO. Soybean oil, sunflower oil, olive oil, and sesame oil were purchased from a local market. Frozen fatty tissue, which was obtained from the renal periphery of beef carcasses, was ground to a powder in a blender with a small amount of dry ice and then melted in a flask in a hot water bath at 70–80 °C. All of the nonfatty tissue, including blood, muscle, and connective tissue, was removed from the liquid fat by filtration. The pure beef fat was then stored in a freezer for future experiments. The standard stock solution of tributylamine for gas chromatographic (GC) analysis was prepared by adding 50 mg of tributylamine to 1 mL of dichloromethane and was stored at 5 °C. The standard stock solution of acrolein for the gas chromatographic calibration curve was prepared by adding 1 g of

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Table I. Spectral Data of 3-Morpholinopropanal

MS, m/z (%)	143 (M^+, 3), 115 (82), 100 (43), 86 (37), 73 (28), 57 (95), 56 (90), 45 (57), 43 (43), 42 (100), 39 (43)
^{1}H NMR ($CDCl_3$), δ	9.70 (1 H, t, CHO), 3.62 (4 H, m, CH_2NCH_2), 2.78 (2 H, m, CH_2), 2.63 (2 H, m, CH_2), 2.41 (4 H, m, CH_2OCH_2)

acrolein to 10 mL of dichloromethane and was stored at 5 °C.

Instrumental Analysis. A Hewlett-Packard Model 5880A gas chromatograph (GC) equipped with a HP thermionic detector and a 30 m × 0.25 mm (i.d.) bonded phase DB-1 fused silica capillary column (J & W Scientific, Folsom, CA) was used for quantitative analysis of 3-morpholinopropanal derived from acrolein and morpholine. The oven temperature was programmed from 80 to 200 °C at 4 °C/min, and peak areas were integrated with an HP 5880A series GC terminal. The injector and detector temperatures were 250 °C. The gas flow rates in milliliters/minute were 1 for carrier gas (helium), 3 for the hydrogen, 60 for the air, and 20 for the makeup gas (nitrogen).

Reaction of Acrolein and Morpholine. Morpholine (300 mg) was added to 25 mL of dichloromethane in a 50-mL Erlenmeyer flask. The flask was sealed with a rubber septum and cooled in an ice bath for 5 min while the solution was stirred with a magnetic stirrer. The standard acrolein solution (1 mL) was injected into the flask through the rubber septum by a gas-tight syringe. The reaction mixture was stirred for 20 min with a magnetic stirrer. The gas chromatogram of the reaction mixture showed two peaks identified as unreacted morpholine and 3-morpholinopropanal by gas chromatography/mass spectrometry (GC/MS) analysis. Structure of 3-morpholinopropanal was also characterized with nuclear magnetic resonance (NMR), and the spectral data are shown in Table I.

Calibration Plot for Acrolein Analysis. Morpholine (300 mg) was reacted with various amounts of acrolein (25–150 mg) in 25 mL of dichloromethane as described above. The tributylamine standard solution (1 mL) was added to each reaction solution prior to GC analysis. The GC peak area ratio of 3-morpholinopropanal to the standard was plotted against quantity of acrolein reacted. The calibration curve for 3-morpholinopropanal shows an ideal linear relationship between the peak area ratio (3-morpholinopropanal/tributylamine) and the quantity of acrolein reacted. This linear relationship indicates the reaction between acrolein and morpholine proceeded quantitatively and reproducibly.

Analysis of Acrolein Formed in the Headspace of Heated Corn Oil. Corn oil (120 g) was placed in a 500-mL round-bottom, two-neck flask. The flask was heated at 180, 240, 280, 300, or 320 °C on a mantle heater. The mantle heater was adjusted to raise the temperature of oil to each specified temperature within 1 h, and then the oil was heated for an additional 2 h at each of these temperatures. The headspace volatiles formed were purged with a purified nitrogen stream (1 mL/s) into a 500-mL round-bottom two-neck flask containing 300 mg of morpholine in 250 mL of deionized water. The morpholine solution was stirred with a magnetic stirrer. 3-Morpholinopropanal formed from acrolein in the morpholine solution was extracted with 25 mL of dichloromethane for 5 h on a liquid–liquid continuous extractor. An extraction time of 5 h was used because more than 99% of 3-morpholinopropanal is extracted during this time. Tributylamine (1 mL) was added to the extract as an internal standard, and 2 μL of the extract was injected onto a GC. The GC of the extract showed only three peaks: solvent, unreacted morpholine, and 3-morpholinopropanal. Other volatile products formed from heated corn oil did not appear on the GC because a thermionic nitrogen–phosphorus specific detector was used. Nitrogen-containing compounds that may be formed from heated corn oil were too dilute in the extract to appear on the GC. The effect of temperature on acrolein formation is shown in Table II.

Table II. Amounts of Acrolein Determined in a Headspace of Corn Oil Heated at Various Temperatures for 2 h

temp, °C	amt, mg	temp, °C	amt, mg
180	0.00	300	54.08
240	0.42	320	141.56
280	5.36		

Table III. Amounts of Acrolein Determined in a Headspace of Corn Oil with Various Heating Times at 300 °C

time, h	amt, mg	time, h	amt, mg
1	24.16	5	140.52
2	54.08	6	165.01
3	86.82		

Table IV. Amounts of Acrolein Determined in the Headspace of Cooking Oils and of Beef Fat Heated at 300 °C for 2 h

sample	SP[a]	I[b]	amt determined, mg: N_2	amt determined, mg: air
corn oil	187–193	103–128	54.08	81.05
soybean oil	189–195	120–141	29.55	76.11
sunflower oil	188–194	125–136	36.90	57.61
olive oil	188–196	80–88	72.01	103.63
sesame oil	188–195	103–195	58.98	85.51
beef fat	193–202	38–45	45.45	75.16

[a] Saponification value (Codd et al., 1975). [b] Iodine value (Codd et al., 1975).

Corn oil was also heated at 300 °C for 1, 2, 3, 5, or 6 h and the acrolein formed analyzed. The effect of time on acrolein formation is shown in Table III.

Analysis of Acrolein in the Headspace of Heated Soybean Oil, Sunflower Oil, Olive Oil, Sesame Oil, and Beef Fat. Each sample of oil or beef fat (120 g each) was placed in a 500-mL round-bottom, two-neck flask. Beef fat was weighed after being melted with a water bath. The flask was heated at 300 °C for 2 h. The rest of the experimental procedure for the analysis of acrolein formed was the same as that previously described for corn oil except that headspace samples were also purged with air (1 mL/s), and amounts of acrolein formed under air purging were determined. The results are shown in Table IV along with saponification and iodine values of the oils and beef fat used.

RESULTS AND DISCUSSION

Isolation of volatile chemicals from a fatty sample is one of the most difficult analytical procedures, especially in the case of highly volatile compounds such as acrolein, which is also highly reactive and capable of self-polymerization (Hess et al., 1978). A review of available analytical methods for acrolein found in air, water, and food was published in 1980 (U.S. EPA, 1980).

Most methods for collecting acrolein in air involve the use of adsorbents or derivatives (Suzuki and Imai, 1982; Krost et al., 1982; Hoshika et al., 1981). Love and Bratzler (1966), for example, trapped acrolein formed from wood smoke in a (2,4-dinitrophenyl)hydrazine solution, and the derivative was identified by GC, but was not quantified.

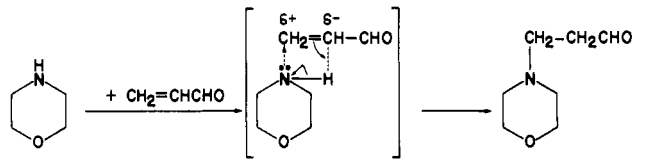

Figure 1. Reaction mechanisms of acrolein and morpholine.

Acrolein emitted from a commerical coffee roaster was trapped in Greenberg-Smith impingers, containing a 1% sodium bisulfite solution, and was quantified by the 4-hexylresorcinol colorimetric method (Levaggi and Feldstein, 1970). All of these methods require several tedious procedures. In the present study, acrolein formed in a headspace was reacted with morpholine to yield 3-morpholinopropanal. This reaction occurs under mild conditions at room temperature, and the product, 3-morpholinopropanal, is much more stable than acrolein and yet is sufficiently volatile for GC. Moreover, because 3-morpholinopropanal contains a nitrogen atom, the highly sensitive and selective thermionic specific detector can be used for analysis. Figure 1 shows proposed mechanisms of 3-morpholinopropanal formation from acrolein and morpholine.

Formation of acrolein from corn oil increased sharply when heated above 300 °C (Table III). Further experiments were, therefore, performed at 300 °C. The amount of acrolein formed from heated corn oil at this temperature showed an almost linear relationship with heating time (Table II). Heating times of 2 h were used for further experiments.

The saponification values of oils used in the present study indicate that the mean molecular weight values of fatty acids present in the oil are quite similar. Iodine values, which show the degree of unsaturation of fatty acid moieties, are somewhat variable among the oils used (Table IV). The amounts of acrolein formed seem to correspond negatively to iodine values. Olive oil, which had the lowest iodine value among the oils used, produced the most acrolein; soybean oil, which had the highest iodine value, produced the least acrolein. Three possible pathways of acrolein formation in heated fats or oils exist. The dehydration of glycerol is the main source of acrolein (Adkins and Hartung, 1935; pathway I, Figure 2). Acrolein is also formed from formaldehyde and acetaldehyde according to the general mechanisms of aldol condensation and croton condensation (Fishbein, 1972; pathway II, Figure 2). Free-radical mechanisms involving homolytic fission of R–O bonds are proposed because acrolein was formed under a nitrogen stream (pathway III, Figure 2). Unsaturated fatty acids are more chemically reactive than the saturated fatty acids. If pathway I predominates in acrolein formation, the water required for glyceride hydrolysis may be consumed by unsaturated fatty acid moieties. This result indicates decreased formation of acrolein with an oil of high iodine value when compared with an oil of low iodine value. That more acrolein formed under an air stream than under a nitrogen stream suggests that the presence of oxygen promoted common fatty acid degradations.

Fassett (1963) reported that exposure to 1 ppm (2.3 mg/m^3) acrolein vapor in the air caused lachrymation and marked eye, nose, and throat irritation to a human within a period of minutes. Acrolein caused severe pulmonary irritation at a level of 3 ppm (7 mg/m^3) and damaged the upper respiratory tract (Prentiss, 1937). Inhalation studies using experimental animals suggest that acrolein causes some damage to respiratory systems at levels of 2.3–13.8 mg/m^3 (Lyon et al. 1970; Bouley et al., 1975). Bauer et al. (1977) reported that accidental exposure of a human subject to vapors from an overheated frying pan containing fat and food items resulted in symptoms similar to those reported in cases of acrolein intoxication.

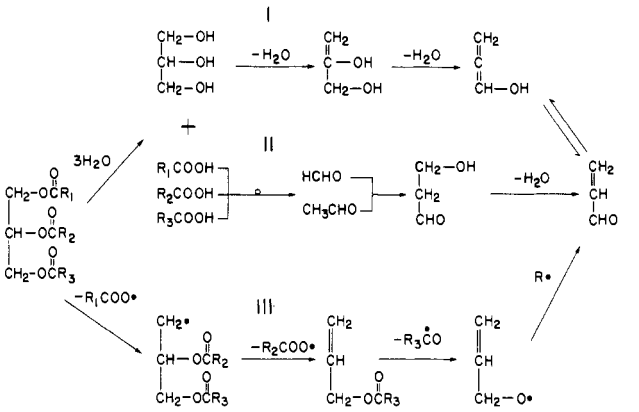

Figure 2. Proposed formation pathways of acrolein from triglyceride in cooking oils and in beef fat.

When corn oil was heated at 180 °C for 2 h, acrolein was not found in the headspace. In common home-cooking practices such as deep-fat frying, temperatures higher than 200 °C are rarely used. Thus, acrolein formation in home cooking may not be a serious problem. But in cooking some foods, broiled beef rib steaks for example, contact temperatures approach 300 °C (Paul and Palmer, 1972). Many studies on fats heated over 300 °C have been done with what were called "laboratory-heated fats" in order to determine chemicals formed from fat by high-temperature treatment (Crampton et al., 1956; Firestone et al., 1961; Schultz, 1962). In the present study, the acrolein concentration in the headspace of corn oil heated at 240 °C was 583 mg/m^3. This value seems very high, but during cooking the acrolein would be distributed into an open ambient area so that the actual concentration of acrolein to which persons in a kitchen would be exposed should be much lower. Because acrolein is reportedly toxic to animals, plants, and unicellular organisms, however, prolonged exposure may be hazardous; further research on acrolein is in order.

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Improved Synthesis of the Food Mutagen 2-Amino-3,7,8-trimethyl-3*H*-imidazo[4,5-*f*]quinoxaline and Activity in a Mammalian DNA Repair System

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A simplified, direct synthesis of 2-amino-3,7,8-trimethyl-3*H*-imidazo[4,5-*f*]quinoxaline (7,8-Me_2IQ_x) is described. Reaction of butane-2,3-dione with 4-nitro-1,2-phenylenediamine yielded 87% 2,3-dimethyl-6-nitroquinoxaline, which in three convenient steps gave 6-(methylamino)-2,3-dimethylquinoxaline (51% yield). Nitration and separation of the two nitrated isomers provided the needed 5-nitro derivative (42%) that upon catalytic reduction and reaction with cyanogen bromide gave 7,8-Me_2IQ_x in 65% yield. 7,8-Me_2IQ_x has about 50% of the mutagenicity of 2-amino-3-methyl-3*H*-imidazo[4,5-*f*]quinoline (IQ) in *Salmonella typhimurium* TA98 with biochemical activation and also in the Williams test, the induction of unscheduled DNA synthesis in rat hepatocytes. Thus, 7,8-Me_2IQ_x is genotoxic, and most likely carcinogenic, as are most chemicals with reliable activity in both tests.

The process of frying or broiling meat or fish under realistic conditions produces a series of chemicals generally belonging to the class of 2-amino-3-methyl-3*H*-imidazo-[4,5-*f*]quinolines or -quinoxalines (Figure 1). Depending on structure, some of these chemicals demonstrate high mutagenic activity in the Ames *Salmonella typhimurium* assay system (Hatch et al., 1986; Sugimura et al., 1986). In addition, those heterocyclic amines that have been tested in other bacterial or mammalian in vitro test systems uniformly exhibit activity. This includes activity in the selective and critical unscheduled DNA synthesis test (UDS) in freshly explanted rodent liver cells, the Williams test (Barnes et al., 1985). Hatch (1986) has reviewed the genotoxicity data base of these new heterocyclic amines.

The production of this class of mutagens has been thought to occur via Maillard-type reactions. The groups of Matsushima (1982), Jägerstad et al. (1986), and Taylor et al. (1986) have utilized in vitro systems to study mechanistic aspects and have found that the chemicals isolated from the surface of fried fish or meat could also be formed under these in vitro conditions. One of the chemicals so formed is 2-amino-3,7,8-trimethyl-3*H*-imidazo[4,5-*f*]quinoxaline (7,8-Me_2IQ_x) (Negishi et al., 1984; Jägerstad et al., 1986; Sugimura et al., 1986).

A number of syntheses for this class of chemicals have been reported (Kasai et al., 1980a,b, 1981; Adolfsson and

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