nitrogen, blended to a tissue powder, and stored at −20 °C. Phenylbutazone was added to the plasma which was then filtered through membrane cones. Plasma filtrates were stored at −20 °C. The concentration of sulfamethazine in plasma was determined using the method described in this report. Kidney tissue from 60 animals whose plasma contained from 0 to 2.8 ppm sulfamethazine was analyzed for sulfonamide content (Bevill et al., 1977). The coexistent plasma and tissue concentrations determined for each animal are presented in Figure 3. A line with slope of 2.51 and intercept of 0.01 was obtained when the data were analyzed by least-square linear regression. The coefficient of correlation of the plasma–tissue points with the least-square regression line was 0.916.

The assay of sulfamethazine in swine plasma has been given primary attention in this report. However, the assay of other sulfonamides appears feasible since the TLC system described earlier in this paper provided a distinct separation of sulfathiazole, sulfamethazine, and sulfadimethoxine which had average and standard deviations of *R*st values of 2.6 ± 0.2, 4.9 ± 0.3, and 5.3 ± 0.3, respectively.

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Effects of Air, Ozone, and Nitrogen Dioxide Exposure on the Oxidation of Corn and Soybean Lipids

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This study was conducted to determine the oxidative effects induced by exposure of corn and soybean seeds to air, nitrogen dioxide (NO_2), and ozone (O_3). Whole, halves, and ground samples of soybean seeds and whole corn kernels were exposed to air, 15 ppm NO_2, or 1.5 ppm O_3 continuously for 100 h at room temperature. Lipid oxidation was measured by polyunsaturated fatty acid (PUFA) and tocopherol destruction and formation of fluorescent lipofuscin-like pigments. Exposure of whole soybean and corn seeds to air, 15 ppm NO_2, or 1.5 ppm O_3 was found to induce no PUFA and tocopherol destruction and no formation of lipofuscin-like pigments. Tocopherol and PUFA destruction and lipofuscin-like pigment formation were detected in samples of soybean seed halves exposed to 15 ppm NO_2 or 1.5 ppm O_3; however, only tocopherol destruction occurred in soybean halves exposed to air. Ground soybean samples exposed to air, 15 ppm NO_2, or 1.5 ppm O_3 incurred the greatest PUFA and tocopherol destruction and lipofuscin-like pigment formation.

Nitrogen dioxide and ozone are two of the most abundant oxidants found in polluted urban air (Stern, 1976). Each is capable of free radical formation, these free radicals being highly potent oxidizing agents in biological systems (Goldstein and Balchum, 1967; Dowell et al., 1971).

Studies on the effects of gaseous oxidants have focused upon their in vivo effects in animals and plants (Goldstein et al., 1969; Thomas et al., 1968; Tingey et al., 1973; Heagle, 1972) and their in vitro effects in animal and plant tissue homogenates (Fletcher and Tappel, 1973; Ting and Heath, 1968). Ozone and nitrogen dioxide have each been shown to cause serious physiological and biochemical damage to plants and animals (Menzel, 1976). Although cereal grains and oil seeds are commonly stored and air-dried in polluted urban environments for several months prior to processing, little is known about the deteriorative biochemical effects of exposure of these edible oil-bearing grains to gaseous oxidants. In addition to loss in nutritional value, oxidation of edible corn and soybean lipids also can result in adverse effects on the flavor, color, texture, and economic value of food products and animal feeds.

Corn contains approximately 3.5–5% oil and soybeans contain from 20–25% oil. Both oils are high in polyunsaturated fatty acid (PUFA) content. High oil contents and high levels of PUFA make these grains potentially susceptible to gaseous oxidant-induced oxidative damage, this being especially possible in commercial soybeans where an appreciable percentage of the seeds have cracked or broken seedcoats.

Tocopherols have been shown to exhibit antioxidant effects on the in vivo and in vitro oxidation of polyunsaturated lipids (Witting, 1975; Tappel, 1965). A delicate balance apparently exists between the amounts and distributions of PUFA and tocopherols in an oil-containing system. Nitrogen dioxide and ozone, being strong oxidizing agents, may upset this delicate balance and induce oxidation of PUFA and tocopherols. Therefore, a study was conducted to determine PUFA and tocopherol destruction induced by short-term, continuous exposure of corn and soybean seeds to concentrations of nitrogen dioxide and ozone approximately tenfold greater than those normally found in polluted urban air. In addition, lipid oxidation

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in corn and soybean seeds was also measured by the quantitation of lipofuscin-like pigments, compounds believed to be metabolic end products of lipid oxidation in biological systems.

MATERIALS AND METHODS

Samples of Illinois High Oil, Illinois Low Oil, and R802A corn varieties were obtained from the University of Illinois, Urbana, Wayne and Amsoy soybean samples from the U.S. Regional Soybean Laboratory, Urbana, Ill., and M65-442 soybean samples from the University of Minnesota, St. Paul.

Whole corn samples and whole, halves, and 30/50 mesh ground soybean samples (30 g/sample) were placed in a glass exposure chamber and exposed to filtered air, 15 ppm NO_2, or 1.5 ppm O_3 continuously for 100 h at room temperature. Fifteen ppm NO_2 was produced by a 1:9 dilution of 150 ppm pressurized NO_2 in N_2 (Matheson Company, Inc., Joliet, Ill.) with filtered pressurized air using a rotameter gas proportioner also made by Matheson Company, Inc. Ozone was generated by an Airox Ozonator obtained from Pollution Control Industries, Stanford, Conn., and diluted to 1.5 ppm with filtered pressurized air using a glass dual-jet mixing chamber. The exposure apparatus was made of glass and stainless steel, and all connections were made using stainless steel Swagelok fittings. Samples were placed in ventilated glass petri dishes (5.5 in. diameter × 1 in. depth) having 45 holes each 0.25 cm diameter. Nine petri dishes containing the samples were stacked in a glass exposure chamber (6 in. diameter × 18 in. height) having a conical gas inlet and outlet. Nitrogen dioxide and O_3 were analyzed at 12-h intervals for 20 min using the methods of Saltzman (1954) and Saltzman and Gilbert (1959), respectively.

Samples were ground and extracted with acetone for 5 h in a Soxhlet apparatus, and the solvent removed using a rotary evaporator at 40 °C. Aliquots of the oil samples were transesterified by the method of Glass (1971) and analyzed for fatty acid methyl ester distributions using a Model 5830A Hewlett-Packard gas–liquid chromatograph equipped with dual-flame ionization detectors. Fatty acid methyl esters were separated using a 6 ft × 0.125 in. o.d. stainless steel column packed with 10% Silar 5CP on 100/120 Gas-Chrom Q and a helium carrier gas flow rate of 35 mL/min. Column temperature was programmed from 180 to 220 °C at a rate of 6 °C/min with a 15-min hold at 220 °C. Percent fatty acid methyl ester distributions were calculated using a slope sensitivity setting of 1.00 and an area rejection setting of 1000 units.

Tocopherols were determined using the method of Csallany and Draper (1961) which separates tocopherols from crystallizable lipids using a dry ice–acetone bath (−70 °C). After filtration under vacuum, washing with precooled acetone and drying under N_2 at 40 °C, free tocopherols in the residues were purified on silica gel G by two-dimensional thin-layer chromatography using chloroform and 20% isopropyl ether in hexane, respectively, as mobile phases (Pennock et al., 1964). Tocopherol isomers were located under ultraviolet light, scraped off, eluted with peroxide-free diethyl ether, and spectrophotometrically measured by the modified Emmerie-Engel procedure of Tsen (1961). Standard α-tocopherol (α-T), α-tocotrienol (α-T-3), γ-tocopherol (γ-T), γ-tocotrienol (γ-T-3), and δ-tocopherol (δ-T) were furnished by Hoffman-LaRoche, Inc., Nutley, N.J.

Lipofuscin-like pigments were isolated and measured using the method of Csallany and Ayaz (1976). Three grams of finely ground samples were extracted with 100 mL of 2:1 chloroform–methanol, the organic layer was washed twice with 50 mL of distilled water, and the combined water layers were back-washed four times with 30 mL of 2:1 chloroform–methanol. The combined organic layers were dried with anhydrous sodium sulfate and filtered, and the solvent was removed at 40 °C, using a rotary evaporator, to approximately 2 mL and then evaporated to dryness under N_2.

The samples were made up to 5 mL with 1:9 chloroform–methanol and 0.5-mL aliquots chromatographed on 1.5 cm × 35 cm Sephadex LH-20 columns using 1:9 chloroform–methanol as the mobile phase. Two-milliliter fractions were collected, and the relative fluorescence of each fraction was measured at an excitation of 365 nm and an emission of 435 nm using a Aminco-Bowman ratio spectrophotofluorometer calibrated daily with standard quinine sulfate solution (1 μg/mL in 0.1 N H_2SO_4) to read 100 relative fluorescence units. The slit arrangements of the instrument were 5, 5, 5, and 5 mm for slits 1, 3, 4, and 6, respectively.

RESULTS

Fatty Acid Distributions of Soybean Samples. The fatty acid distributions of whole, halves, and ground samples of Wayne, Amsoy, and M65-442 soybean varieties continuously exposed for 100 h to air, 15 ppm NO_2, or 1.5 ppm O_3, and of whole unexposed control samples, are shown in Table I. No changes in fatty acid distributions, compared to the whole unexposed control samples, were induced by exposure of whole soybean seeds to air, 15 ppm NO_2, or 1.5 ppm O_3 in any of the three varieties with three exceptions. The exceptions occurred as increases in the stearic and oleic acid percentages of Amsoy and M65-442 variety samples exposed to 1.5 ppm O_3.

The fatty acid distributions of soybean seed halves of all three varieties were not affected by exposure to air, with the exception of an increased stearic acid percentage in the Amsoy variety sample. Exposure of soybean seed halves of all three varieties to 15 ppm NO_2 induced no changes in palmitic or stearic acid percentages, but did cause decreased linoleic acid percentages in each variety. Increased oleic acid percentages, induced by exposure of soybean seed halves to 15 ppm NO_2, were found only in Wayne and M65-442 varieties while decreased linolenic acid percentages occurred only in M65-442 variety samples. Exposure of soybean seed halves to 1.5 ppm O_3 induced no change in the palmitic acid percentage of any of the three varieties, produced increased stearic acid percentages in only the Amsoy variety, and caused decreased linolenic acid percentages in all three varieties. Increased oleic acid percentages were found in Wayne and Amsoy variety seed halves after exposure to 1.5 ppm O_3 while decreased linoleic acid percentages occurred in only the Wayne variety seed halves.

The exposure of Wayne variety ground soybean samples to air induced decreases in only the linolenic acid percentages while air exposure of ground M65-442 variety samples induced increases in only the oleic acid percentages. All other fatty acid distributions in the ground Wayne and M65-442 samples exposed to air remained unchanged. Exposure of ground Amsoy variety samples to air caused increased palmitic acid percentages and decreased linoleic and linolenic acid percentages; however, no changes in stearic or oleic acid percentages were produced. Exposure of ground samples of all three soybean varieties to 15 ppm NO_2 or 1.5 ppm O_3 caused changes in the distributions of all five principal fatty acids with two exceptions. No changes were found in the stearic acid percentages of ground Wayne samples exposed to 15 ppm NO_2, and no changes were found in the palmitic acid

Table I. Fatty Acid Composition of Wayne, Amsoy, and M65-442 Soybean Varieties Continuously Exposed to Air, 15 ppm NO_2, or 1.5 ppm O_3 for 100 h

Sample	Fatty acid content (% + SD)[a]				
	16:0	18:0	18:1	18:2	18:3
Wayne whole					
Control	11.5 ± 0.63	4.4 ± 0.27	24.0 ± 0.18	53.2 ± 0.46	6.7 ± 0.24
Air	11.7 ± 0.14	4.5 ± 0.06	24.2 ± 0.15	52.8 ± 0.35	6.6 ± 0.53
15 ppm NO_2	11.5 ± 0.20	4.5 ± 0.06	24.3 ± 0.27	52.8 ± 0.40	6.4 ± 0.11
1.5 ppm O_3	11.4 ± 0.47	4.7 ± 0.06	24.3 ± 0.30	53.2 ± 0.37	6.4 ± 0.09
Wayne halves					
Control	11.5 ± 0.63	4.4 ± 0.27	24.0 ± 0.28[A b]	53.2 ± 0.46[A]	6.7 ± 0.24[A]
Air	11.8 ± 1.25	4.5 ± 0.13	24.6 ± 0.33[A]	52.3 ± 0.75[A]	6.7 ± 0.08[A]
15 ppm NO_2	11.5 ± 0.19	4.5 ± 0.05	25.3 ± 0.03[B]	51.1 ± 0.13[B]	6.6 ± 0.10[A]
1.5 ppm O_3	12.3 ± 0.35	4.6 ± 0.03	25.6 ± 0.04[B]	51.7 ± 0.28[B]	5.7 ± 0.06[B]
Wayne ground					
Control	11.5 ± 0.63[A]	4.4 ± 0.27[A]	24.0 ± 0.18[A]	53.2 ± 0.46[A]	6.7 ± 0.24[A]
Air	12.5 ± 0.51[A]	4.5 ± 0.07[A]	24.3 ± 0.15[A]	52.5 ± 0.28[A]	6.2 ± 0.12[B]
15 ppm NO_2	17.6 ± 0.42[B]	4.6 ± 0.08[A]	24.8 ± 0.17[B]	47.4 ± 0.16[B]	5.3 ± 0.06[B]
1.5 ppm O_3	13.4 ± 0.21[B]	4.8 ± 0.03[B]	25.2 ± 0.13[B]	51.2 ± 0.10[B]	5.2 ± 0.30[B]
Amsoy whole					
Control	13.2 ± 0.64	4.1 ± 0.09[A]	27.2 ± 0.25	48.5 ± 0.30	7.0 ± 0.08
Air	13.4 ± 1.12	4.1 ± 0.27[A]	27.0 ± 0.46	48.1 ± 0.51	7.0 ± 0.19
15 ppm NO_2	13.4 ± 0.45	4.2 ± 0.14[A]	27.4 ± 0.40	48.4 ± 0.33	7.0 ± 0.09
1.5 ppm O_3	12.9 ± 0.35	4.5 ± 0.09[B]	27.5 ± 0.29	48.3 ± 0.16	6.8 ± 0.15
Amsoy halves					
Control	13.2 ± 0.65	4.1 ± 0.09[A]	27.2 ± 0.25[A]	48.5 ± 0.30[A]	7.0 ± 0.08[A]
Air	12.8 ± 1.09	4.4 ± 0.10[B]	27.5 ± 0.06[A]	48.2 ± 0.76[A]	7.0 ± 0.15[A]
15 ppm NO_2	13.4 ± 0.35	4.2 ± 0.12[A]	27.3 ± 0.15[A]	47.9 ± 0.15[B]	7.1 ± 0.05[A]
1.5 ppm O_3	13.0 ± 0.21	4.5 ± 0.07[B]	27.9 ± 0.26[B]	48.0 ± 0.19[A]	6.5 ± 0.15[B]
Amsoy ground					
Control	13.2 ± 0.64[A]	4.1 ± 0.09[A]	27.2 ± 0.25[A]	48.5 ± 0.30[A]	7.0 ± 0.08[A]
Air	14.7 ± 0.64[B]	4.1 ± 0.07[A]	27.0 ± 0.17[A]	47.6 ± 0.36[B]	6.7 ± 0.18[B]
15 ppm NO_2	15.4 ± 0.31[B]	5.6 ± 0.13[B]	28.4 ± 0.38[B]	42.0 ± 0.88[B]	6.2 ± 0.18[B]
1.5 ppm O_3	14.8 ± 0.27[B]	5.0 ± 0.02[B]	28.0 ± 0.21[B]	45.9 ± 0.20[B]	6.3 ± 0.24[B]
M65-442 whole					
Control	12.4 ± 0.41	3.3 ± 0.10[A]	24.1 ± 0.20[A]	53.5 ± 0.21	6.7 ± 0.15
Air	12.5 ± 0.64	3.3 ± 0.15[A]	24.5 ± 0.29[A]	53.1 ± 0.25	6.6 ± 0.03
15 ppm NO_2	11.8 ± 0.39	3.5 ± 0.12[A]	24.0 ± 0.05[A]	53.7 ± 0.31	6.7 ± 0.14
1.5 ppm O_3	12.2 ± 0.18	3.6 ± 0.04[B]	24.8 ± 0.25[B]	53.4 ± 0.06	6.8 ± 0.28
M65-422 halves					
Control	12.4 ± 0.41	3.3 ± 0.10	24.1 ± 0.20[A]	53.5 ± 0.21[A]	6.7 ± 0.15[A]
Air	12.6 ± 0.50	3.3 ± 0.07	24.0 ± 0.19[A]	53.4 ± 0.29[A]	6.6 ± 0.06[A]
15 ppm NO_2	12.6 ± 0.26	3.4 ± 0.03	24.6 ± 0.17[B]	52.7 ± 0.15[B]	6.4 ± 0.05[B]
1.5 ppm O_3	12.3 ± 0.33	3.4 ± 0.03	24.2 ± 0.11[A]	53.4 ± 0.52[A]	6.5 ± 0.03[B]
M65-422 ground					
Control	12.4 ± 0.41[A]	3.3 ± 0.10[A]	24.1 ± 0.20[A]	53.5 ± 0.21[A]	6.7 ± 0.15[A]
Air	12.3 ± 0.26[A]	3.4 ± 0.03[A]	24.4 ± 0.04[B]	53.2 ± 0.13[A]	6.7 ± 0.06[A]
15 ppm NO_2	15.8 ± 0.43[B]	4.1 ± 0.11[B]	26.3 ± 0.32[B]	47.5 ± 0.45[B]	5.4 ± 0.03[B]
1.5 ppm O_3	12.0 ± 0.44[A]	3.6 ± 0.10[B]	25.8 ± 0.15[B]	52.6 ± 0.11[B]	6.0 ± 0.26[B]

[a] Average and standard deviation of three sample determinations. [b] Letter superscripts: Values in the same column (fatty acid) that do not share a common letter superscript with the control are significantly different from the control ($P < 0.05$) by an analysis of variance and a two-tailed t test. Values with no letter superscripts are not significantly different.

percentages of ground M65-442 samples exposed to 1.5 ppm O_3. It should be noted that the observed increase in the proportion of saturated and monoenoic acids are probably the reflections of the decrease in polyunsaturated fatty acid content of the seeds.

Tocopherol Distributions of Soybean Samples. The total tocopherol contents, the α-T, γ-T, and δ-T distributions, and the total oil contents of whole, halves, and ground samples of Wayne, Amsoy, and M65-442 soybean varieties continuously exposed for 100 h to air, 15 ppm NO_2, or 1.5 ppm O_3, and of whole unexposed control samples are shown in Table II. Exposure of whole soybean seeds of all three varieties to air, 15 ppm NO_2, or 1.5 ppm O_3 induced no oxidative destruction of any of the three tocopherol isomers when compared to whole unexposed control samples. Exposure of soybean seed halves to air, 15 ppm NO_2, or 1.5 ppm O_3 was found to induce significant destruction of all three tocopherol isomers in each of the three varieties with the exception of Amsoy seed halves, where no significant destruction of α-T occurred after exposure to air. Exposure of ground samples to air, 15 ppm NO_2, or 1.5 ppm O_3 induced significant destruction of each of the three tocopherol isomers in all three soybean varieties.

Fatty Acid Distributions of Corn Samples. The fatty acid distributions of samples of whole, perfect kernels of Illinois High Oil (IHO), Illinois Low Oil (ILO), and R802A corn varieties continuously exposed to air, 15 ppm NO_2, or 1.5 ppm O_3 for 100 h at room temperature, and of whole unexposed control samples, are shown in Table III. No changes in fatty acid distributions were induced in any of the three corn varieties exposed to air, 15 ppm NO_2, or 1.5 ppm O_3 when compared to the whole unexposed control samples.

Tocopherol Distributions of Corn Samples. The total tocopherol contents, the α-T, α-T-3, γ-T, and γ-T-3 distributions, and the total oil contents of samples of whole, perfect kernels of IHO, ILO, and R802A corn varieties continuously exposed to air, 15 ppm NO_2, or 1.5 ppm O_3 for 100 h at room temperature, and of whole unexposed control samples, are shown in Table IV. No significant destruction of any of the four tocopherol iso-

Table II. Tocopherol and Oil Content of Wayne, Amsoy, and M65-442 Soybean Varieties Continuously Exposed to Air, 15 ppm NO_2, or 1.5 ppm O_3 for 100 h

Sample	Tocopherol content, (μg/g of oil + SD)[a]				
	α-Tocopherol	γ-Tocopherol	δ-Tocopherol	Total tocopherol	Oil content, %
Wayne whole					
Control	58 ± 0.9	626 ± 14.6	284 ± 10.4	968	20.7
Air	58 ± 0.0	621 ± 17.0	283 ± 7.8	962	20.8
15 ppm NO_2	58 ± 0.3	620 ± 21.9	276 ± 14.1	954	20.1
1.5 ppm O_3	56 ± 2.5	615 ± 5.0	278 ± 9.2	949	20.7
Wayne halves					
Control	58 ± 0.9[A b]	626 ± 14.6[A]	284 ± 10.4[A]	968	20.7
Air	48 ± 0.0[B]	573 ± 22.6[B]	252 ± 14.1[B]	873	20.3
15 ppm NO_2	37 ± 1.1[B]	486 ± 8.5[B]	244 ± 2.1[B]	767	20.6
1.5 ppm O_3	45 ± 3.7[B]	544 ± 28.3[B]	244 ± 11.3[B]	833	21.0
Wayne ground					
Control	58 ± 0.9[A]	626 ± 14.6[A]	284 ± 10.4[A]	968	20.7
Air	38 ± 0.0[B]	372 ± 10.6[B]	164 ± 19.8[B]	574	20.8
15 ppm NO_2	26 ± 0.9[B]	305 ± 1.4[B]	145 ± 10.6[B]	476	21.1
1.5 ppm O_3	27 ± 0.7[B]	354 ± 0.0[B]	146 ± 15.6[B]	527	20.5
Amsoy whole					
Control	77 ± 4.0	638 ± 12.3	366 ± 10.4	1081	22.5
Air	77 ± 5.7	632 ± 25.5	364 ± 10.6	1073	22.3
15 ppm NO_2	70 ± 5.7	628 ± 7.8	352 ± 7.8	1050	22.8
1.5 ppm O_3	74 ± 0.6	630 ± 3.5	356 ± 12.7	1060	22.5
Amsoy halves					
Control	77 ± 4.0[A]	638 ± 12.3[A]	366 ± 10.4[A]	1081	22.5
Air	74 ± 1.4[A]	596 ± 5.0[B]	307 ± 15.6[B]	977	22.8
15 ppm NO_2	56 ± 2.1[B]	580 ± 15.6[B]	257 ± 7.8[B]	893	23.1
1.5 ppm O_3	64 ± 0.6[B]	582 ± 7.1[B]	293 ± 4.2[B]	939	22.7
Amsoy ground					
Control	77 ± 4.0[A]	638 ± 12.3[A]	366 ± 10.4[A]	1081	22.5
Air	52 ± 2.8[B]	447 ± 5.0[B]	192 ± 5.7[B]	691	22.1
15 ppm NO_2	50 ± 3.5[B]	418 ± 2.1[B]	179 ± 0.7[B]	647	22.6
1.5 ppm O_3	52 ± 0.3[B]	460 ± 0.7[B]	205 ± 0.7[B]	717	22.2
M65-442 whole					
Control	98 ± 4.1	708 ± 8.2	349 ± 9.2	1155	25.2
Air	98 ± 7.4	704 ± 16.9	347 ± 18.4	1149	24.9
15 ppm NO_2	92 ± 4.2	697 ± 3.5	338 ± 7.8	1128	24.7
1.5 ppm O_3	95 ± 0.7	702 ± 4.2	339 ± 1.4	1136	25.1
M65-442 halves					
Control	98 ± 4.1[A]	708 ± 8.2[A]	349 ± 9.2[A]	1155	24.9
Air	88 ± 5.0[B]	668 ± 14.1[B]	303 ± 1.4[B]	1059	25.1
15 ppm NO_2	81 ± 0.7[B]	659 ± 6.4[B]	298 ± 2.8[B]	1038	24.9
1.5 ppm O_3	84 ± 2.4[B]	667 ± 10.6[B]	313 ± 8.5[B]	1064	25.3
M65-442 ground					
Control	98 ± 4.1[A]	708 ± 8.2[A]	349 ± 9.2[A]	1155	24.9
Air	73 ± 7.8[B]	531 ± 6.4[B]	196 ± 14.9[B]	800	25.3
15 ppm NO_2	67 ± 1.4[B]	490 ± 0.7[B]	178 ± 7.1[B]	735	25.2
1.5 ppm O_3	73 ± 0.1[B]	536 ± 7.1[B]	196 ± 1.4[B]	805	24.7

[a] Average and standard deviation of two sample determinations. [b] Letter superscripts: Values in the same column (tocopherol isomer) that do not share a common letter superscript with the control are significantly different from the control ($P < 0.05$) by an analysis of variance and a two-tailed t test. Values with no letter superscripts are not significantly different.

mers were found in IHO, ILO, and R802A varieties exposed to air, 15 ppm NO_2, or 1.5 ppm O_3 when compared to the whole unexposed control samples.

Lipofuscin-like Pigments (LLP) in Soybean Samples. Figure 1 shows the Sephadex LH-20 elution profiles of the organic solvent-soluble fluorescent compounds found in ground Wayne variety soybean samples continuously exposed to air, 15 ppm NO_2, or 1.5 ppm O_3 for 100 h at room temperature, and the elution profiles of the organic solvent-soluble fluorescent compounds extracted from mouse heart tissue. The first peak of each of the ground Wayne samples exposed to air, NO_2, or O_3 eluted from the column in fractions 8–14, the same region as purified mammalian organic solvent-soluble lipofuscin pigments extracted from mouse heart tissue. Fluorescent emission and excitation spectra of ground Wayne samples exposed to air, NO_2, or O_3 and of purified mammalian organic solvent-soluble lipofuscin pigments are shown in Figure 2. Soybean samples exhibited excitation maxima at 350 nm and emission maxima at 420 nm while purified mammalian lipofuscin pigments had an excitation maximum at 345–350 nm and an emission maximum at 435 nm. Identical elution profiles and fluorescence excitation and emission maxima were obtained from all three soybean varieties following exposure to air, 15 ppm NO_2, or 1.5 ppm O_3.

The total concentrations of LLP (expressed as total relative fluorescence in fractions 8–14 per gram of sample) in whole, halves, and ground samples of Wayne, Amsoy, and M65-442 soybean varieties continuously exposed to air, 15 ppm NO_2, or 1.5 ppm O_3 for 100 h at room temperature, and of whole unexposed control samples, are presented in Figure 3. No increases in the concentrations of LLP eluting in fractions 8–14 were induced by exposure of whole soybean seeds to air, NO_2, or O_3 in any of the three varieties. The concentrations of LLP of Wayne and Amsoy seed halves were unaffected by air exposure; however, increased concentrations of LLP occurred in M65-442 seed halves exposed to air. Exposure of soybean seed halves of each variety to 15 ppm NO_2 or 1.5 ppm O_3

Table III. Fatty Acid Composition of Illinois High Oil, Illinois Low Oil, and R802A Corn Varieties Continuously Exposed to Air, 15 ppm NO_2, or 1.5 ppm O_3 for 100 h

Sample	Fatty acid content, % SD[a]				
	16:0	18:0	18:1	18:2	18:3
Illinois High Oil					
Control	12.2 ± 0.18[b]	2.1 ± 0.03	38.0 ± 0.13	47.1 ± 0.08	0.6 ± 0.02
Air	12.3 ± 0.34	2.1 ± 0.02	38.4 ± 0.27	46.6 ± 0.45	0.6 ± 0.01
15 ppm NO_2	12.2 ± 0.04	2.1 ± 0.02	37.7 ± 0.05	47.3 ± 0.18	0.6 ± 0.04
1.5 ppm O_3	11.7 ± 0.37	2.0 ± 0.04	38.7 ± 0.58	47.0 ± 0.09	0.6 ± 0.02
Illinois Low Oil					
Control	18.4 ± 0.74	1.0 ± 0.10	12.9 ± 0.22	65.4 ± 0.52	2.3 ± 0.13
Air	18.6 ± 0.56	1.1 ± 0.06	13.1 ± 0.05	65.0 ± 0.50	2.2 ± 0.02
15 ppm NO_2	18.0 ± 0.17	1.4 ± 0.36	13.0 ± 0.10	65.5 ± 0.14	2.2 ± 0.12
1.5 ppm O_3	17.3 ± 1.51	0.9 ± 0.20	13.3 ± 0.20	66.1 ± 0.91	2.4 ± 0.24
R802A					
Control	13.8 ± 0.29	1.6 ± 0.12	23.6 ± 0.19	59.8 ± 0.43	1.2 ± 0.13
Air	14.0 ± 0.15	1.7 ± 0.13	23.9 ± 0.18	59.3 ± 0.13	1.1 ± 0.12
15 ppm NO_2	13.4 ± 0.19	1.6 ± 0.11	23.3 ± 0.44	60.5 ± 0.41	1.1 ± 0.13
1.5 ppm O_3	14.2 ± 0.24	1.6 ± 0.12	23.9 ± 0.14	59.2 ± 0.34	1.1 ± 0.12

[a] Average and standard deviation of three sample determinations. [b] Letter superscripts: Values in same column (fatty acid) that do not share a common letters superscript with the control are significantly different from the control ($P < 0.05$) by an analysis of variance and a two-tailed t test. Values with no letter superscripts are not significantly different.

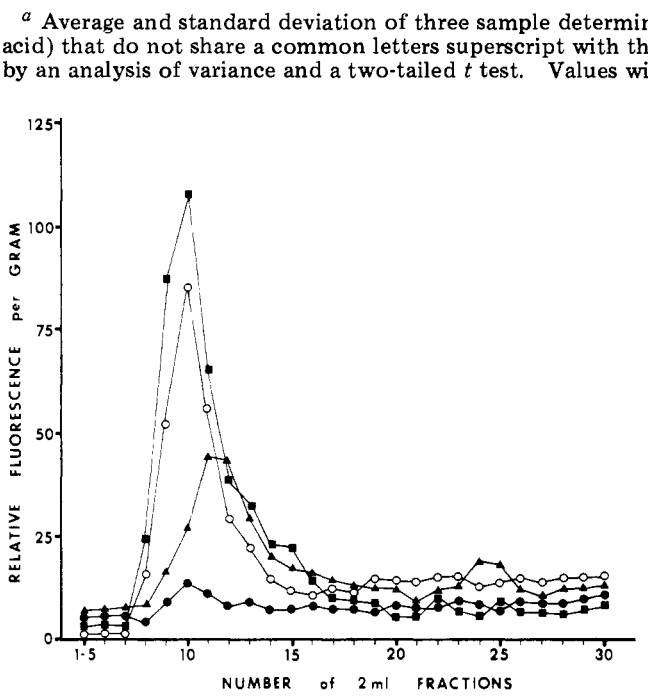

Figure 1. Elution profiles of the organic solvent-soluble fluorescent compounds of ground Wayne variety soybean samples continuously exposed to air (●—●), 15 ppm NO_2 (▲–▲), or 1.5 ppm O_3 (■—■) for 100 h at room temperature and of fluorescent mammalian lipofuscin pigments extracted from mouse heart tissue (○—○).

induced significantly higher concentrations of LLP. Exposure of ground samples to air, 15 ppm NO_2, or 1.5 ppm O_3 induced significantly higher concentrations of LLP in all three soybean varieties.

Lipofuscin-Like Pigments in Corn Samples. No LLP were detected in samples of whole, perfect kernels of IHO, ILO, and R802A corn varieties continuously exposed to air, 15 ppm NO_2, or 1.5 ppm O_3 for 100 h at room temperature.

DISCUSSION

The fact that exposure of whole soybean and whole corn seeds to air, 15 ppm NO_2, or 1.5 ppm O_3 did not induce detectable oxidative destruction of PUFA and tocopherols, and induced no formation of LLP, indicates that under these experimental conditions seeds were effectively protected from oxidative damage by their intact seedcoats. Chow and Draper (1969) reported similarly no detectable oxidation of PUFA and tocopherols in whole, perfect corn kernels dried in air at 30–60 °C for 48 h.

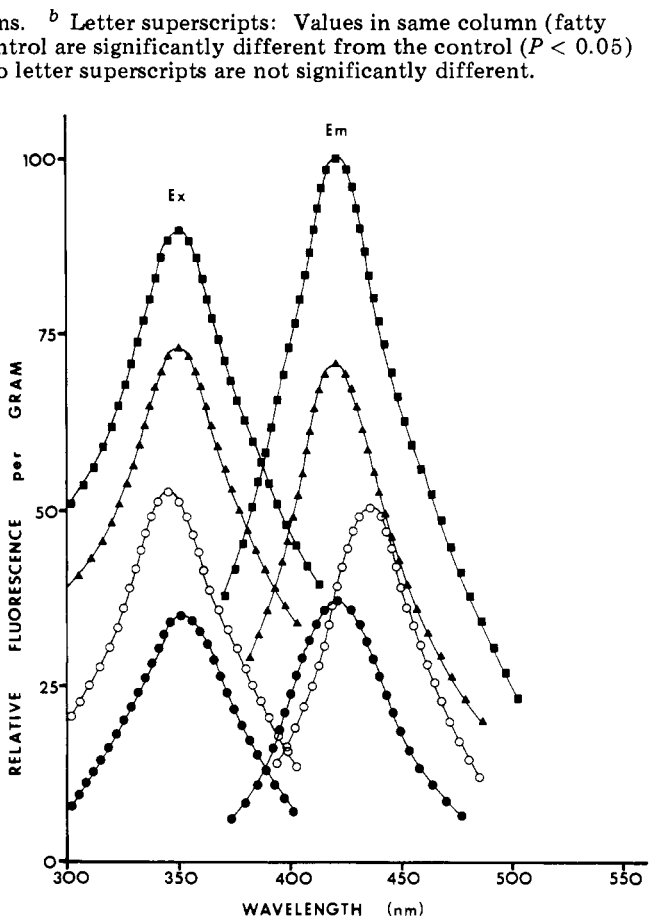

Figure 2. Fluorescent emission (Em) and excitation (Ex) spectra of the 2-mL fraction (no. 10 or no. 11) possessing maximum relative fluorescence of ground Wayne variety soybean samples continuously exposed to air (●—●), 15 ppm NO_2 (▲–▲), or 1.5 ppm O_3 (■—■) for 100 h at room temperature and of mammalian lipofuscin pigments extracted from mouse heart tissue (○—○).

However, when the intact seedcoats of the soybeans were destroyed by splitting the seeds into halves, the seeds were found to have increased susceptibility to gaseous oxidants. The severity of oxidation was found to be dependent upon the oxidizing ability and the concentration of the gases. Air, being a milder oxidant than NO_2 or O_3, induced no detectable PUFA destruction in soybean seed halves under the experimental conditions. Air exposure, however, did cause destruction of tocopherols in all varieties of soybean seed halves and also caused formation of significantly higher quantities of LLP in one of the three varieties,

Table IV. Tocopherol Content and Oil Content of Illinois High Oil, Illinois Low Oil, and R802A Corn Varieties Continuously Exposed to Air, 15 ppm NO_2, or 1.5 ppm O_3 for 100 h

Sample	Tocopherol content, μg/g of oil + SD[a]					
	α-Tocopherol	α-Tocotrienol	γ-Tocopherol	γ-Tocotrienol	Total tocopherol	Oil content, %
Illinois High Oil						
Control	307 ± 4.5[b]	144 ± 5.2	805 ± 21.0	226 ± 2.6	1482	16.2
Air	304 ± 6.4	141 ± 1.4	795 ± 23.3	224 ± 0.0	1464	15.9
15 ppm NO_2	303 ± 1.4	133 ± 7.8	789 ± 17.7	219 ± 7.1	1444	15.9
1.5 ppm O_3	304 ± 5.7	140 ± 6.4	794 ± 21.9	223 ± 0.7	1461	16.3
Illinois Low Oil						
Control	122 ± 5.2	64 ± 3.5	574 ± 14.6	76 ± 5.2	836	1.0
Air	121 ± 1.4	64 ± 6.4	568 ± 17.7	76 ± 5.0	829	1.1
15 ppm NO_2	116 ± 6.4	60 ± 2.1	556 ± 20.5	72 ± 0.7	804	1.0
1.5 ppm O_3	119 ± 7.8	63 ± 2.1	562 ± 5.7	68 ± 9.9	812	1.1
R802A						
Control	215 ± 2.8	101 ± 5.0	811 ± 18.9	147 ± 5.4	1274	7.3
Air	213 ± 4.2	100 ± 6.4	804 ± 27.6	146 ± 2.1	1263	7.1
15 ppm NO_2	212 ± 2.1	94 ± 7.8	795 ± 18.4	143 ± 4.2	1244	6.9
1.5 ppm O_3	216 ± 2.1	101 ± 0.7	801 ± 10.6	142 ± 9.9	1260	7.0

[a] Average and standard deviation of two sample determinations. [b] Letter superscripts: Values in the same column (tocopherol isomer) that do not share a common letter superscript with the control are significantly different from the control ($P < 0.05$) by an analysis of variance and a two-tailed t test. Values with no letter superscripts are not significantly different.

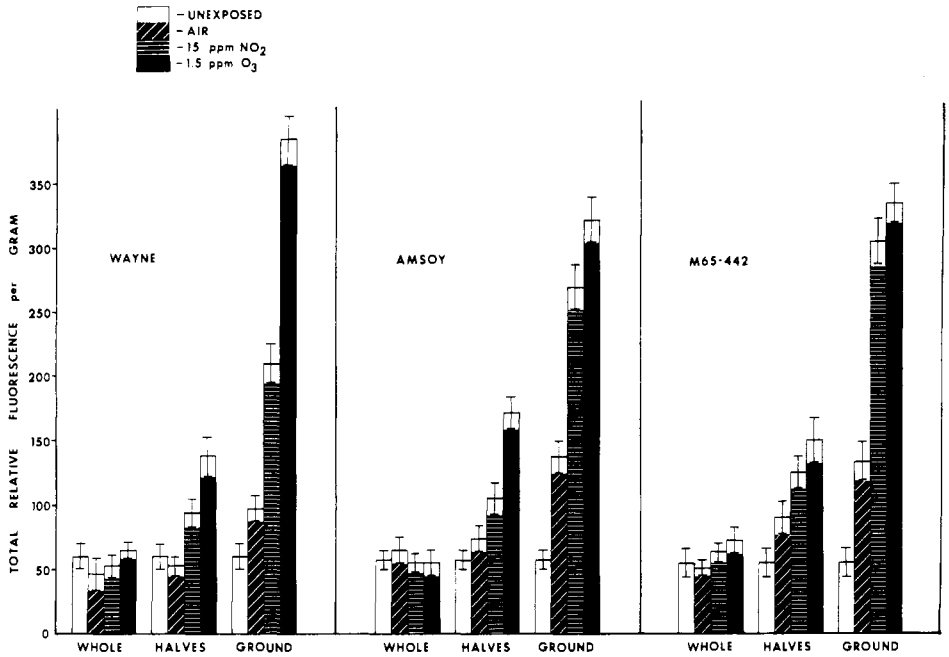

Figure 3. Total concentrations of lipofuscin-like pigments in whole, unexposed control soybean samples and in whole, halves, and ground samples of Wayne, Amsoy, and M65-442 soybean varieties continuously exposed to air, 15 ppm NO_2, or 1.5 ppm O_3 for 100 h at room temperature. Concentration of lipofuscin-like pigments is expressed as total relative fluorescence in fractions 8–14 per gram of sample.

indicating that tocopherols are more oxidatively labile than PUFA. As expected, greater tocopherol and PUFA destruction and increased LLP formation occurred due to exposure of soybean seed halves to the stronger oxidizing agents, NO_2 and O_3. Exposure of soybean seed halves to 15 ppm NO_2 or 1.5 ppm O_3 was found to induce significant destruction of both tocopherols and PUFA. These findings may indicate that tocopherol destruction in NO_2- or O_3-exposed samples occurred as the additive result of the following two reactions: (1) the oxidation of tocopherols due to their direct reaction with the gaseous oxidants and (2) the oxidation of tocopherols resulting from their hydrogen donation as they function as antioxidants in inhibiting lipid oxidation within the seeds. Samples of soybean seed halves had a 50% increase in total surface area (1155 mm^2/g) compared to the whole intact seeds (770 mm^2/g), and this increase in total surface area also contributed to their increased susceptibility to oxidation.

When the surface area of the ground soybean seeds was increased to 12883 mm^2/g, 16.7 times the total surface area of the whole intact seeds, more severe oxidation occurred due to air, 15 ppm NO_2, or 1.5 ppm O_3 exposure. This resulted in increased tocopherol and PUFA destruction and increased LLP formation. As also expected, air appeared to be a weaker oxidizing agent than either 15 ppm NO_2 or 1.5 ppm O_3 as shown by PUFA destruction results. Air exposure of ground soybean samples caused 1.3–1.9% destruction of linoleic acid and 4.3–7.5% destruction of linolenic acid. Exposure of ground soybean samples to 15 ppm NO_2 resulted in 10.9–13.4% destruction of linoleic acid and 11.4–20.4% destruction of linolenic acid. Exposure of ground soybean samples to 1.5 ppm O_3 induced 1.7–5.4% destruction of linoleic acid and 10.0–22.4% destruction linolenic acid.

The increased formation of fluorescent, organic solvent-soluble lipofuscin-like pigments (LLP) in soybean seed samples exposed to gaseous oxidants is also an indication of increased lipid oxidation. Since NO_2 and O_3 induce and accelerate free radical-type lipid oxidation, increased formation of secondary oxidation products, such as malondialdehyde, could also be expected, leading to increased formation of Schiff-base containing fluorescent LLP.

The elution profiles of mammalian lipofuscin pigments (LP) and soybean LLP on Sephadex LH-20 columns are identical (Figure 1) and the excitation maxima at 345 nm and 350 nm, respectively, are also almost identical; however, the emission maximum at 435 nm for mammalian LP appears to be slightly higher than the emission maximum at 420 nm for soybean LLP. The observed slight shift of 15 nm in emission maximum may possibly be caused by the compositional differences in the moieties attached to the Schiff-base of mammalian LP and soybean LLP.

The fact that increased oxidation of PUFA due to exposure to gaseous oxidants resulted in increased fluorescent LLP concentrations in soybean seeds indicates that these Schiff-base-type secondary oxidation products do not only exist in mammalian tissues, but also exist in oil-containing plant tissues. In addition, the occurrence of gaseous oxidant-induced PUFA and tocopherol destruction and LLP formation all appear to be related, each resulting from free radical-type lipid oxidation induced within the corn and soybean seeds.

The present experiments also confirm the reports of other investigators (Robertson et al., 1973; Chow and Draper, 1969; Weber, 1969) that corn and soybean varieties mainly contain five fatty acids: palmitic, stearic, oleic, linoleic, and linolenic. Only certain tocopherol isomers, α-T, γ-T, and δ-T in soybean varieties and α-T, α-T-3, γ-T, and δ-T-3 in corn varieties, were found in the present experiments which is in agreement with the findings of Chow and Draper (1969, 1974) and Grams et al. (1970).

Comparison of the total tocopherol contents and the total oil contents of the whole unexposed control samples of each of the three soybean and corn varieties, shown in Tables II and IV, respectively, indicate a direct correlation between total oil contents and total tocopherol contents. The total tocopherol concentration and the individual tocopherol isomer concentrations increased when the corn or soybean seeds' total oil content increased. Gutfinger and Letan (1975) studied the concentrations and distributions of tocopherols in soybean, cottonseed, olive, avocado, and coconut oils and also found a direct positive correlation between total oil contents and tocopherol concentrations. It is hypothesized that seeds which synthesize higher amounts of oil also synthesize higher amounts of tocopherols to function as natural antioxidants in protecting their lipids from oxidation.

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