Influence of Free Radicals and Other Factors on Formation of Cholesterol Oxidation Products in Spray-Dried Whole Egg

Shu-Mei Lai,[†] J. Ian Gray,*[,†] D. Joseph Buckley,[‡] and Philip M. Kelly[§]

Department of Food Science and Human Nutrition, Michigan State University, East Lansing, Michigan 48824, Department of Food Technology, University College, Cork, Ireland, and National Dairy Products Research Centre, Fermoy, Ireland

Factors influencing formation of cholesterol oxidation products (COPS) in spray-dried whole eggs, particularly free radicals generated during the combustion process, were investigated. Total COPS formed in products dried in the presence of free radicals (by using a gas burner or addition of prooxidants into an electric heating system) were approximately 2–5 times greater than those in powders processed by an electric heating system. Addition of antioxidants (tertiary butylhydroquinone and oleoresin rosemary) did not significantly affect ($P < 0.05$) the formation of COPS in eggs spray-dried with direct gas-fired heating during processing and/or storage. Room temperature storage significantly promoted ($P < 0.05$) further formation of COPS regardless of the initial content of COPS in the egg powders. The pathway of cholesterol oxidation initiated by oxides of nitrogen appeared to be similar to the hydroperoxide-induced free radical reaction.

Keywords: *Cholesterol oxidation; egg powders; free radicals*

INTRODUCTION

Although dietary cholesterol has long been considered a contributing factor to atherosclerosis in humans (Addis and Park, 1989), recent studies have indicated a possible role of cholesterol oxidation products (COPS) in the initiation of atherosclerotic plaque formation (Peng et al., 1982; Addis et al., 1989; Kubow, 1990; Kumar and Singhal, 1991). Furthermore, plasma COPS concentrations in humans have been found to increase with consumption of oxidized egg powders containing approximately 230 μg/g COPS (Emanuel et al., 1991). Thus, the presence and quantity of COPS in foods resulting from processing and subsequent storage have received considerable attention in recent years (Finocchiaro and Richardson, 1983; Morgan and Armstrong, 1987; Sander et al., 1989).

The method of spray-drying affects the stability of unsaturated lipids, including cholesterol, in dehydrated foods. For example, it has been reported that the concentrations of COPS are greater in egg powders processed by a direct gas-fired heating source than in powders produced with indirect heating (Missler et al., 1985; Tsai and Hudson, 1985; Faulkner et al., 1992). It was suggested that the exposure of foods to oxides of nitrogen (NOx) in the direct gas-fired spray-dryer may be responsible for the elevated COPS concentrations.

NOx, including nitric oxide (NO) and nitrous oxide (NO_2), are produced from air as a result of combustion processes (Wheeler, 1980). NO_2 has been demonstrated to be a free radical initiator of oxidation of unsaturated lipids in model systems (Roehm et al., 1971; Pryor and Lightsey, 1981). Morgan and Armstrong (1992) investigated the effect of NOx on the formation of COPS in egg yolk powders produced with direct gas-fired heating. They manipulated the levels of NOx in the combustion gas by delivery of NO_2 to the gas burner, where NO_2 dissociates into a mixture of oxidizing nitrogen oxide gases. They demonstrated that the quantities of COPS in spray-dried egg yolk powders increased proportionally to the NOx concentrations in the combustion gas. However, there is still no direct evidence that NOx are mainly responsible for the elevated COPS concentrations in egg powders with direct gas-fired heating.

The present study was carried out to investigate factors influencing the formation of COPS in spray-dried egg powders, particularly the role of free radicals generated during the combustion process. The effects of adding prooxidants and antioxidants to the liquid egg before drying on egg product stability were also determined.

MATERIALS AND METHODS

Reagents. Cholesterol (cholest-5-en-3β-ol) and 6-ketocholestanol (6-oxo-5-cholestan-3β-ol) standards were purchased from Sigma Chemical Co. (St. Louis, MO). Cholesterol oxide standards, α- and β-epoxides (5α,6α-epoxycholestan-3β-ol and 5β,6β-epoxycholestan-3β-ol), 7α- and 7β-hydroxycholesterols (5-cholestene-3β,7α-diol and 5-cholestene-3β,7β-diol), 7-ketocholesterol (7-oxo-5-cholesten-3β-ol), 20α-hydroxycholesterol (5-cholestene-3β,20α-diol), 25-hydroxycholesterol (5-cholestene-3β,25-diol), and cholestane-3β, 5α,6β-triol, were purchased from Steraloids Inc. (Wilton, NH). Bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane (BSTFA plus 1% TMCS) was obtained from Pierce Chemical Co. (Rockford, IL). Cumene hydroperoxide (80%) was purchased from Sigma, oleoresin rosemary (Herbalox Seasoning Type WM, 100% activity) was donated by Kalsec, Inc. (Kalamazoo, MI), and tertiary butylhydroquinone (TBHQ) was obtained from Eastman Chemical Products Inc. (Kingsport, TN). Other reagents and solvents used in this study were of analytical grade and/or HPLC grade.

Quantitation of Cholesterol Oxidation Products. Five COPS (cholesterol α- and β-epoxides, 7α- and 7β-hydroxycholesterols, and 7-ketocholesterol) in the egg powders were quantified as described earlier by Lai et al. (1994). Essentially, COPS in a lipid extract of the egg powders were isolated by solid phase extraction, derivatized to their trimethylsilyl (TMS) ether derivatives using the BSTFA/TMCS reagent, and quantitated by capillary gas chromatography.

* Author to whom correspondence should be addressed [fax (517) 353-5406; e-mail 18215JIG@MSU.edu].

[†] Department of Food Science and Human Nutrition.

[‡] Department of Food Technology.

[§] National Dairy Products Research Centre.

0021-8561/95/1443-1127$09.00/0

Table 1. Liquid Egg Treatments and Drying Conditions Used To Prepare Egg Powders

heating source	NOx in drying air (ppm)	liquid egg
indirect electric	0	control
indirect electric	0	cumene-00H 0.5%
indirect electric	15[a]	control
indirect electric	15	OR 0.05%[c]
direct gas-fired	8[b]	control
direct gas-fired	8	OR 0.05%
direct gas-fired	8	OR 0.1%
direct gas-fired	8	TBHQ 0.02%

[a] Amount of NOx added to the drying air which originally contained no detectable NOx. [b] Amount of NOx generated by gas combustion. [c] Concentrations of antioxidants (OR and TBHQ) are based on lipid content of liquid egg.

Statistical Analysis. The experiment was designed as a three-factor (treatment × time × replication) complete randomized model with balanced data. The whole experiment was repeated twice. Analysis of variance (ANOVA) for data was calculated using the MSTATC microcomputer statistical program (Michigan State University, 1991). Bonferroni *t* statistics were performed to analyze specific contrasts among treatments (Gill, 1978).

Preparation of Liquid Egg. Freshly processed liquid whole egg was purchased from a commercial supplier in Cork, Ireland, and stored under refrigeration (4 °C) until dried (within 8 h).

Cumene hydroperoxide was added directly to the liquid egg to achieve a concentration of 0.05% (w/w). Oleoresin rosemary (OR) was diluted with distilled water to prepare a 15% (w/v) stock solution. A stock solution (0.6% w/v) of TBHQ was prepared in 50% (v/v) ethanol solution. The stock solutions of OR and TBHQ were then added to the liquid egg in appropriate amounts to produce the desired final concentrations based on the lipid content (12%) of the liquid egg (Table 1).

Preparation of Spray-Dried Egg Powders. Two spray-drying systems, indirect (electric) heating and direct gas-fired heating, were used to dehydrate liquid whole egg (Table 1). Prooxidants were added during processing by introducing NOx gas to the drying air and by adding cumene hydroperoxide into the liquid egg immediately before drying. Antioxidants were also added to the liquid egg before drying.

All egg samples were spray-dried using a pilot scale Anhydro Lab3 spray-dryer with inlet and outlet temperatures of 200 and 95 °C, respectively, at the National Dairy Products Research Centre (Fermoy Co., Cork, Ireland). The Anhydro Lab3 model is a single-stage, conical dryer equipped with a pneumatic nozzle and electric heating elements for indirect air heating. Conversion to direct gas-fired heating was achieved by the attachment of a gas burner to the extended air inlet duct (Kelly et al., 1989).

NOx gases consisting of 50% NO and 50% NO_2 (B.O.C. Ltd., London, England) were introduced into the drying air in the indirect heating system through the duct between the electric heater and the inlet of the drying chamber. The concentrations of NOx in the drying air were measured by Draeger tubes (Draegerwerk AG, Lubeck, Germany) at the inlet of the drying chamber. A similar location was used to quantify the levels of NOx in the direct gas-fired heating system. A sample of the air was drawn through the Draeger tube and any NOx present reacted with *N,N'*-diphenylbenzidine in the tube to form bluish-gray compounds (Leichnitz, 1987). NOx (NO plus NO_2) concentration was determined from the scale on the tube at the point where the color reaction terminated. The range of measurement for NOx with this method was 0.5–50 parts per million (ppm).

The egg powders were vacuum packaged in polyethylene-laminated nylon pouches (Koch, Kansas City, MO) and immediately air freighted to Michigan. These pouches (90 μm thickness) have a water-vapor transmission rate of 0.041 mL m^{-2} day^{-1} $mmHg^{-1}$ and an oxygen transmission rate of 0.124 mL m^{-2} day^{-1} $mmHg^{-1}$ at 22.7 °C and 50% relative humidity. Once received (within 2 weeks of processing), the egg samples were repacked in low-density polyethylene bags (8 in. × 10 in., 75 μm thickness, WhirlPak, Fisher Scientific, Fair Lawn, NJ) without heat sealing and stored at ambient temperature (22 ± 2 °C) in the dark for 6 months. Sampling for analysis of the egg samples was performed randomly after the egg powder was thoroughly mixed in the bags with a spatula (approximately 100 g per bag). Total solids were determined using the AOAC (1984) vacuum oven method. COPS in the samples were determined at time 0 (corresponding to 2 weeks postprocessing) and after storage for 3 and 6 months.

RESULTS AND DISCUSSION

Cholesterol is an unsaturated lipid and susceptible to oxidation by a free radical mechanism (Smith, 1981). Many factors including elevated temperatures, prolonged storage, and processing in the presence of prooxidants can influence the rate of cholesterol oxidation as well as the quantities and distribution of COPS in foods (Sander et al., 1989). Although the presence of several COPS in egg powders have been reported (Missler et al., 1985; Tsai and Hudson, 1985; Morgan and Armstrong, 1987; Nourooz-Zadeh and Appelqvist, 1987), the effects of prooxidants produced during processing on the formation of COPS have not been conclusively investigated.

In this study, whole liquid egg was spray-dried with two spray-drying systems, electrical heating and gas-fired heating, with or without the addition of prooxidants and/or antioxidants. The average total solid contents of egg powders was 96.47% ± 0.56% (standard errors for means). The mean values of the total solids did not differ significantly ($P < 0.01$) among samples from different treatments. Results of ANOVAs for total COPS as well as individual COPS in egg samples showed significant effects ($P < 0.05$) of treatments and storage times. On the other hand, replication, as well as interactions of each combination from these factors, did not significantly ($P < 0.05$) affect the formation of COPS.

Effects of Drying Method. No measurable quantities of NOx in the drying air were detected by the Draeger tubes at the inlet of the drying chamber (minimum detection = 0.5 ppm) when the dryer was used in the indirect heating mode. However, when this system was converted to direct gas-fired heating, 8 ppm of NOx was found in the drying air.

The concentrations of total COPS in the egg samples spray-dried with direct gas-fired heating were significantly ($P < 0.01$) greater than concentrations in samples dried with indirect heating. This trend in concentrations in the samples was observed initially (2 weeks after processing) and after 3 and 6 months of storage (Table 2). Similar findings have been reported in the literature. For example, Missler et al. (1985) found that scrambled egg mixes dried using a direct gas-fired heating system contained greater amounts of COPS than those dried by the indirect (electric) heating process. Tsai and Hudson (1985) surveyed commercial egg powders from 15 plants and reported that egg samples dried by air heated directly with a gas burner contained greater amounts of cholesterol epoxides (up to 74 μg/g) than those dried by air heated indirectly with steam (up to 5 μg/g). These investigators suggested that exposure to NOx may be responsible for the greater quantities of COPS in the egg samples processed by the direct heating process.

Five COPS (cholesterol α- and β-epoxides, 7α- and 7β-hydroxycholesterols, and 7-ketocholesterol) were found in samples spray-dried with direct gas-fired heating.

Table 2. Effects of Drying Method on the Concentrations of Cholesterol Oxidation Products (Micrograms per Gram) in Egg Powders Stored at 22 ± 2 °C for 6 Months[a]

COPS	month 0[b]		month 3		month 6	
	direct	indirect	direct	indirect	direct	indirect
cholesterol α-epoxide	1.76	–[c]	4.35	3.21	6.10	4.21
cholesterol β-epoxide	5.46	2.96	21.29	14.05	26.69	20.43
7α-hydroxycholesterol	2.57	1.81	7.25	4.92	6.89	6.00
7β-hydroxycholesterol	2.13	2.32	7.79	4.08	10.70	6.90
7-ketocholesterol	2.44	1.48	3.54	2.71	5.48	5.00
total	14.36	8.57	44.22	28.97	55.86	42.54

[a] All values represent the average of two replicated experiments analyzed in duplicate. [b] Month 0 = 2 weeks after processing due to shipping. [c] Not detectable (minimum detection limit = 0.1 μg/g).

Table 3. Effects of Adding Prooxidants and Antioxidants to Liquid Egg on the Concentrations of Cholesterol Oxidation Products (Micrograms per Gram) in Egg Powders Produced with an Indirect Heating System[a,b]

COPS	control[c]	cumene-OOH (0.5%)	NOx (15 ppm)	NOx (15 ppm) + OR (0.05%)[d]
7α-hydroxycholesterol	1.81	3.49	5.07	3.59
7β-hydroxycholesterol	2.32	2.77	6.15	3.77
7-ketocholesterol	1.48	3.03	2.45	2.70
cholesterol α-epoxide	–[e]	2.08	3.83	2.19
cholesterol β-epoxide	2.96	8.76	21.32	12.65
total	8.57	20.13	38.82	24.90

[a] All values represent the average of two replicated experiments analyzed in duplicate. [b] Two weeks after processing due to shipping. [c] Egg powder produced by the indirect heating system without any adjustment to the drying air or prior addition of antioxidants/prooxidants to the liquid egg. [d] Based on lipid content of liquid egg. [e] No detectable cholesterol α-epoxide (minimum detection limit = 0.1μg/g).

However, α-epoxide was not detected in samples dried with indirect electric heating before storage but appeared after 3 months of storage. The effects of storage times and the mechanism of formation of cholesterol epoxides (α- and β-epoxides) will be discussed later.

Effects of Prooxidants. Cumene hydroperoxide and NOx gases (NO and NO_2) were used to investigate the influence of free radicals on the formation of COPS in spray-dried eggs. Cumene hydroperoxide, added to liquid egg (0.5%) prior to spray-drying, will undergo decomposition by heat to generate alkoxyl or peroxyl radicals (Hiatt, 1975). These radicals will accelerate the chain reaction of lipid oxidation, including cholesterol oxidation (Smith, 1981). NOx gases, as free radical initiators (Pryor and Lightsey, 1981), were introduced into the drying air heated with the indirect electric heating source. The amount of NOx in drying air measured at the inlet of the drying chamber was increased from the original nondetectable amount (<0.5 ppm) to 15 ppm.

Results presented in Table 3 suggest that the presence of prooxidants plays an important role in the oxidation of cholesterol in spray-dried egg powders. The amounts of COPS in egg powders produced by indirect heating were increased 2.3- and 4.5-fold by the addition of cumene hydroperoxide and NOx gases, respectively. Morgan and Armstrong (1987) also demonstrated that the addition of prooxidants such as hydrogen peroxide to liquid egg before spray-drying promoted formation of measurable quantities of cholesterol epoxides in egg yolk powder produced by indirect heating. Hydroxyl radical, a product from thermolytic cleavage of hydrogen peroxide (Simic and Taylor, 1987), like NOx, is highly reactive with unsaturated lipids such as cholesterol.

Similarly, results of the indirect heating studies, with and without cumene hydroperoxide and NOx suggest that the presence of prooxidants is necessary to explain the greater quantities of COPS in egg powders produced by the indirect heating system. These results support the hypothesis that cholesterol oxidation in spray-dried egg powders is initiated by NOx formed during the combustion process.

Effects of Antioxidants. Antioxidants, OR and TBHQ, which function as free radical scavengers, were added to liquid egg to ascertain their effectiveness in preventing cholesterol oxidation in powders produced by direct gas-fired spray-drying. Although there appeared to be a trend toward reduced COPS in samples dried in the presence of antioxidants (data not shown), the observed differences in COPS concentrations were not statistically significant ($P < 0.05$). The ineffectiveness of TBHQ and OR may be due to their volatilization and subsequent loss during spray-drying.

On the other hand, OR reduced the formation of COPS by 36% in the egg powders produced with the indirect spray-dryer with the addition of 15 ppm of NOx in the drying air. It is possible that lower temperatures are attained during the drying operation using indirect heating, thus reducing the loss of antioxidant principles from OR during egg processing. However, further studies on the effect of temperature of the drying operation on the effectiveness of antioxidants are needed to evaluate this suggestion.

Effects of Storage Times. The length of storage time significantly ($P < 0.05$) affected the formation of COPS in all egg samples. This result is predictable because the samples were stored in an oxygen-permeable package (polyethylene bags without sealing) at ambient temperature to facilitate cholesterol oxidation. Concentrations of COPS increased more rapidly in the first 3 months than in the subsequent 3 months.

The presence of cholesterol α- and β-epoxides, 7α- and 7β-hydroxycholesterols, and 7-ketocholesterol in the egg powders is consistent with literature reports. However, 20α-hydroxycholesterol and 25-hydroxycholesterol, the products of side chain oxidation of cholesterol, as well as cholestane-3β,5α,6β-triol, the hydration products of cholesterol epoxides, were not detected. These compounds have been reported in egg powders stored for more than 5 years (Missler et al., 1985; Nourooz-Zadeh and Appelqvist, 1987).

The most abundant COP in the egg powders was β-epoxide. The ratios of this compound to its α-isomer in the samples ranged from 4:1 to 4.9:1 after 3 and 6

Table 4. Effects of Storage Time on the Concentrations of Cholesterol Oxidation Products (Micrograms per Gram) in Egg Powders Produced with an Indirect Heating System[a]

storage time	control[b]	cumene-OOH (0.5%)	NOx (15 ppm)	NOx (15 ppm) + OR (0.05%)[c]
month 0[d]				
total COPS	8.57	20.13	38.82	24.90
% C-7	65.5	46.2	35.2	40.4
% epoxides	34.5	53.9	64.8	59.6
ratio β/α[e]	–[f]	4.2	5.6	5.8
month 3				
total COPS	28.97	50.56	63.20	42.18
% C-7	40.4	42.1	39.0	41.5
% epoxides	59.6	57.9	61.0	58.5
ratio β/α	4.4	4.3	4.1	4.5
month 6				
total COPS	42.54	58.93	79.38	47.52
% C-7	42.1	48.0	39.7	48.4
% epoxides	57.9	52.0	60.3	51.6
ratio β/α	4.9	4.0	4.9	4.6

[a] All values represent the average of two replicated experiments analyzed in duplicate. [b] Egg powder produced by the indirect heating system without any adjustment to the drying air or prior addition of antioxidants/prooxidants to the liquid egg. [c] Based on lipid content of liquid egg. [d] Two weeks after processing due to shipping. [e] Ratio of cholesterol β-epoxide to α-epoxide. [f] No detectable cholesterol β-epoxide (minimum detection limit = 0.1 μg/g).

months of storage (Table 4). The relative abundance of β-epoxide compared to its α-isomer can be explained by the greater thermodynamic stability of the equatorial conformation over that of the axial conformation (Smith, 1981). Tsai and Hudson (1985) reported a ratio of 4:1 in commercial dried egg powders. Nourooz-Zadeh and Appelqvist (1987) also reported ratios of 5:1 for egg powders and 10:1 for scrambled egg mixes.

Cholesterol oxidation is initiated by the abstraction of the allylic C-7 hydrogen, with the subsequent formation of C-7 COPS such as 7α- and 7β-hydroxycholesterols and 7-ketocholesterol (Smith, 1981). On the other hand, cholesterol α- and β-epoxides are the products of attack by cholesterol 7-hydroperoxide on the 5,6-double bond of cholesterol and therefore are the secondary oxidation products (Maerker, 1987). Other lipid hydroperoxides and some organic hydroperoxides such as cumene hydroperoxide and hydrogen peroxide likewise favor epoxidation of cholesterol and form cholesterol epoxides (Smith, 1981).

Many investigators have claimed C-7 COPS as the predominant cholesterol oxides in foods such as beef, tallow, and milk powders (Park and Addis, 1986, 1987; Chan et al., 1993). However, data presented in Table 4 indicate that under conditions of NOx- or cumene hydroperoxide-induced oxidation of cholesterol, epoxides were the predominant COPS. These findings support the results of other studies in which epoxides were identified as the major COPS formed in egg powders spray-dried with a direct heating source (Missler et al., 1985; Morgan and Armstrong, 1992).

In conclusion, this study has demonstrated that the presence of NOx, free radicals generated during the combustion process, is responsible for the rapid formation of COPS in egg powders produced by the direct gas-fired spray-dryer. The pathways of cholesterol oxidation initiated by NOx appear to be similar to the hydroperoxide-induced free radical chain reaction. More specific studies on the mechanism of NOx-initiated cholesterol oxidation in a lipid model system are currently in progress.

LITERATURE CITED

AOAC. *Official Methods of Analysis*, 14th ed.; Association of Official Analytical Chemists: Arlington, VA, 1984.

Addis, P. B.; Park, S.-W. Role of lipid oxidation products in atherosclerosis. In *Food Toxicology, A Perspective on the Relative Risks*; Taylor, S. L., Scanlan, R. A., Eds.; Dekker: New York, 1989; Chapter 11, pp 297–330.

Addis, P. B.; Emanuel, H. A.; Bergmann, S. D.; Zavoral, J. H. Capillary GC quantification of cholesterol oxidation products in plasma lipoproteins of fasted humans. *Free Radical Biol. Med.* **1989**, *7*, 179–182.

Chan, S. H.; Gray, J. I.; Gomaa, E. A.; Harte, B. R.; Kelly, P. M.; Buckley, D. J. Cholesterol oxidation in whole milk powders as influenced by processing and packaging. *Food Chem.* **1993**, *47*, 321–328.

Emanuel, H. A.; Hassel, C. A.; Addis, P. B.; Bergmann, S. D.; Zavoral, J. H. Plasma cholesterol oxidation products (oxysterols) in human subjects fed a meal rich in oxysterols. *J. Food Sci.* **1991**, *56*, 843–847.

Faulkner, J. A.; Gray, J. I.; Buckley, D. J.; Monahan, F. J.; Kelly, P. M. Influence of spray drying method and vitamin E on cholesterol oxidation in whole egg powder. Presented at the 52nd Annual Meeting of the Institute of Food Technologists, New Orleans, LA, 1992; Paper 40.

Finocchiaro, E. T.; Richardson, T. Sterol oxides in foodstuffs: A review. *J. Food Prot.* **1983**, *46*, 917–925.

Gill, J. L. *Design and Analysis of Experiments in the Animal and Medical Science*; Iowa State University Press: Ames, IA, 1978; Vol. 1.

Hiatt, R. R. Hydroperoxide destroyers and how they work. *Crit. Rev. Food Sci. Nutr.* **1975**, *7*, 1–12.

Kelly, P. M.; Gray, J. I.; Slattery, J. Direct "low-NOx" gas combustion heating of a spray drier during milk powder manufacture. *J. Soc. Dairy Technol.* **1989**, *42*, 14–18.

Kubow, S. Toxicity of dietary lipid peroxidation products. *Trends Food Sci. Technol.* **1990**, *1* (3), 67–71.

Kumar, N.; Singhal, O. P. Cholesterol oxides and atherosclerosis: A review. *J. Sci. Food Agric.* **1991**, *55*, 497–510.

Lai, S.-M.; Gray, J. I.; Zabik, M. E. Evaluation of solid phase extraction and gas chromatography for determination of cholesterol oxidation products in spray-dried whole egg. *J. Agric. Food Chem.* **1995**, *43*, 1122–1126.

Leichnitz, K. *Detector Tube Handbook*, 6th ed.; Draegerwerk AG: Lubeck, Germany, 1987.

Maerker, G. Cholesterol autoxidation—current status. *J. Am. Oil Chem. Soc.* **1987**, *64*, 388–392.

Missler, S. R.; Wasilchuk, B. A.; Merritt, C. Separation and identification of cholesterol oxidation products in dried egg preparations. *J. Food Sci.* **1985**, *50*, 595–598.

Morgan, J. N.; Armstrong, D. J. Formation of cholesterol 5,6-epoxides during spray-drying egg yolk. *J. Food Sci.* **1987**, *52*, 1224–1227.

Morgan, J. N.; Armstrong, D. J. Quantification of cholesterol oxidation products in egg yolk powder spray-dried with direct heating. *J. Food Sci.* **1992**, *57*, 43–45, 107.

Nourooz-Zadeh, J.; Appelqvist, L.-A. Cholesterol oxides in Swedish foods and food ingredients: Fresh eggs and dehydrated egg products. *J. Food Sci.* **1987**, *52*, 57–62, 67.

Park, S. W.,; Addis, P. B. Identification and quantitative estimation of oxidized cholesterol derivatives in heated tallow. *J. Agric. Food Chem.* **1986**, *34*, 653–659.

Park, S. W.; Addis, P. B. Cholesterol oxidation products in some muscle foods. *J. Food Sci.* **1987**, *52*, 1500–1503.

Peng, S-K.; Taylor, C. B.; Mosbach, E. H.; Huang, W. Y.; Hill, J. C.; Mikkelson, B. Distribution of 25-hydroxy cholesterol in plasma lipoprotein and its role in atherogenesis. *Atherosclerosis* **1982**, *41*, 395–402.

Pryor, W. A.; Lightsey, J. W. Mechanism of nitrogen dioxide reactions: Initiation of lipid peroxidation and the production of nitrous acid. *Science* **1981**, *214*, 435–437.

Roehm, J. N.; Hadley, J. G.; Menzel, D. B. Oxidation of unsaturated fatty acids by ozone and nitrogen dioxide: A common mechanism of action. *Arch. Environ. Health* **1971**, *23*, 142–148.

Sander, B. D.; Addis, P. B.; Park, S. W.; Smith, D. E. Quantification of cholesterol oxidation products in a variety of foods. *J. Food Prot.* **1989**, *52*, 109–114.

Simic, M. G.; Taylor, K. A. Free radical mechanisms of oxidation reactions. In *Warmed-Over Flavor of Meat*; St. Angelo, A. J., Bailey, M. E., Eds.; Academic Press: Orlando, FL, 1987; pp 69–117.

Smith, L. L. *Cholesterol Autoxidation*; Plenum Press: New York, 1981.

Tsai, L. S.; Hudson, C. A. Cholesterol oxides in commercial dry egg products: Quantitation. *J. Food Sci.* **1985**, *50*, 229–237.

Wheeler, W. H. Chemical and engineering aspects of low NOx concentration. *Chem. Eng.* **1980**, *362*, 693–699.

Received for review October 24, 1994. Accepted March 16, 1995.[⊗] We gratefully acknowledge the financial support of the MSU Crop and Food Bioprocessing Center and the Michigan Agricultural Experiment Station for this study.

JF940604K

[⊗] Abstract published in *Advance ACS Abstracts,* May 1, 1995.