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THE INFLUENCE OF SPRAY DRYING METHOD ON THE FORMATION OF CHOLESTEROL OXIDATION PRODUCTS IN EGG POWDER

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THE INFLUENCE OF SPRAY DRYING METHOD ON THE FORMATION OF CHOLESTEROL OXIDATION PRODUCTS IN EGG POWDER

BY

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ABSTRACT

THE INFLUENCE OF SPRAY DRYING METHOD ON THE FORMATION OF CHOLESTEROL OXIDATION PRODUCTS IN EGG POWDER

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The influence of spray drying method and the effect of selected antioxidants on the formation of cholesterol oxidation products (COP's) in egg powders were examined. Eggs dried using a direct gas-fired dryer, high in nitrogen oxides (NOx), contained greater concentrations of cholesterol oxides than those dried using an indirect, electric or direct, low NOx dryer. No significant difference ($p<0.05$) was found between COP concentrations in egg powder dried using the latter two systems. Cholesterol oxides detected in descending order of concentration were: cholesterol β-epoxide >> 7 β-hydroxycholesterol > cholesterol α-epoxide and 7-ketocholesterol. During storage for four months at 20 and 40 °C, the concentrations of COP's increased. In addition, incorporation of α-tocopherol into the egg yolk by dietary supplementation increased the oxidative stability of egg yolk lipids. In spray dried egg powder, the extent of COP formation was reduced by dietary supplementation of the feed with α-tocopherol.

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INTRODUCTION

The oxidation of cholesterol will result in the formation of over eighty products, some of which have been shown to be atherogenic and carcinogenic (Smith, 1981). In egg powder, the formation of cholesterol oxides is of considerable importance because of the high concentration of cholesterol in the yolk and the widespread use of dried egg products, particularly in military rations.

Free radicals, enzymes and light have all been postulated as initiators of cholesterol oxidation (Smith, 1981). Of the free radicals, oxides of nitrogen (NOx) are of concern in spray drying operations, since these are formed during the combustion process (Wheeler, 1980). Cholesterol oxidation initiated by NOx in rat lung tissue and in model systems has been demonstrated (Roehm et al., 1971; Sevanian et al., 1979; Prior and Lightsey, 1981). Recently, the influence of spray drying method on the formation of cholesterol oxides in egg powder has been examined. Comparisons between the concentrations of cholesterol oxides in egg powders dried using an indirect, electric heater and a direct, gas-fired, high NOx burner have demonstrated that NOx increases oxidation (Tsai and Hudson, 1985; Missler et al., 1985).

The focus of this study was to examine the influence of three methods of spray drying (indirect, electric; direct, low NOx; dirct, high NOx) on the formation of cholesterol oxides in egg powder. The underlying premise was that NOx initiates cholesterol oxidation in spray dried egg powder; and that by reducing the concentration of NOx in the drying chamber, a lowering of cholesterol oxidation products in the egg powder would be achieved.

In addition, the effect of dietary supplementation of α-tocopherol in the feed of laying hens was examined, in order to determine if high levels of α-tocopherol could be incorporated into the yolk. Hence, the oxidative stability of the lipids in the dried eggs would be increased.

Other food-approved antioxidants (butylated hydroxy-toulene and bisulfite) were studied to determine the extent of cholesterol oxide reduction in egg powder. An attempt to generate NOx by the addition of sodium nitrite and perchlorate to the liquid egg yolk before drying was also made, in order to demonstrate the effect of NOx on cholesterol oxidation in eggs dried using an electric heater.

LITERATURE REVIEW

Distribution and composition of lipids in egg

Chicken eggs consist of approximately 9.5 % shell, 63.4 % albumen, and 27.5 % yolk (Cotterill and Geiger, 1977). The total lipid content in whole egg ranges from 10.0 % to 12.3 % and varies according to breed, strain, age of hen, and egg size (Edwards, 1964; Marion et al., 1964; Posati et al., 1975). Essentially all of the lipid in egg is contained in the yolk.

The major fractions of yolk fat are listed in Table 1 (Tullet, 1987). Triacylglycerols, phospholipids, and cholesterol constitute 63.1, 29.7 and 6.2 %, respectively, of the egg total lipid. Approximately 21.0 % of the cholesterol is found in the esterified form.

Lipids in the yolk may exist in the free form or bound to protein as a lipoprotein complex. Parkinson (1975) quantified the proportions of the major lipid groups in the free and bound state and reported that the majority of triacylglycerols and cholesterol exist in the free form. On the other hand, the phospholipid fraction was almost completely bound.

Table 1. The major fractions of yolk fat.

Component	Percent of total yolk fat
Triacylglycerols	63.1
Phospholipids	29.7
Free cholesterol	4.9
Cholesteryl esters	1.3
Free fatty acids	0.9

Adapted from Tullett (1987).

Oleic acid is the most abundant fatty acid present in egg yolk, followed by palmitic and linoleic acid (Privett, 1962). The ratio of polyunsaturated fatty acids (i.e., those containing two or more double bonds) to saturated fatty acids (P/S ratio) is high (0.59), and exceeds the recommended 0.45 P/S ratio established by the Department of Health and Social Services (1984) for dietary fat consumption. It is important to recognize that the relative proportions of fatty acids can be significantly altered by changing the type of fat in the diet of laying hens. Research has shown that when the levels of polyunsaturated fatty acids are increased in the diet, the amount of linoleic acid in egg lipids increases at the expense of oleic acid (Coppock and

Daniels, 1962; Posati et al., 1975). The percent of saturated fatty acids remains relatively constant, regardless of diet, except when coconut oil is added to the diet (Chen et al., 1965). Coconut oil will increase the level of palmitic acid in the yolk, when present in the diet in high amounts.

Health risks associated with cholesterol and its oxidation products

It is well established that elevated serum cholesterol poses a major risk in the development of coronary heart disease (CHD). However, the relationship between dietary cholesterol and CHD remains controversial. Many studies have demonstrated a lowering of serum cholesterol by reducing the amount of cholesterol consumed in the diet, but have failed to show a direct correlation between dietary cholesterol alone and CHD (Kris-Etherton, 1988). Recently, it has been suggested that lowering the dietary intake level affects only a small proportion of the population, and that most individuals compensate for increases in dietary cholesterol by a combination of the following mechanisms: feedback regulation of cholesterol synthesis, increased catabolism and re-excretion of cholesterol, and decreasing fractional absorption at high levels of intakes (McGill, 1979).

Although the major focus has been on cholesterol, especially in the mass media, considerable attention has

also been given to the presence of cholesterol oxides in foods because of their reported effects on biological activity.

Cholesterol oxides have been linked with carcinogenesis, cytotoxicty, inhibition of cholesterol synthesis, and atherogenesis. Cholesterol α-oxide has been reported to induce tumor formation in mice (Bischoff, 1969). Cox et al. (1988) demonstrated the cytotoxic effect of 25-hydroxycholesterol using sparse and confluent cultures of rabbit thoracic aorta smooth muscle cells and human umbilical vein endothelial cells. Kandutsch et al. (1978) reported loss of DNA synthesis and cell division when 25-hydroxycholesterol or 7-ketocholesterol was added to L-cell cultures. In addition, cholesterol synthesis was inhibited by suppression of 3-hydroxy-3-methlyglutaryl coenzyme A (HMG-CoA) reductase activity.

Most of the research, though, has centered directly on the role of cholesterol oxides in coronary artery disease. Early reports indicated that animals fed diets high in cholesterol developed arterial damage. Anitschkow (1913) was one of the first to demonstrate this phenomenon in rabbits. Recently, however, Peng et al. (1978) suggested that cholesterol oxides rather than cholesterol were responsible for the initiation of atherosclerosis in many of the previous studies since cholesterol is known to spontaneously decompose when

exposed to air and light. On the other hand, Higley et al. (1986) reported that rabbits fed U.S.P. cholesterol, purified by recrystallization and analyzed by high performance liquid chromatography (HPLC), exhibited six times more arterial lesions than rabbits fed a mixture of cholesterol oxides. Conversely, Peng (1979) demonstrated that 25-hydroxycholesterol and cholestanetriol were toxic to aortic smooth muscle, while purified cholesterol had no effect. Peng et al. (1982) later showed that 25-hydroxycholesterol was preferentially incorporated into atherogenic LDL (low density lipoproteins) and VLDL (very low density lipoproteins) but bound only slightly to HDL (high-density-lipoprotein) which is anti-atherogenic. Cholesterol α-oxide has also been found in the sera of patients with hypercholesterolemia, but not in normal patients (Gray et al., 1971).

Clearly, cholesterol oxides play a role in atherogenesis and possibly other diseased conditions, such as cancer. More research is needed in order to firmly establish the specific toxicological activites of cholesterol oxidation products. In view of this, the occurrence of cholesterol oxides in food is of considerable importance.

Occurrence of cholesterol oxides in egg

It is well established that cholesterol readily oxidizes in the presence of air. Reports on the instability of cholesterol date back to the beginning of the twentieth century. In 1902, Schulze and Winterstein (cited by Smith, 1980) observed that cholesterol underwent decomposition resulting in the production of a pungent aroma and off-color during shelf storage.

Many reports on the autoxidation of cholesterol have since followed, and over eighty cholesterol oxidation products (COP's) have now been identified (Smith, 1981). Cholesterol oxides have been detected in foods such as cheese, butteroil, french fries (processed in tallow), processed meats, and egg products (Finocchiaro et al., 1984; Lee et al., 1985; Higley and Taylor, 1986; Morgan and Armstrong, 1987; Nourooz-Zadeh and Appelqvist, 1987). Among these, egg products are of considerable interest because of their high contents of cholesterol.

Acker and Greve (1963) were the first to detect the presence of oxidation products of cholesterol in egg. Fine powders of egg-containing foods, exposed to sunlight, were analyzed for cholesterol oxides using thin layer chromatography (TLC). Cholesterol hydroperoxide and 7-hydroxycholesterol were detected. The isomers of 7-hydroxycholesterol were not distinguished by this method.

In addition, when egg products were stored for two years in the dark, no change in cholesterol content or formation of cholesterol oxidation products was observed.

Chicoye et al. (1968) undertook a more detailed study of cholesterol oxidation in spray-dried egg powders, exposed to both fluorescent and direct sunlight. Although the isomers of 7-hydroxycholesterol were resolved well by gas liquid chromatography, they could not separate cholesterol 5,6-epoxide into the alpha and beta isomers (henceforth, known as cholesterol β-epoxide and cholesterol α-epoxide). Infrared spectroscopy (IR) was subsequently used to determine the isomers of cholesterol epoxide. Both the egg powders exposed to fluorescent and direct sunlight showed similar patterns of oxidation with the major products being: 7-ketocholesterol, 7 α-hydroxycholesterol, 7 β-hydroxycholesterol, and cholesterol β-epoxide. Cholestane-3β,5α,6β-triol was also present in trace amounts.

In another study, cholesterol α-epoxide and cholesterol β-epoxide were isolated from commercial egg powders, and characterized by IR, mass spectroscopy, and nuclear magnetic resonance (Tsai and Hudson, 1984). However, the amounts present were not quantified.

Naber and Biggert (1985) also identified cholesterol oxides in fresh egg and yolk heated at 100 °C for four hours in air. They observed a four-fold increase in 25-

hydroxycholesterol, 7-hydroxycholesterol, and 7-keto-cholesterol using both reverse phase and direct adsorption chromatography. Isomers of 7-hydroxy-cholesterol were not resolved by this method.

One of the first reported studies quantifying cholesterol oxides in egg powder was by Tsai and Hudson (1985). They measured the levels of cholesterol α-epoxide and cholesterol β-epoxide in commercially dried egg products from 15 dehydration plants in the United States. Sixty-four percent of the samples tested contained between 6-49 μg/g whole egg cholesterol epoxide. The ratio of α/β was found to be 1:4.

These investigators also compared the effect of different drying methods on cholesterol epoxide formation. Egg samples dried by air heated directly with a gas burner contained a greater amount of cholesterol epoxides than samples dried by air heated indirectly with steam.

Missler et al. (1985) also quantified the levels of cholesterol oxides in egg powder. Several cholesterol oxides were identified in a scrambled egg mix stored for five years in cans under a nitrogen atmosphere. Cholesterol α-epoxide, cholesterol β-epoxide, and 7-ketocholesterol were present in the greatest concentrations. It is worth noting that the levels of cholesterol α-epoxide were five times higher than that of cholesterol β-epoxide. Minor amounts of 25-hydroxy-

cholesterol, 5 α-cholestane-3 β, 5,6 β-triol, and the isomers of 7-hydroxycholesterol were also detected. Like Tsai and Hudson (1985), Missler et al.(1985) compared the influence of spray-dryer design (direct fired, high levels of nitrogen oxide versus indirect, electric) on the formation of cholesterol oxides in egg powder. The theory behind this investigation was that nitrogen oxides present in the flue gas of direct gas-fired dryers were involved in the initiation of cholesterol oxidation. They found that egg mixes dried with the direct heat source contained significantly higher levels of cholesterol oxides than those prepared by indirect electric spray drying.

In a Swedish study, Nourooz-Zadeh and Appelquist (1987) quantified the concentrations of a number of cholesterol oxides in fresh egg yolk, freeze-dried yolk powder, and commercially dried egg yolks produced by indirect spray drying, and Petit-Choux mix. Petit Choux mix is a Swedish brand name for an egg yolk blend containing high levels of vegetable oil. Gas chromatographic analysis revealed no cholesterol oxides in the fresh egg yolk and freeze-dried yolk powder, at the detection limit of 0.2 μg/g of total lipids. Although the same cholesterol oxides were found in the spray-dried yolk as in Missler's study (1985) (with the exception of 20 α-diol which was not reported by Missler), the relative concentrations differed.

Interestingly, Nourooz-Zadeh and Appelquist (1987) found 7-hydroxycholesterol to be in greatest concentration, and the ratio of cholesterol α-epoxide to cholesterol β-epoxide to be 1 to 5 after prolonged storage from 12 to 18 months. However, the Petit-Choux mix contained predominately α-epoxide with a ratio of 10 to 1.

The effect of operating conditions and antioxidants on the formation of cholesterol epoxides in spray dried egg powder have also been investigated. No correlation between inlet or outlet temperature and cholesterol epoxide formation was found by Morgan and Armstrong (1987). The addition of BHA and BHT at 67 μg/g yolk solids did not affect the concentrations of cholesterol epoxides. However, at 200 μg/g yolk solids, both antioxidants reduced the extent of epoxide formation by approximately 50 percent.

General Mechanism of Cholesterol Oxidation

The autoxidation of lipids, including cholesterol, is a self-propagating, non-catalytic reaction involving molecular oxygen in which peroxyl radicals are formed. The reaction of lipid oxidation is generally broken down into three stages: initiation, propagation, and termination (Farmer et al., 1943).

Smith (1981) described two distinct cholesterol autoxidation processes. The major process, Equation 1, involves the initial abstraction of the allyl C-7

hydrogen and reaction with triplet oxygen to form a 7-hydroperoxyl radical. Stabilization of the 7-peroxyl radical by hydrogen abstraction yields two epimeric cholesterol 7-hydroperoxides. The quasi-axial 7 α-hydroperoxide is thermodynamically less stable than the beta isomer, and readily interconverts to the more stable quasi-equatorial 7 β-hydroperoxide. In the minor process of formol alcohol dehydrogenation, peroxyl radicals are formed at the C-3 position and stabilized by the elimination of hydrogen peroxide, yielding cholest-5-ene-3-one, as shown in Equation 2.

$$RH + O_2 \longrightarrow ROOH \quad \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

$$RCH(OH) + O_2 \longrightarrow RC{=}O + H_2O_2 \quad \ldots\ldots\ldots (2)$$

The hydroperoxides formed at the C-7 positions can undergo subsequent transformation to produce a variety of oxidation products, only a few of which directly pertaining to egg will be discussed. These transformations can be grouped as formol reduction, dehydration, thermal decomposition, and epoxide formation reactions, and are summarized in Figure 1.

Formol reduction of 7-hydroperoxides proceeds by thermal or metal ion catalyzed homolysis of the peroxide bond, yielding a 7-oxyl radical, that in turn abstracts a hydrogen from an adjacent molecule to form the 7-hydroxycholesterol (Smith, 1981). The 7α-hydroxycholesterol isomer may epimerize to the more stable 7β-hydroxycholesterol. Dehydration of the

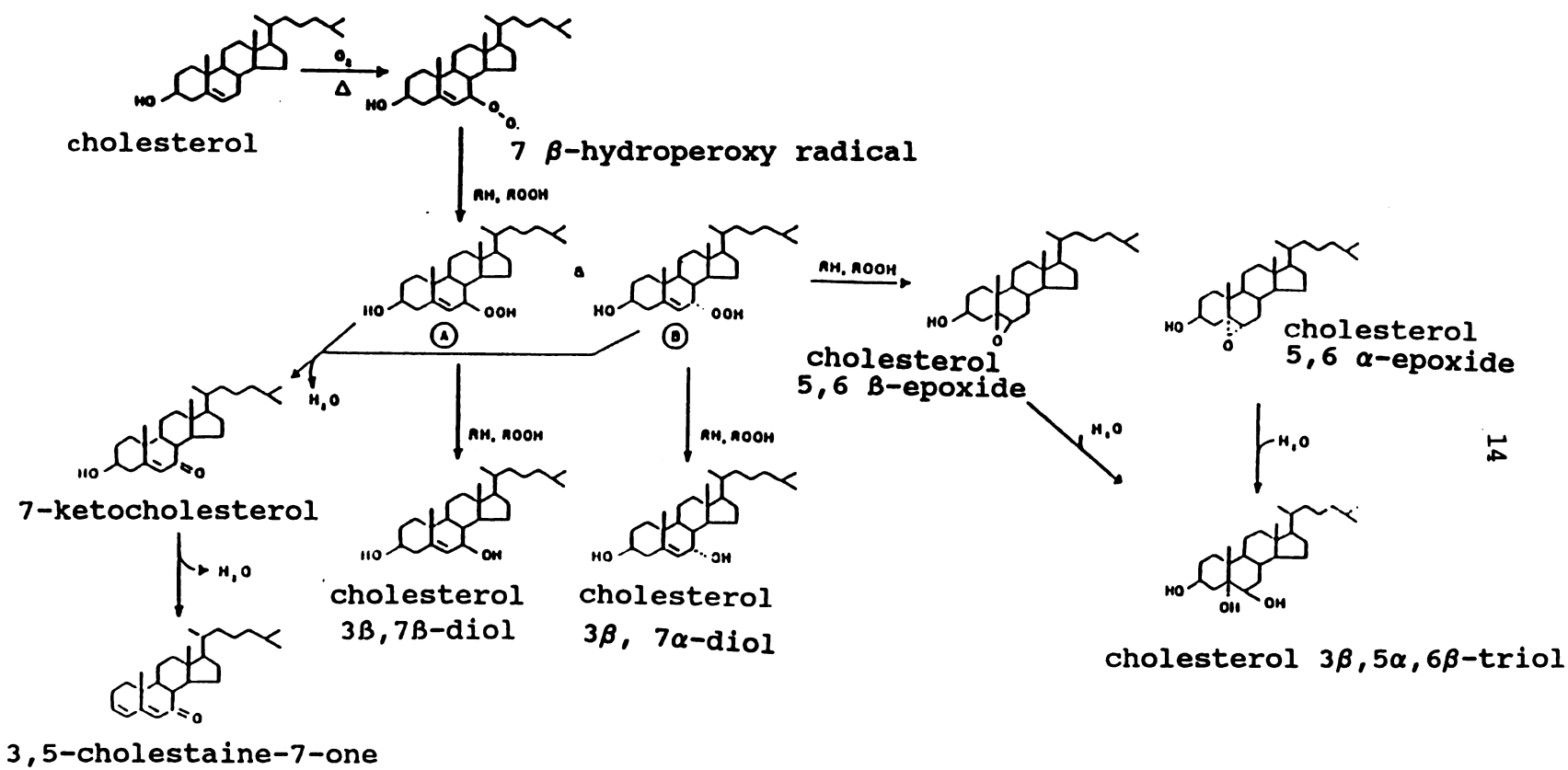


Figure 1. Major pathway for cholesterol oxidation (Adapted from Luby, 1982).

cholesterol 7-hydroperoxide results in the formation of 7-ketocholesterol. Thermal dehydration of 7-keto-cholesterol produces 3,5-cholestane-7-one. In addition, cholesterol 3β,7-diol and 7-ketocholesterol may be formed indirectly by singlet oxygen (Smith, 1981). Attack by singlet oxygen on cholesterol results in the formation of 5α-hydroperoxide that is readily isomerized to 7α-hydroperoxide, which can then undergo the aformentioned reactions.

Epoxides are thought to be secondary oxidation products of cholesterol. Smith and Kulig (1975) established that singlet and triplet oxygen attack on cholesterol resulted in only the formation of hydroperoxides, but not epoxides. However, when cholesterol was treated with 7-hydroperoxy-cholesterol or 5-hydroperoxyl-6-ene, epoxide formation was observed. Smith (1981) further stated that cholesterol may be epoxidized by hydrogen peroxide, hydroxy radicals, perbenzoic acid, soybean lipoxygenase, and X-radiation. Hydration of cholesterol epoxide yields cholesterol-3β,5α,6β-triol.

Nitrogen oxides as initiators of cholesterol oxidation

There are many known initiators of lipid oxidation. Smith (1981) described a variety of promoters such as free radicals, chemical reactions, enzyme

transformations, radiolysis,and photolysis. Free radicals included nitrogen oxides, azo compounds, hydroperoxides, hydrogen peroxide and transition metals, and oxygen.

In egg powder, oxides of nitrogen are of importance since they are thought to form as a result of the combustion process in gas-fired spray dryers and lead to the formation of cholesterol oxides. Oxides of nitrogen include nitric oxide (NO) and nitrogen dioxide (NO_2).

During combustion of natural gas, methane is oxidized forming carbon dioxide and water:

$$CH_4 + 2O_2 \quad ---> \quad CO_2 + 2H_2O$$

Nitrogen present in the natural gas or from the air may react with oxygen through a series of reactions forming nitrogen oxides (Wheeler, 1980) [Tables 2 and 3]. Reactions (1/2) and (3/4) are referred to as the "Zeldovitch exchange reactions". The rate of the reaction, "k", is temperature dependent. At 1600 °C, the breakdown of diatomic nitrogen to form nitrogen oxide and nitrogen atoms (reaction 1) proceeds at a much slower rate than the reverse reaction (reaction 2). It might, therefore, be expected that reaction 1 does not occur to any significant amount. However, this is not the case, since the mole fractions of diatomic nitrogen and oxygen far outweigh those of nitrogen oxide and atomic nitrogen, in the system (Wheeler, 1980).

Table 2. Reactions involved in nitrogen oxide formation.

(1)	$N_2 + O \rightarrow NO + N$
(2)	$NO + N \rightarrow N_2 + O$
(3)	$O_2 + N \rightarrow NO + O$
(4)	$NO + O \rightarrow O_2 + N$
(5)	$OH + N \rightarrow NO + H$

Adapted from Wheeler (1980).

Table 3. Zeldovitch reaction-rate constants at 1600 °C.

Constant	Rate
k_1	2.5×10^5
k_2	2.8×10^{13}
k_3	2.3×10^4
k_4	9.6×10^4
k_5	4.0×10^{13}

Adapted from Wheeler (1980).

Similarly, the rate of reaction 3 is much greater than that of reaction 1. It might then be assumed that reaction 3 principally accounts for the majority of nitrogen oxide formed during combustion. However, the thermal dissociation of oxygen proceeds at a higher rate than that of nitrogen so that early in the reaction, atomic oxygen is more abundant than atomic nitrogen. As a result, both reactions contribute substantially to the formation of nitrogen oxide (Wheeler, 1980).

Nitrogen dioxide decomposes at normal flame temperatures, and is scarcely detected at the mouth of the combustion chamber. It is formed by the reaction of NO with diatomic oxygen:

$$NO + O_2 \longrightarrow NO_2 + 1/2\ O_2$$

The rate of the reaction increases as the temperature falls, so that it is likely to be formed in the drying chamber (Wheeler, 1980). The rate of the reaction is also dependent on the square of the nitrogen oxide concentration (Lightsey, 1982). This means that the level of nitrogen oxide present contributes substantially to the rate of formation of nitrogen dioxide.

NOx gases may also dissolve in the moist environment of the spray dryer to form nitrous and nitric acid that can be detected as nitrite and nitrate in dried powder (Kelly et al., 1989):

$$2NO_2 + H_2O \longrightarrow HNO_2 + HNO_3$$

Nitrate, absorbed in the gastrointestinal tract of humans, reacts with hemoglobin to form methemoglobin (Kelly et al., 1989). In healthy adults, methemoglobin is converted enzymatically to oxyhemoglobin (Kelly et al., 1989). In infants, less than three months of age, the enzyme system is not complete. Excess amounts of methemoglobin may result in a condition of methemoglobinemia (Kelly et al., 1989). Microorganisms present in the gastrointestinal tract are capable of converting nitrate to nitrite. Nitrite is also absorbed in the gastrointestinal tract, and can react with amines formed by protein metabolism resulting in the formation of n-nitrosamines (Kelly et al., 1989).

In addition, nitrite has also been proposed to react with the unsaturated lipids in model systems resulting in the formation of nitro-nitroso compounds (Liu et al., 1988). During frying, oxides of nitrogen are released which are capable of nitrosating secondary amines.

The biological toxicity of nitrogen oxide is much less than that of nitrogen dioxide (National Academy of Sciences, 1977), and therefore, will not be discussed. Nitrogen dioxide, on the other hand, has been postulated as an initiator of cholesterol and fatty acid oxidation (Lightsey, 1982; Missler et al., 1985).

The mechanism by which nitrogen dioxide initiates cholesterol oxidation is not yet fully understood. One of the earlier studies by Kamel et al. (1971) focused on

the formation of products when NO_2 was bubbled over a monomolecular layer of cholesterol. The investigators found that exposure of the cholesterol film to 95 to 175 parts per million (ppm) NO_2 resulted in the condensation of the film, and also in the formation of cholesteryl nitrate. They postulated that the condensation occurred as a result of the difference in polarities of cholesterol and cholesteryl nitrate. The latter, being more polar, had a greater affinity for the aqueous solution, and was consequently desorbed from the cholesterol layer. When the monomolecular film of cholesterol was exposed to air, expansion of the film was observed. In addition, oxidation products were detected using TLC, in only the film of cholesterol that was not exposed to NO_2.

The condensation and expansion effects of NO_2 and air were also measured using monolayers of dihydroxycholesterol, 5-cholesten-3-one,7 β-hydroxycholesterol and 7-ketocholesterol in order to establish the mechanism of attack. The same effects of condensation and expansion were observed, with the exception of 5-cholesten-3-one exposed to NO_2. From their findings, Kamel et al. (1971) theorized that nitrogen dioxide attacks the 3-hydroxy group, not the 5,6 double bond, to form cholesteryl nitrate.

Conversely, Roehm et al. (1971) postulated that nitrogen dioxide adds directly to the double bonds of

unsaturated fatty acids to produce a nitroso free radical, which, in turn abstracts an allylic hydrogen from another molecule. This creates a second free radical which would then react with oxygen to form a peroxy radical. These investigators found that trace quantities of NO_2 (0.5 to 5.4 ppm) rapidly catalyzed the oxidation of methyl linoleate and methyl linolenate, both as thin films and in aqueous dispersions. Interestingly, the products (measured as thiobarbaturic reactive substances (TBARS) and conjugated diene formation at 235 nm were formed at a fast rate with no appreciable induction phase and at high concentration. Roehm et al. (1971) also found that vitamin E prolonged the induction period, thereby slowing down the oxidation process.

Studies have also been performed to demonstrate the effect of inhaling NO_2 on lung lipid tissue. Sevanian et al. (1979) observed that rats exposed to 6.5 ppm NO_2 for 24 hours resulted in significantly higher amounts of cholesterol epoxide than the control group.

Thomas et al. (1968) demonstrated that alpha-tocopherol (vitamin E) had a partial protective effect in preventing oxidation of lung lipid in rats exposed to NO_2 as measured by diene conjugation. Roehm et al. (1971) conducted a similar study exposing rats fed a vitamin E free diet or 100 mg/kg of alpha-tocopherol acetate per day, to 10 ppm NO_2 for four weeks. They reported reduced but not significantly different ($p<0.5$) levels of C18:3,

C18:2, and C22:5 fatty acids in the vitamin E-supplemented group, compared to the levels found in the supplemented control not exposed to NO_2. Significant reductions in C18:2, C18:3, C20:5, C22:5, and C22:6 fatty acids were found in the vitamin E-deficient group.

More recently, Pryor and Lightsey (1981) have shown that the mechanism of NO_2-initiated oxidation is concentration dependent. At low concentrations, NO_2 reacts with unsaturated fatty acids primarily by abstraction of the allylic hydrogen to form nitrous acid and a free radical. At high concentrations, nitrogen dioxide adds directly to the double bond.

Nitrogen dioxide-initiated cholesterol oxidation has also been studied in egg powder as a function of spray drying method. Missler et al. (1985) reported that scrambled egg mix dried using a direct gas-fired heat source contained higher amounts of cholesterol oxidation products than those dried using an indirect, electric heat source. In the direct gas-fired spray dryer, oxides of nitrogen would be generated as a result of the combustion of natural gas. Missler et al. (1985) theorized that NO_2 was responsible for the high levels of cholesterol oxides found in the egg powder processed using the direct gas-fired burner.

Similarily, Morgan and Armstrong (1987) demonstrated that the addition of hydrogen peroxide or low levels of NO_2 (3 ppm in exhaust air) promoted the formation of

cholesterol epoxides initially and after storage for 7 months at ambient temperature.

These investigations suggest that the formation of cholesterol oxides in egg powder can be reduced by lowering or eliminating the amount of nitrogen oxides present during spray drying.

Antioxidant activity of alpha-tocopherol in egg

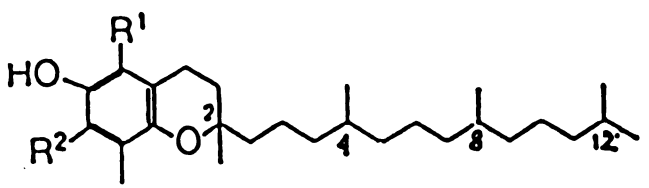
Antioxidants function by delaying the onset of oxidation by acting as free radical scavengers or metal chelating agents. Antioxidants may be classified as either synthetic or natural. Commonly used food grade synthetic antioxidants are butylated hydroxyanisole, butylated hydroxytoluene, tertiary butylhydroquinone, and propyl gallate (Dugan, 1976). These all act as free radical scavengers. In contrast, citric acid, phosphoric acid, and ethylenediamine tetraacetic acid (EDTA) act as metal complexing agents (Fennema, 1985). Citric acid and phosphoric acid are naturally occurring, while EDTA is synthetic. Although these are important in food, the focus of this review will be on alpha-tocopherol.

Alpha-tocopherol is a naturally occurring antioxidant that belongs to a class of compounds exhibiting vitamin E activity. All the compounds in this class have a 6-chromanal ring structure and a side chain that may be saturated (tocopherols) or unsaturated at positions 3, 7 and 11 (tocotrienols) (Shearer, 1986).

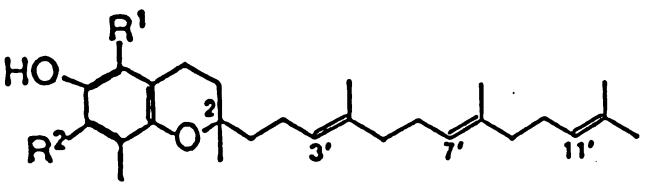
Within each group, there are four compounds that differ in the number and the position of the methyl groups on the 6-chromanal ring (Figure 2).

Tocopherols differ in antioxidant activity. In general, antioxidant activity increases with decreasing vitamin E activity. The antioxidant activity of tocopherols increases in the following order: delta (δ) > gamma (γ) > beta (β) > alpha (α) (Fennema, 1985). Although alpha-tocopherol has the lowest level of activity, it is the most predominant tocopherol in egg yolk (Sasago et al., 1974). Unlike the synthetic antioxidants mentioned above, the side chain of alpha-tocopherol can readily interact with the phospholipid component of cell membranes, thereby anchoring itself to the membrane. This may help facilitate antioxidant activity (Buttriss and Diplock, 1984).

Animals do not synthesize vitamin E, but acquire it from their diet. Unlike most animal products, eggs contain about one-third of the vitamin E in the gamma-tocopherol form (McLaughlin and Weihrauch, 1979). Tsugo et al. (1968) reported the concentrations of α-, γ-, and δ- tocopherol in white Leghorn eggs to be 4.0, 2.1, and 0.1 mg/ 100 g lipid, respectively. Syvaoja et al. (1985) analyzed whole egg, yolk, white, and boiled egg yolk for vitamin E content using two different extraction methods. They demonstrated that all of the vitamin E was contained in the yolk, and that boiling had



R^1	R^2	
CH_3	CH_3	α-Tocopherol or 5, 7, 8-Trimethyl-tocol
CH_3	H	β-Tocopherol or 5, 8-Dimethyl-tocol
H	CH_3	γ-Tocopherol or 7, 8-Dimethyl-tocol
H	H	δ-Tocopherol or 8-Monomethyl-tocol



R^1	R^2	
CH_3	CH_3	α-Tocotrienol or 5, 7, 8-Trimethyl-tocotrienol
CH_3	H	β-Tocotrienol or 5, 8-Dimethyl-tocotrienol
H	CH_3	γ-Tocotrienol or 7, 8-Dimethyl-tocotrienol
H	H	δ-Tocotrienol or 8-Monomethyl-tocotrienol

Modified from BASF Doc. MEA 3207 AA 0388-10.

Figure 2. The structures of the tocopherols and tocotrienals.

no significant effect on the concentrations. Using direct solvent extraction, the concentrations of three yolk tocopherols (α,β,andγ) and α-tocotrienol were present in the concentrations of: 5.50, 0.12, 0.23, and 0.72 mg/100 g of yolk, respectively. Analysis by saponification resulted in slightly higher values.

The values reported by Syvaoja et al. (1985) are higher than those cited by other researchers. In Finland, the feed is supplemented with alpha-tocopherol acetate. It appears that there is transfer from the feed into the egg. The theory of transfer of tocopherol from feed into egg is supported by the work of Sasago et al. (1974). A single oral dose of 30 mg of α-, γ-, and δ-tocopherol given to hens resulted in the transfer of 18.0 %, 4.8 % and 1.0 % of the tocopherol dosed, respectively. It took four to six days to reach these levels. When 10 mg/day of alpha-tocopherol acetate was given to hens daily, it took twelve days to reach the maximum value, with a transfer efficiency of 18.2 percent.

Sasago et al. (1974) also noted a great variation in the tocopherol content in eggs. Tocopherol levels in the yolk were found to be: α-tocopherol, 19.3 ± 7.6 mg/100g; δ-tocopherol, 10.0 ± 4.0 mg/100 g. Interestingly, they found that the tocopherol levels in eggs taken consecutively from a single hen were similar, but the

levels in the eggs among hens of the same flock varied considerably.

Further evidence of the relationship between the tocopherol content of feed and egg yolk is cited in a review article by BASF (Doc. MEA 3207 AA 0388-1.0). Three levels of increasingly higher amounts of α-tocopherol in the feed (8.6, 12.4 and 16.6 mg/kg) resulted in increasingly greater concentrations of α-tocopherol in egg yolk (119 ± 11, 214 ± 16 and 304 ± 30 μg/g yolk, respectively).

No evidence was found in the literature regarding the stabilization of egg yolk lipids by α-tocopherol. Stabilization of poultry fat and meat tissue by dietary vitamin E supplementation has been demonstrated by Webb et al. (1972) and Bartov and Bornstein (1976, 1977a). In addition, Asghar et al. (1989) determined that dietary supplementation of α-tocopherol reduced the extent of lipid peroxidation in the microsomes of dark broiler meat. It has also been demonstrated that a feed concentration of 200 mg α-tocopherol/kg stabilized the membranal lipids in pork and reduced the rate of lipid oxidation in pork products during storage (Buckley et al., 1989).

Heater design of spray dryers

Egg powders are traditionally produced by spray drying. There are five types of air heaters ultilized in the spray drying process (Masters, 1976):

(1) indirect, steam air heaters
(2) indirect oil or gas air heaters
(3) direct oil or gas air heaters
(4) electric air heater
(5) liquid phase air heaters

The review will be limited to only gas and electric air heaters since they are most commonly used in drying eggs.

Electric heaters are commonly found only in laboratories or pilot plant settings. Fixed costs of electric heaters are less expensive than others types of heaters, but are more expensive to operate (Jansen and Elgersma, 1985).

In general, gas heaters work on an aerating principle. The gas flows from a jet, often a venturi, at high velocity causing a reduction in pressure surrounding the jet. Air readily flows into this area resulting in intimate mixing. Depending on design, the gas may be mixed with all or part of the air required for combustion (Masters, 1976).

Gas mixtures have limits of inflammability. Natural gas must contain between 5% and 14% oxygen, by volume, in order for combustion to occur (Masters, 1976).

Jansen and Elgersma (1985) compared the economics of direct and indirect gas heaters. During the combustion process, water vapor is produced. They found that in order to maintain the same moisture content in the powder, as in indirect heating, the inlet and outlet temperatures have to be increased. Because of the increase in drying temperatures and higher specific heat of the drying air, the heating efficiency of the direct heater is less than 100 percent.

On the other hand, Jansen and Elgersma (1985) reported that the heating efficiency of indirect heating increased as the flue gas temperature decreased up to the dew point temperature (about 57 °C). At the dew point, condensation occurs. At 220 °C and a relative humidity of 9 %, the heating efficiency of the indirect heater was 7.6 % less than that of direct heator under comparable conditions. In addition, Jansen and Elgersma (1985) indicate that the capital costs associated with indirect heaters are higher than with direct heaters.

Although direct gas heaters offer economic advantages, biologically toxic oxides of nitrogen formed during combustion come in direct contact with the powder. Newer designs reduce the level of NOx (low NOx burners) produced. Wheeler (1980) described two heater designs that lower the level of nitrogen oxides in the flue gas. The first design is based on substoichiometric staged combustion and cooling. In substoichiometric combustion,

fuel is burnt under starved oxygen conditions so that oxygen becomes the limiting factor in nitrogen oxide formation. The combustion is staged so that incomplete combustion products do not leave the chamber. Incomplete combustion results in unburnt hydrocarbons, carbon monoxide , formaldehyde, and polycylic aromatic hydrocarbons such as 3,4-benzapyrene (Jansen and Elgersma, 1985). The gas is cooled between combustions so that nitrogen oxide formation is reduced.

The second design described by Wheeler (1980) is a single stage burning with excess air. In this design, the combustion temperature is kept low and the duration of the highest temperature phase as short as possible to limit NOx formation.

EXPERIMENTAL

Materials

Cholesterol oxide standards

The cholesterol oxide standards listed below were purchased from Steraloids Inc., Wilton, NH: Cholesterol-5,6 α-epoxide, 7-ketocholesterol, 5α-cholestane-3β, 5,6β-triol, 25-hydroxycholesterol, and 7β-hydroxycholesterol.

Reagents

All reagents used in the experiments were of analytical grade. Alpha-tocopherol acetate was donated by BASF Corporation (Detroit, MI).

Source of eggs

For the α-tocopherol feeding study, fresh eggs were obtained from the Poultry Research and Teaching Center at Michigan State University.

In the cholesterol oxidation study, eggs were supplied from a local producer in Ireland, spray dried at the Agricultural Institute (Moorepark Research Center, Fermoy, Ireland), vacuum packaged, and air freighted to MSU. The egg powders were stored in a sealed carton at -20°C until required for analysis.

Methods

Influence of dietary α-tocopherol on the oxidative stability of egg yolk lipids

Supplementation of the diet of laying hens with α-tocopherol

Forty-eight White Leghorn hens were divided into three groups receiving one of three levels of α-tocopherol acetate in the feed: 10 mg/kg feed (control), 100 mg/kg feed, or 200 mg/kg feed. All groups were fed the basal level of 10 mg α-tocopherol/kg feed for one month prior to the study in order to establish the baseline level of α-tocopherol in the egg yolk.

Each of the three groups was further subdivided into four sets of four hens each for feeding purposes. The four hens in each set were caged separately, but shared a common feeder. Additional feed was added daily so that a level of approximately 2.5 kg was maintained in the feeder. Feed consumption and the general health of the birds were maintained throughout egg production.

Eggs were collected every afternoon between 4:00 and 6:00 pm for twenty-eight days and stored in a 40 °F walk-

The yolk was initially separated from the white of the egg with an egg separator, and then placed on a damp paper towel to remove any residual white. The membranes surrounding the yolk were punctured, and the free flowing yolks from each group were combined. Analysis for α-tocopherol was performed in duplicate for each group using a high performance liquid chromatography procedure (Monaham, private communication).

Approximately 5 g of the yolk were diluted 1:4 with deionized water in order to reduce the viscosity of the liquid yolk. From this suspension, 0.5 g aliquots were weighed into test tubes. The weights were recorded to five significant digits. No attempt was made to weigh the exact amount each time.

The yolks were saponified by the following procedure (modified from Monaham, unpublished data): Two milliliters of a 1% (w/v) ethanolic solution of pyrogallol, and 0.5 ml of 50 % (w/v) potassium hydroxide were added to the test tubes, which were then nitrogen flushed, capped, and vortexed for 2 minutes. The tubes were then placed in a 70 ± 2 °C water bath for 45 minutes, and then cooled to room temperature before the addition of 2 ml of deionized water.

Alpha-tocopherol was extracted from the saponified layer with three 5 ml portions of hexane. The hexane extracts were combined, concentrated under nitrogen, and the residue redissolved in 500 μl of ethanol. The

concentration of α-tocopherol in the yolks was ascertained by reverse phase high performance liquid chromatography, with a Beckman Ultrasphere ODS column (4.6 mm x 15 cm), methanol mobile phase (1 ml/min), and an ultraviolet detector (280 nm). .

Lipid oxidation studies

(a) pH study

Twenty four egg yolks from each of the groups fed varying amounts of α-tocopherol (10 mg/kg feed, 100 mg/kg feed, and 200 mg/kg feed) were combined following the feeding trial. Sixty grams of yolk from each group were weighed into four flasks, to which were added 10 ml of deionized water and 0.012% (w/w) sodium azide. The pH of the egg yolk suspension was adjusted to 3.0 ± 0.05 in half the flasks using 1 N hydrochloric acid. The yolks in the remaining flasks were left at their natural pH (6.0 ± 0.05), and deionized water was added so that the final volume in all groups was equal. The flasks were then covered with parafilm (American Can Company, Greenwich,CT) and agitated with a wrist action shaker for twelve days at room temperature. Thiobarbituric acid (TBA) values were determined every four days.

Thiobarbituric acid test

Ten grams of the yolk suspension from each flask were mixed with 100 ml of deionized water, and added to a distillation flask containing antifoam, glass beads, and 2.5 ml of hydrochloric acid (diluted 2 parts H2O : 1 part HCl). Approximately 50 ml of distillate were collected. A 5 ml aliquot of the distillate was added to a test tube containing 5 ml of TBA reagent (Tarladgis et al., 1960). (TBA reagent consisted of 0.3 % (w/v) thiobarbituric acid in deionized water.) The capped tubes were then vortexed, heated in a water bath at 100 °C for 35 minutes, and then cooled in ice water for ten minutes. Absorbance was measured at 532 nm using a double beam Bausch and Lomb spectrometer (Rochester, N.Y.)

(b) Liposome peroxidation test

A lipid peroxidation test was performed using an initial egg yolk suspension similar to that used in the pH experiment. Fourteen milliliters of the yolk suspension from the three groups (10 mg/kg feed, 100 mg/kg feed, and 200 mg/kg feed) at the natural pH were added to a series of test tubes . After incubation in a water bath at 34 °C for five minutes, 1.0 ml of a 30 μM metmyoglobin solution and 0.1 ml of a 30 μM hydrogen peroxide solution were added. One milliliter of each sample was removed from the test tube at the following time intervals : 0, 5, 10, 15, 20, 30, 60,

90, 120, 150, and 180 minutes, and placed in a test tube containing 2 ml of TBA reagent. The TBA reagent consisted of 0.4 % (w/v) thiobarbituric acid, 10 % (w/v) trichloroacetic acid, and 2.5 % (v/v) of concentrated hydrochloric acid. The reactants in the test tubes were heated in a boiling water bath for 15 minutes to facilitate color development. The samples were allowed to cool to room temperature, and then centrifuged for 10 minutes at speed 4 in a IEC Clinical Centrifuge (Damon/IEC Division, Needham Hts., MA). Absorbance was read at 532 nm using the double beam spectrometer previously mentioned.

Cholesterol Oxidation Products of Egg Powder

Synthesis and identification of cholesterol 5,6 β-epoxide

Cholesterol 5,6 β-epoxide (cholesterol β-epoxide) was synthesized according to the method developed by Chicoye et al. (1967). Cholestane-3β,5α,6β-triol (0.2 g) and 5 ml 2 N potassium hydroxide in ethanol were refluxed for 2 hours. After cooling, 300 ml deionized water were added to the solution, and carbon dioxide gas was then bubbled into the solution until the pH of the solution reached 6.0. Cholesterol β-epoxide was then extracted succesively with three portions of ethyl ether (200, 100, and 100 ml). The ether extracts were combined, vacuum evaporated, and the β-epoxide

recrystallized in a small amount of methanol by holding overnight in a 4 °C cooler.

Cholesterol β-epoxide was identified using infrared spectroscopy. The IR spectra for potassium bromide pellets of cholesterol α-epoxide and the synthesized compound were compared in the region of 800-1600 cm-1 frequency using a Perkin Elmer 337 Model Infrared Spectrometer. (Norwalk, CT) In addition, the spectrum for the synthesized compound was compared to spectra reported in the literature.

Experimental design of cholesterol oxidation study

At the Agricultural Institute Fermoy, Ireland, liquid whole egg magma was spray dried using three types of air heaters: indirect, electric; direct gas-fired, low NOx; direct gas-fired, high NOx. The egg samples were spray dried using a pilot scale Anhydro Spray drier Lab3 with an inlet and outlet temperature of 200 and 95 °C, respectively. The Anhydro Lab 3 model is a single stage, conical drier equipped with a pneumatic nozzle and electrically air heated. Conversion to direct gas-fired air heaters was achieved by the attachment of gas burners. The concentrations of nitrogen oxides and carbon monoxide (CO) were measured for the low NOx and high NOx dryers. Attachment of the low NOx burner resulted in a concentration of 0.05 ppm NOx and 1 ppm CO at the burner, whereas 8 ppm NOx and 10 ppm CO were

detected at the inlet of the drying chamber using the high NOx dryer. A description of the low NOX and high NOX burners are found in the appendix.

A variety of food approved ingrediants were added to the liquid egg yolk before drying: BHT (200 μg/g fat); HSO3 (0.1 w/v %); and 14.68 g NaNO2 / 10 ml of 1.2 N HCLO4 to 66 kg liquid egg. In addition, α-tocopherol-enriched eggs and a control group were dried using all three methods of drying.

The egg powder was stored in vacuum-pckaged bags for seven months at -20 °C before analysis. After the initial analysis, duplicate samples from all treatments and drying methods were stored for four months at 20°C and 40°C, and analyzed for the formation of COP's.

Extraction of Cholesterol Oxides From Egg Powder

One gram of egg powder was weighed into an Erylenmyer flask, to which was added 50 ml of chloroform. The flasks were then nitrogen flushed, stoppered and shaken by hand for two thirty second intervals to ensure mixing. The extract was filtered through Whatman filter paper (Number 4) to remove any egg particles, and the flask was washed twice with 10 ml portions of chloroform. After washing, the extract was evaporated to approximately 20 ml using a rotary evaporator, and placed on an Extraluet column (Anspec, Ann Arbor, MI) that had been previously wetted with 15 ml of ethyl acetate. The

Extraluet column is a silica column, and was used to remove any highly polar substances. The cholesterol oxides were eluted off the column with three 15 ml portions of ethyl acetate, evaporated to near dryness and brought up to 2 ml in ethyl acetate.

High Performance Liquid Chromatography

After optimizing the conditions for separating cholesterol oxides and cholesterol, the retention times for the cholesterol oxides of interest were ascertained. In order to reduce collection time, the flow rate of the mobile phase (ethyl acetate) was increased from 0.5 ml/min to 1 ml/min, after twenty-five minutes, to give a total run time of 40 minutes. A sample volume of 250 μl was injected onto a Waters μ-Porasil column (3.9 mm x 15 cm) and collection of the eluant begun after twenty-one minutes. This corresponded in time to the end of the elution of cholesterol as indicated by a refractive index detector. It is important to note that 25-hydroxycholesterol was not resolved well from cholesterol when a 250 μl of sample was injected, and was "sacrificed" in order to reduce the number of collection fractions. Two fractions from each sample were collected in a flat-bottomed flask immersed in ice, vacuum evaporated to near dryness, quantitatively transferred to a one dram vial with ethyl acetate (final volume of 1 ml), and nitrogen flushed. Between fractions, the flow

rate was reduced back to 0.5 ml/min and allowed to equlibrate for 5 minutes.

Gas Liquid Chromatography

Samples were evaporated to dryness in a stream of nitrogen, derivatized with 50 µl of bis-(trimethyl silyl)- trifluoroacetamide (BSTFA, Pierce Chemical Co., Rockford, IL) and 100 µl of pyridine, and placed in the dark for thirty minutes. Capillary gas chromatographic analysis was performed using a Hewlett Packard 5840A gas chromatograph (Avondale, PA) equipped with a flame ionization detector. The temperature was programmed from 180° C to 230 °C at a rate of 10°C per minute, held for 2 minutes , and then increased to 240°C at a rate of 0.2°C per minute. The temperature of the injection and detector ports were 270°C and 300°C, respectively.

Nitrite Analysis

The extraction procedure to determine nitrite levels in egg was adapted from the International Dairy Federation 95 (1980) procedure. Five grams of egg powder were dissolved in 100 ml of warm deionized water in a 250 ml flask, and agitated for one hour. Six milliliters of 53 % (w/v) zinc sulfate solution was then thoroughly mixed with the contents of the flask, and filtered through Whatman filter paper (Number 5). Ten milliliters of the filtrate were collected. Nitrite analysis was

performed using a Lachat Quikchem Automated Flow Injection Ion Analyzer (Mequon, WI) in which nitrite was diazotized with sulfanilamide, and then coupled with N-(1-naphthyl)ethylenediamine dihydrochloride to produce a wather-soluble magenta dye. Absorbance was measured at 520 nm.

Statistical Analysis

Statistical analysis of the data from the feeding trial, pH study, and the metmyoglobon / hydrogen peroxide-initiated lipid oxidation test was performed using linear regression and the Bonferroni t-test. For the cholesterol oxide data, one, two, and three-way analysis of variance were performed using MSTAT. MSTAT is a program developed at Michigan State University (Departments of Crop and Soil Sciences, and Agricultural Economics) and the Agricultural University of Norway (1989).

RESULTS AND DISCUSSION

STABILIZATION OF EGG YOLK LIPIDS THROUGH DIETARY SUPPLEMENTATION

(a) Incorporation of α-tocopherol in egg yolk by dietary supplementation

The first phase of this study focused on the effect of dietary supplementation of vitamin E in the feed of laying hens on the concentration of α-tocopherol in the egg yolk. Laying hens were fed one of three levels of vitamin E (10 mg/kg, 100 mg/kg, and 200 mg/kg) in the feed. Dietary levels used in this study were based on the results of a recent investigation by Buckley (unpublished data, 1989) in which approximately 180 μg α-tocopherol/g were incorporated into the egg yolk by supplementation of the feed with 100 mg/kg α-tocopherol acetate.

At least two other reports in the literature have addressed the increase in α-tocopherol content in the yolk through dietary supplementation. Sasago et al. (1974) reported that after 12 days of a daily dose of 10 mg of α-tocopherol acetate, a concentration of 120 μg α-tocopherol/g yolk was achieved. A second study (cited in BASF Doc. MEA 3207 AA 0388-10), author unknown) revealed

that the α-tocopherol content in the yolk increased to approximately 304 μg per whole yolk, when the concentration in the feed was 16.6 mg/kg α-tocopherol.

The results of the feeding study are shown in Figure 3. There are several missing data points because of procedural difficulties discovered at a later date. In these samples, 2 ml of deionized water were added to the test tubes and the lipid portion extracted with hexane. The extract was not washed with deionized water. Washing would have removed any alkaline contamination of the aqueous layer in the hexane extract.

A white precipitate was noticed in some samples after evaporation of the extract. The precipitate may have been potassium hydroxide. Later work by Asghar (unpublished data, 1989) indicates the instability of α-tocopherol under these conditions. Desai (1980) recommended washing of the hexane extract when saponification is an integral part of the procedure, although Buttriss and Diplock (1983) did not mention this step.

As a result, it is likely that the α-tocopherol content in many samples was reduced or destroyed. Therefore, these data were omitted from the data analysis. The decision to exclude a sample from subsequant data analysis was made if the concentration of α-tocopherol was one-half the amount determined for a replicate sample. This increases the error

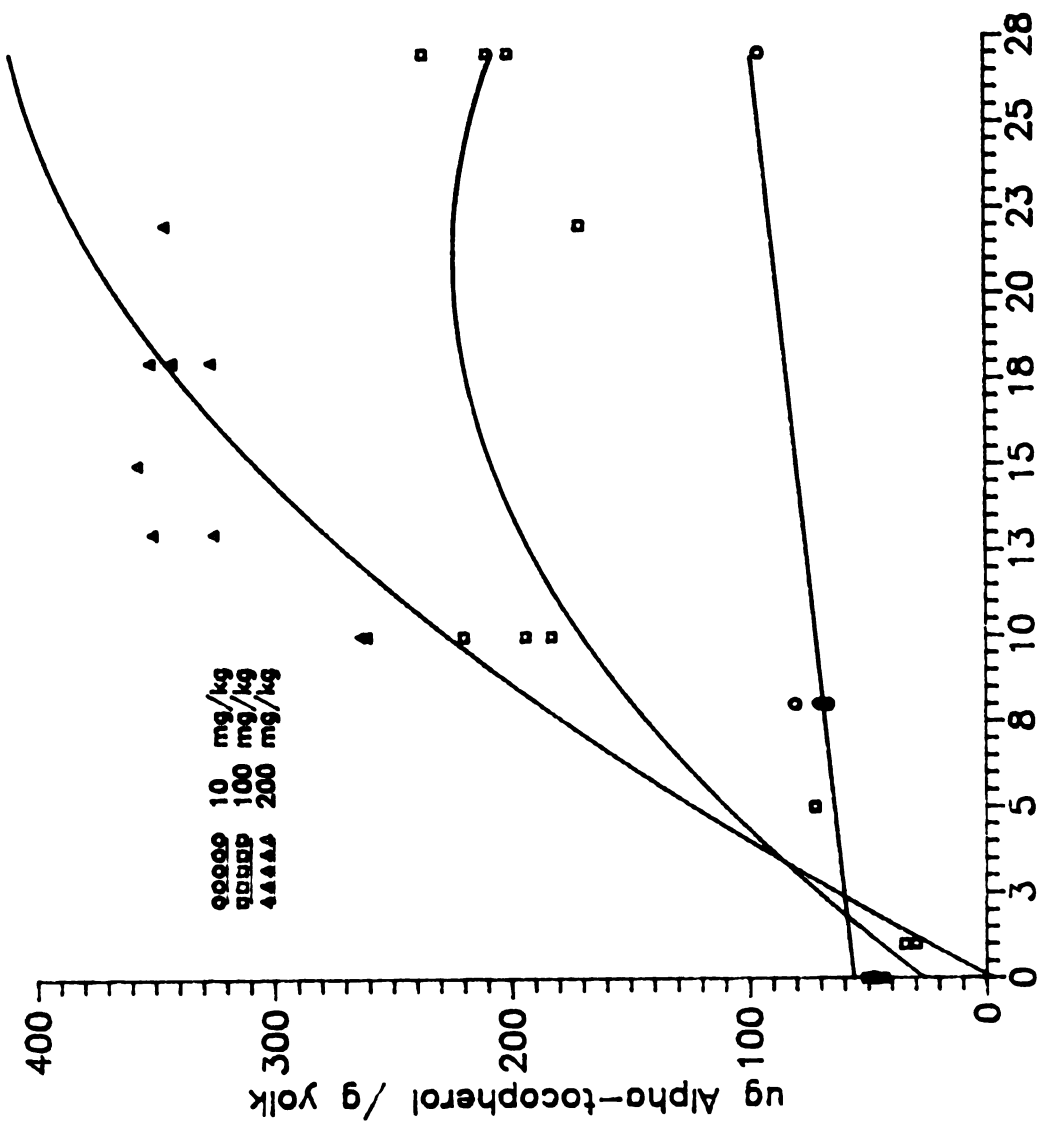


Figure 3: Effect of dietary supplementation on the alpha-tocopherol concentrations in egg yolk

associated with the analysis and based on this study alone, erroneous conclusions are possible. On the other hand, general trends are evident and supported by the results of a concurrent study by Buckley (unpublished data, 1989).

Significant differences ($p<0.05$) between the α-tocopherol contents in the yolk were found for all three feeding levels. Feeding the basal level resulted in approximately 80 μg/g α-tocopherol in the yolk after 28 days. This value is somewhat similar to the total tocopherol levels (65.5 μg/g yolk) reported by Syvaoja et al. (1985). In Finland, feeds are supplemented with α-tocopherol acetate. The basal level in the Finnish study was not reported and differences between concentrations may account for the slight variation between the two studies.

For the 100 and 200 mg/kg supplementation levels, the α-tocopherol concentrations in the yolk increased to 200 and 350 μg/g, respectively. These concentrations were significantly different ($p<0.05$) from each other. After 10-12 days, the concentrations of α-tocopherol tended to level off for both levels of dietary supplementation.

This study did not indicate a maximum level of incorporation of α-tocopherol in the egg yolk as related to the level of dietary supplementation. Theoretically, it may be possible to further increase the α-tocopherol

content in the yolk by increasing the concentration in the feed.

(b) Stabilization of egg yolk lipids by α-tocopherol

Alpha-tocopherol is a naturally occurring antioxidant that acts as a free radical acceptor during the initial phase of lipid oxidation (Fennema, 1985). In the first part of this project, it was determined that concentrations of α-tocopherol in the egg yolk are increased by dietary supplementation of the feed with α-tocopherol acetate. To determine if these elevated levels of α-tocopherol influence the oxidative stability of yolk lipids, two experiments were conducted in which the lipids were subjected to conditions favoring oxidation.

The effect of α-tocopherol on the oxidative stability of egg yolk under acidic conditions

Eggs from the feeding study were used to determine the effect of α-tocopherol on the oxidative stability of egg yolk during storage under acidic conditions (pH 3) and at the natural pH of egg yolk (pH 6). The stability of egg yolk containing approximately 80, 200 or 350 μg α-tocopherol/g yolk was determined using the thiobarbituric acid test (Tarladgis et al., 1960).

At pH 3, lipid oxidation proceeded at a significantly ($p<0.05$) greater rate for the yolks containing the lowest concentration of α-tocopherol (Figure 4). Concentrations of 200 and 350 μg α-tocopherol /g yolk effectively reduced lipid oxidation in the egg yolks. On the other hand, no increase in TBARS occurred at the natural pH of 6 during the storage period (Figure 5).

The susceptibility of egg yolk to lipid oxidation under acidic conditions was also reported by Pike and Peng (1988b). They observed that the degree of oxidation increased in the following order: pH 3 >> pH 2 > pH 4. Pike and Peng (1988a) theorized that the loss of oxidative stability in egg yolk was a result of the denaturation of low density lipoproteins (LDL). Since LDL contains about 95 % of the lipid in egg yolk, any loss of structure would expose the lipid fraction to pro-oxidants in the surrounding yolk, thus, accelerating oxidation (Powrie and Nakai, 1986).

Egg yolk contains approximately 60 μg iron /g yolk and trace amounts of other minerals, such as copper and manganese, that are known to catalyze lipid oxidation (Cotterill and Glauert, 1979). The addition of EDTA to egg yolk reduces the interaction of lipid and pro-oxidants by chelation of the metal ions (Pike and Peng, 1988b). The sequestering effect of EDTA has also been demonstrated in meat systems. For example,Igene et al.

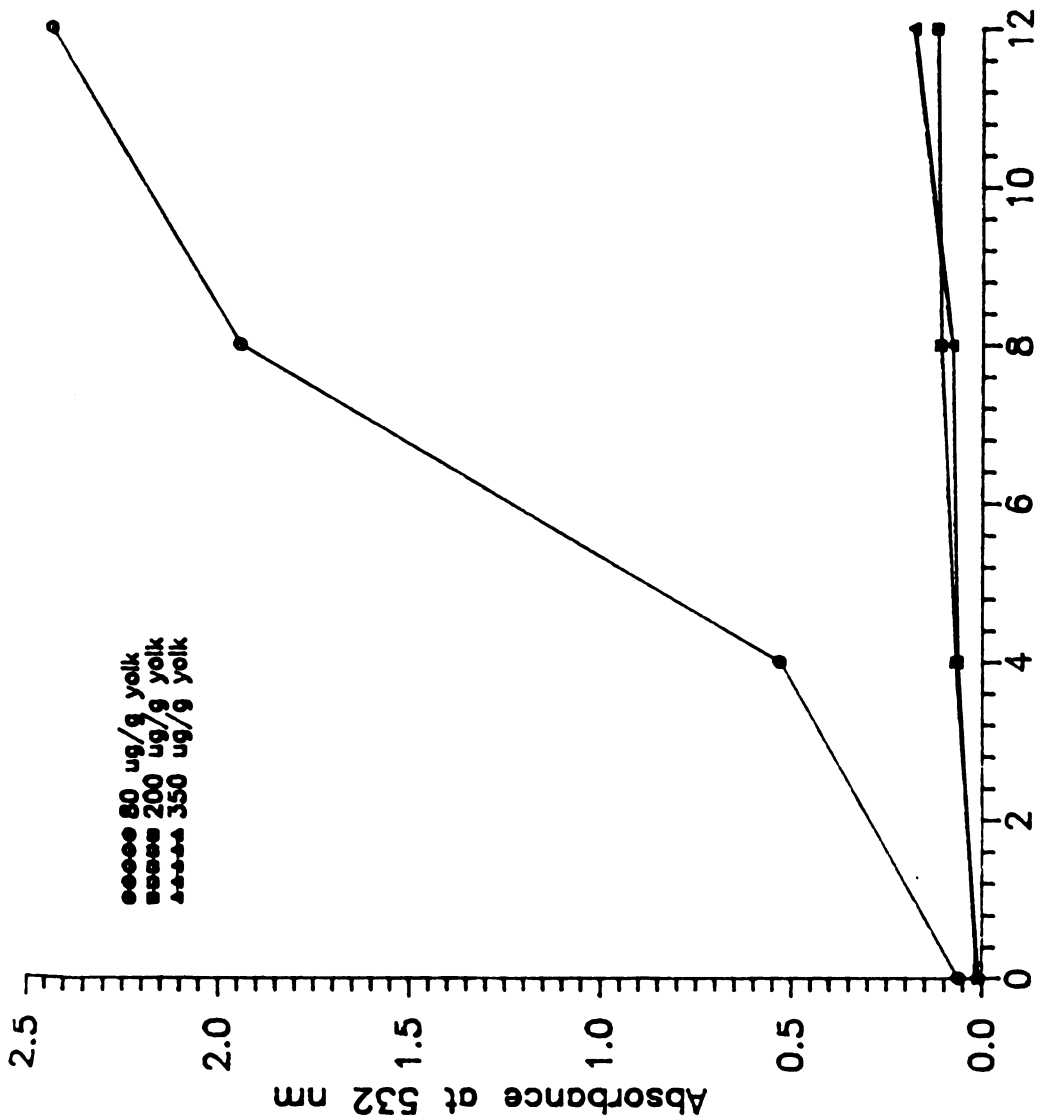


Figure 4: Effect of storage at room temperature on the rate of lipid oxidation as measured by change in absorbance in egg yolks (pH 3.0) containing various concentrations of alpha-tocopherol.

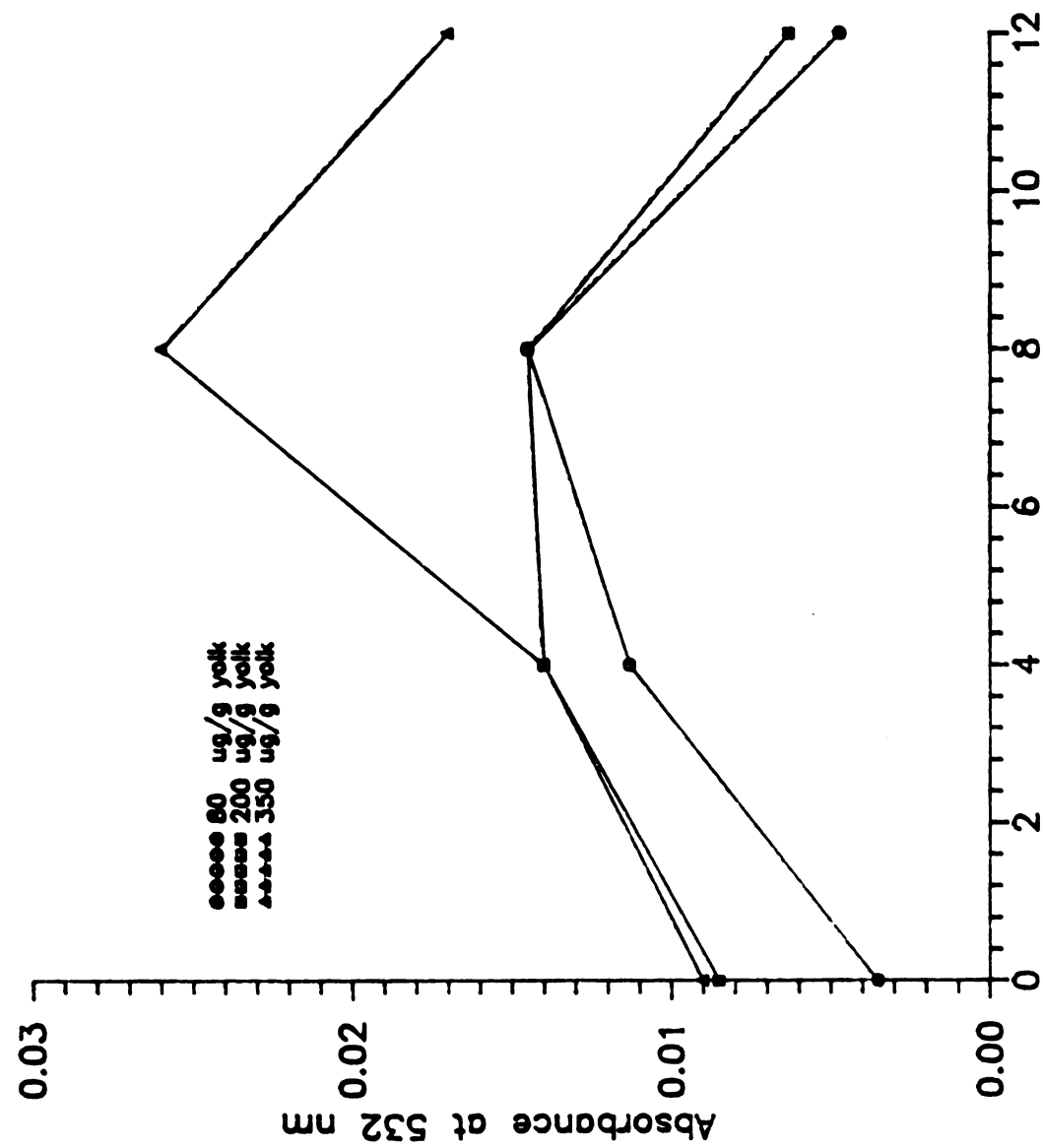


Figure 5: Effect of storage at room temperature on the rate of lipid oxidation as measured by change in absorbance at 532 nm in egg yolks (pH 6.0) containing various concentrations of alpha-tocopherol.

(1979) reported that the level of free iron in meat increased during cooking, thus accelerating oxidation by catalyzing the breakdown of preformed lipid hydroperoxides. The addition of EDTA reduced lipid oxidation by chelating most of the free iron present in the cooked meat.

Metmyoglobin / hydrogen peroxide-induced lipid peroxidation

Metmyoglobin and hydrogen peroxide were used to further assess the stability of egg yolk containing the three concentrations of α-tocopherol. The peroxidation assay is based on the interaction of metmyoglobin and hydrogen peroxide generating an activated species, which has been suggested as a possible initiator of peroxidation in biological systems (Harel and Kanner, 1985). The activated species has been postulated to be a porphyrin cation (ferryl) radical with an iron oxidation number of four. Initiation of lipid peroxidation occurs by a two-electron reduction via the porphyrin cation radical generating a peroxy radical (Harel and Kanner, 1985):

$$P^{+}\text{-}Fe^{4+}\text{=}O + LH \longrightarrow P\text{-}Fe^{4+}\text{=}O + L\cdot + H^{+}$$

$$L\cdot + O_2 \longrightarrow LOO\cdot$$

$$LOO\cdot + LH \longrightarrow LOOH + L\cdot$$

$$P\text{-}Fe^{4+}\text{=}O + LOOH \longrightarrow P\text{-}Fe^{3+} + LOO\cdot + -OH$$

$$LOO\cdot + LH \longrightarrow LOOH + L\cdot$$

The data represented in Figure 6 again indicate that the degree of peroxidation is influenced by the concentration of α-tocopherol in the egg yolk. In general, the extent of oxidation was greatest in the egg yolks containing the smallest level of α-tocopherol. By increasing the α-tocopherol concentration in the egg yolk through dietary supplementation, the oxidative stability of the egg yolk was increased. Although the trends were evident, no significant difference ($p<0.05$) was found in the rates between the three concentrations (Table 4). This may, in part, be due to the difference in fit of the second degree polynomial equation used in expressing the data.

Molenaar et al. (1980) proposed that the integrity of sub-cellular membranes from peroxidation was preserved by the presence of α-tocopherol in the membrane. The redox potential of the phenolic group on the chromanol ring of α-tocopherol, if located in the range of free radicals (range being 3 nm), would protect the membrane from peroxidation. Location of α-tocopherol near mixed-function oxidases capable of generating free radicals, such as reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, would therefore, enhance the stability of the membrane (Molenaar et al., 1980). The unsaturated fatty acids comprising the phospholipids in the membrane adjacent to the enzymes would be protected from oxidation with such location. In addition, the

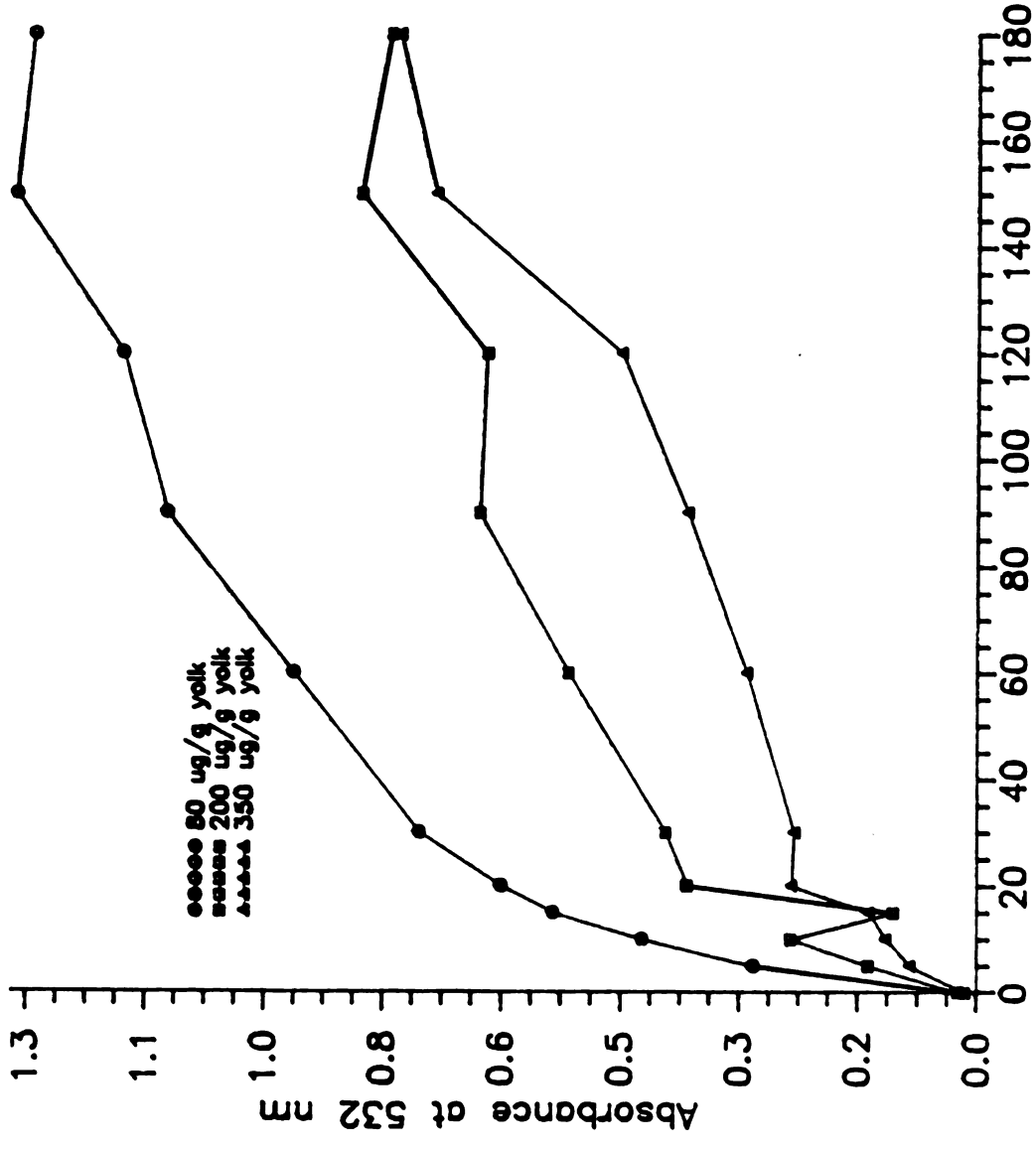


Figure 6: Metmyoglobin/hydrogen peroxide-initiated lipid oxidation as measured by change in absorbance at 532 nm in egg yolk containing various levels of alpha-tocopherol.

Table 4. Linear regression equations describing metmyoglobin / hydrogen peroxide-initiated oxidation in egg yolk at various levels of α-tocopherol.

α-tocopherol (μg/g yolk)	equation	r^2
100	$Y = 0.19 + 2.3E^{-2} - 1.9E^{-4}$	0.96
200	$Y = 0.08 + 1.3E^{-4} - 1.0E^{-4}$	0.85
350	$Y = 0.05 + 7.5E^{-2} - 0.5E^{-4}$	0.92

No significant differences ($p<0.05$) in rate constants were found between the samples.

phytyl chain of α-tocopherol would interact with the arachidonyl residue of the phospholipids further anchoring the tocopherol molecule in place (Molenaar et al., 1980).

In support of this theory, Harel and Kanner (1985) demonstrated that activated metmyoglobin-catalyzed peroxidation of lipids in microsomes isolates from turkey and chicken tissue was partially inhibited (65%) by the addition of 5 μmoles of α-tocopherol to the peroxidizing system. Asghar et al. (1989) determined that dietary supplementation of α-tocopherol reduced the extent of peroxidation in the microsomes of dark broiler meat. It has also been demonstrated recently that a feed concentration of 200 mg α-tocopherol/kg stabilized the membranal lipids in pork and reduced the rate of lipid oxidation in pork products during storage (Buckley et al., 1989).

COP Formation in Egg Powder as Influenced by Storage Conditions, Method of Drying, and Use of Selective Additives Prior to Drying

(a) Synthesis of cholesterol 5,6 β-epoxide

Cholesterol 5,6 β-epoxide (cholesterol β-epoxide) was synthesized as the compound was not commercially available. Infrared spectroscopy was used to confirm its formation from the hydroysis of cholestane-3β, 5α,6β-triol triacetate, using the method of Chicoye et al. (1968b).

Strong absorption bands at 3600, 2900, 1470, and 1384 cm^{-1} were present in the spectrum of the synthesized compound (Figure 7). The 3600 cm^{-1} band corresponds to the hydroxyl group at the 3 position in the A ring of the cholesterol nucleus. The other three bands are representative of the alkane groups forming the sterol molecules. Absence of bands at 1700 and 1680 cm^{-1} indicate the lack of a carbonyl group and a double bond at the 5,6 position, respectively.

In the fingerprint region (1350 to 600 cm^{-1}), the IR spectrum of the synthesized compound was compared to that of cholesterol α-epoxide (Figures 7 and 8), and with the IR spectra found in the literature (Chicoye et al., 1968b). The position and intensity of the bands in the fingerprint region of the synthesized compound were similar to those reported for cholesterol β-epoxide. A strong absorption band at 1060 cm^{-1} was observed. In addition, the positions and intensities of the three bands between 950 to 900 cm^{-1} corresponded to those in the spectrum of cholesterol β-epoxide as reported by Chicoye et al. (1968b). These bands are readily distinguishable between the α and β isomers. The three bands were located at 950, 933 and 911 cm^{-1} for the synthesized compound, and at 950, 922 and 906 cm^{-1} for cholesterol α-epoxide (Figures 7 and 8). A major band at 800 cm^{-1} present in the α-isomer of cholesterol epoxide,

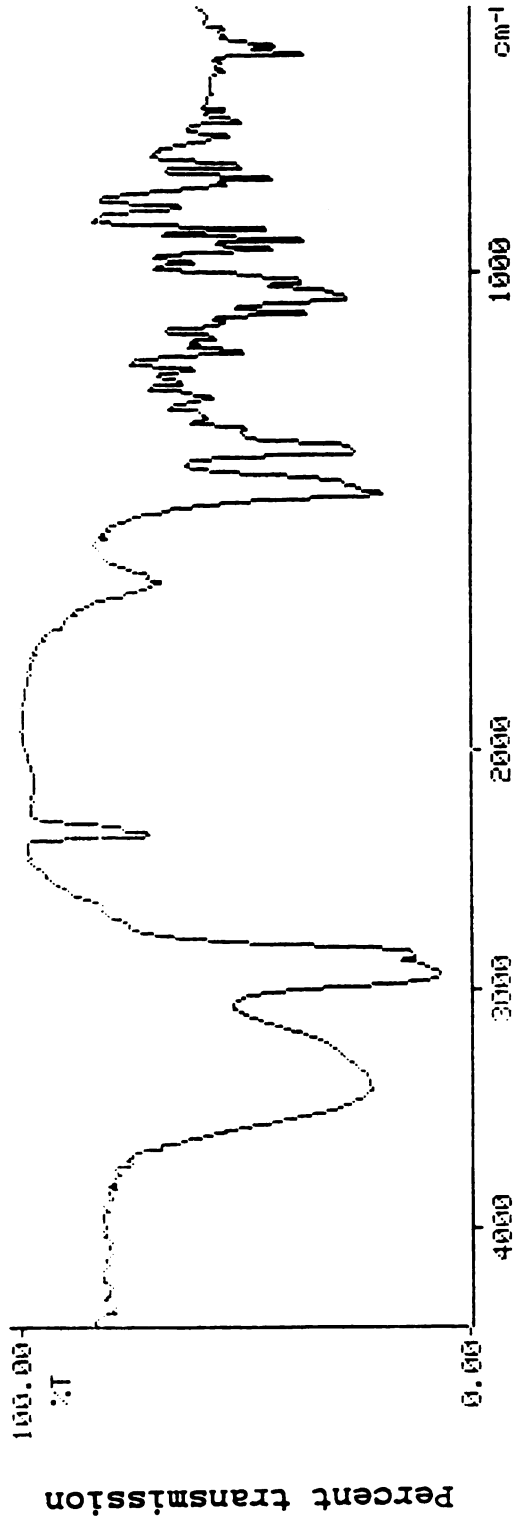


Figure 7. Infrared spectrum of cholesterol β-epoxide synthesized by hydrolysis of 5 α-cholestane-3β,6β-triol triacetate.

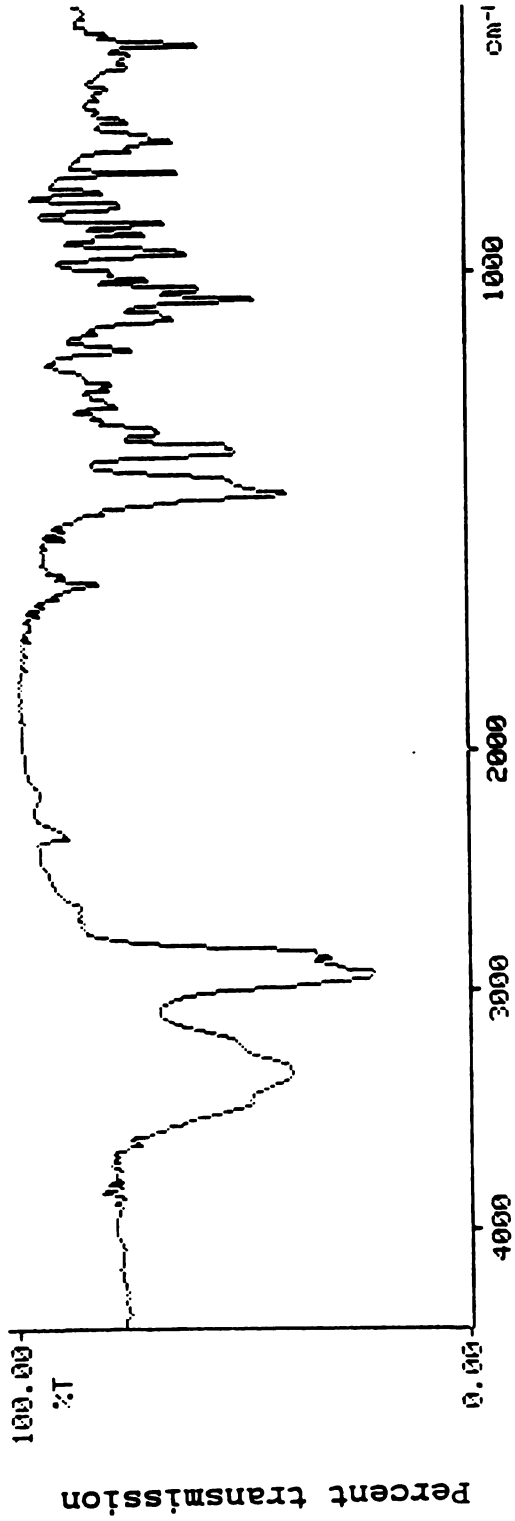


Figure 8. Infrared spectrum of cholesterol α-epoxide.

was not present in the synthesized compound or in cholesterol β-epoxide (Chicoye et al., 1968b).

(b) Analysis of Cholesterol Oxides in Egg Powder

The formation of cholesterol oxidation products (COP's) during the storage of dried egg products is well documented in the literature (Missler et al., 1985; Tsai and Hudson, 1985; Morgan and Armstrong, 1987; Nourooz-Zadeh and Appelqvist, 1987). In this study, egg powders were produced in Ireland, vacuum packaged, air freighted to MSU, and stored at -20 °C for three months before subjecting to various storage treatments. Samples of powders were stored at 20 and 40 °C for four months, and then analyzed for the presence of cholesterol oxidation products. The influence of three spray drying procedures (indirect, electric; direct, low NOx; and direct, high NOx) on COP formation was investigated, as was the effect of various food additives.

Cholesterol oxidation products in egg powder

Vacuum-packaged egg samples were held at -20 °C for seven months until analysis. The concentrations of individual cholesterol oxides in the samples ranged from 1-6 μg/g whole egg powder (Table 5). The method of drying did not significantly ($p<0.05$) affect the initial concentrations of COP's in the egg powders.

Table 5. Intial concentration (μg/g whole egg powder) of cholesterol oxides in vacuum-packaged egg samples storedat -20 °C for seven months.

Cholesterol oxide	Mode of drying		
	Indirect electric	Direct low NOx	Direct high NOx
Cholesterol β-epoxide	4.3	5.1	4.8
Cholesterol α-epoxide	1.4	2.6	1.7
7-Ketocholesterol	4.2	5.1	5.7
7 β-Hydroxycholesterol	2.3	1.5	3.8

No significant difference ($p<0.05$) for the concentrations of any cholesterol oxides in the samples was found among dryers.

Whether the COP's present were the result of storage or initially formed during the drying process can not be determined with these data. Since the samples were vacuum packaged, exposure to oxygen would have been minimal. In addition, the samples were held at -20 °C until the analytical methodology was perfected. Small concentrations of cholesterol epoxides have also been reported by Tsai and Hudson (1985) in egg powder dried using indirect heating, and stored for 1-6 months at -21 °C.

Storage of the samples for four months at 20 and 40 °C increased the concentrations of cholesterol oxides in the egg powder. The major COP's present were cholesterol β-epoxide, cholesterol α-epoxide, 7-ketocholesterol and 7 β-hydroxycholesterol (Figures 9 and 10, Table 6). Trace amounts (less than 2 μg/g powder) of 5 α-cholestane-3β,5,6 β-triol were also identified in many of the samples. The detection limit of the BSTFA-derivatized samples was 0.5 μg/g egg powder by gas liquid chromatography, using a FID detector.

Before injection onto the gas chromatographic column, the COP's were separated from triacylglycerols and cholesterol by HPLC, followed by derivatization (Missler et al., 1985). The elution of 25-hydroxy-cholesterol in close proximity to cholesterol did not allow for the collection and subsequent detection of this oxide by GLC analysis. In addition, HPLC clean-up was

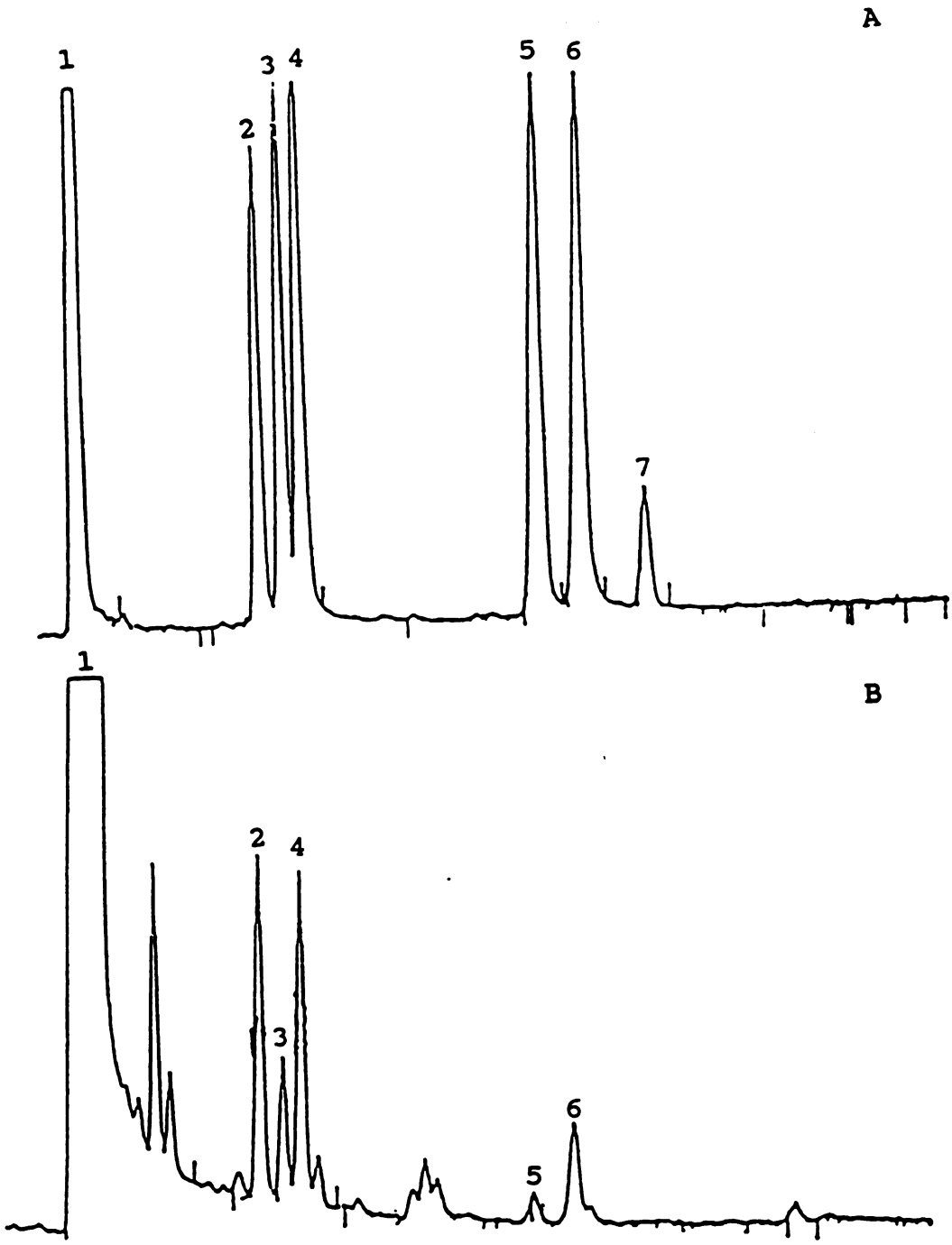


Figure 9. Gas chromatographic analysis of (a) standard mix of cholesterol oxides (b) cholesterol oxides isolated from egg powders dried by an indirect, electric spray dryer.

1) cholesterol 2) cholesterol β-epoxide 3) cholesterol α-epoxide 4) 7 β-hydroxy-cholesterol 5) 5 α-cholestane 3β, 5, 6 β-triol 6) 7-ketocholesterol 7) 25-hydroxycholesterol.

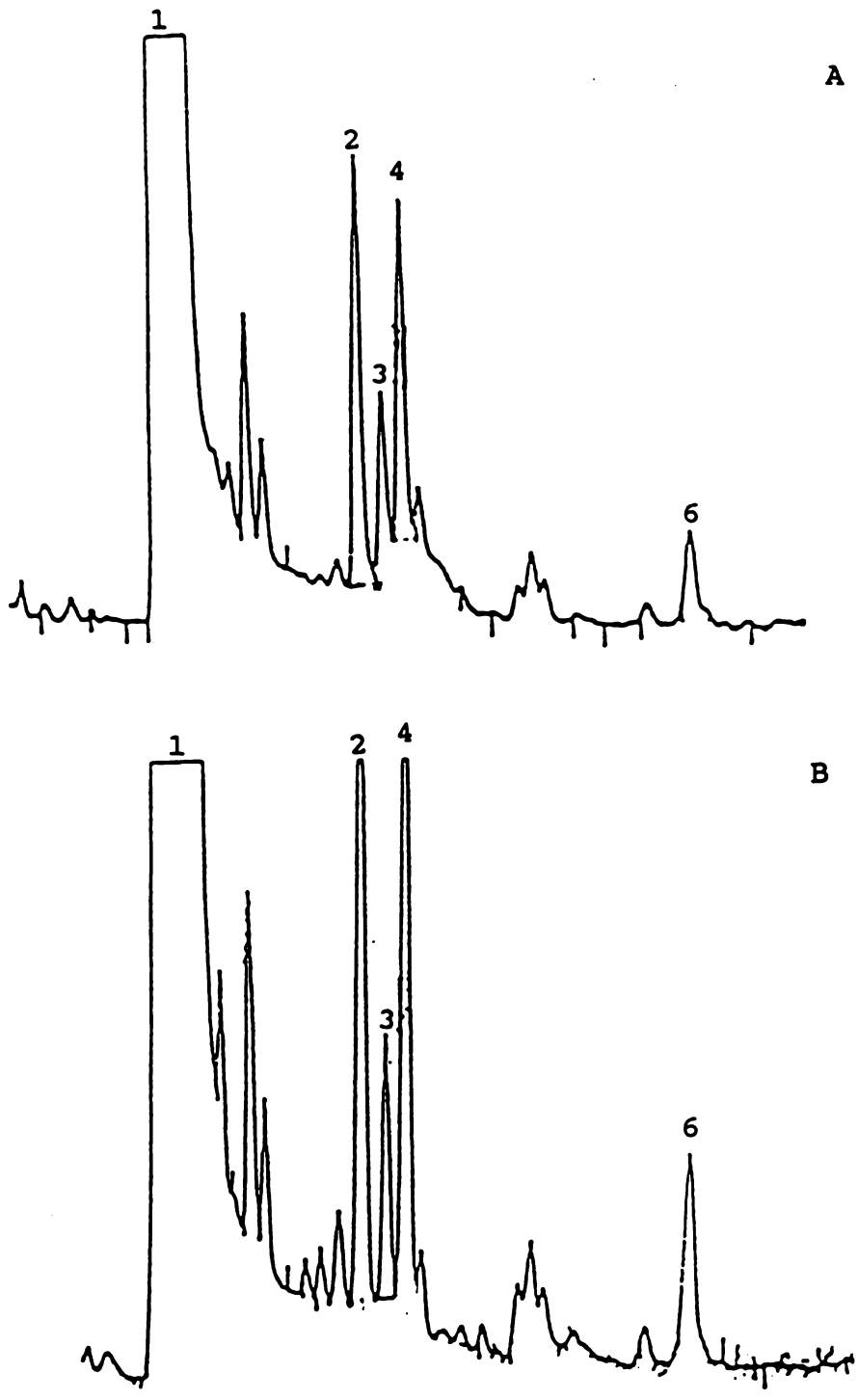


Figure 10. Gas chromatographic analysis of cholesterol oxides isolated from egg powdes dried by (a) direct, low NOx spray dryer (b) direct, high NOx spray dryer.

1) cholesterol 2) cholesterol β-epoxide 3) cholesterol α-epoxide 4) 7 β-hydroxy-cholesterol 6) 7-ketocholesterol.

not complete, and part of the cholesterol present in the sample was collected in the fraction to be injected on the GLC. As a result, 7 α-hydroxycholesterol was poorly resolved from the cholesterol peak on the gas chromatographic column. Therefore, it is possible that 25-hydroxycholesterol and 7 α-hydroxy-cholesterol were present in the egg samples, but not detected.

Missler et al. (1985) also was not able to separate completely 25-hydroxycholesterol from cholesterol by HPLC. The reported concentrations of 25-hydroxy-cholesterol ranged from 1-5 μg/g powder, and were calculated based on a distribution ratio of 4:1 (elution with cholesterol : elution after cholesterol). Although cholesterol was detected using GLC, the amount present did not interfere with the detection of 7 α-hydroxy-cholesterol. This may have been due to better fractionation by HPLC and differences in the type column and programming temperatures of the GLC. Missler et al. (1985) used a more non-polar GLC column (DB-1 versus SE-30, OV-1) and a higher final temperature (300 °C versus 240 °C). As a result, elution order, retention times, and resolution differed. The amount of 7 α-hydroxy-cholesterol reported by Missler et al. (1985) ranged from 1.8 to 7.0 μg/g egg mix. The concentration of α-hydroxy-cholesterol was small in comparison to other cholesterol oxides present in the samples.

The presence of cholesterol α-epoxide, cholesterol β-epoxide, 7-ketocholesterol, 7β-hydroxycholesterol, and trace amounts of 5α-cholestane-3β, 5,6β-triol in the egg products is consistent with similar reports in the literature. The concentrations of cholesterol oxides in the egg samples are shown in Table 6. Cholesterol β-epoxide was the most predominant COP present, followed by 7β-hydroxycholesterol, cholesterol α-epoxide and 7-keto-cholesterol. Depending on spray drying method, concentrations of the four cholesterol oxides in egg powders stored at 40 °C for four months ranged from 23-50, 16-25, 8-17, and 5-8 μg / g dried powder, respectively. Missler et al. (1985) detected α- and cholesterol β-epoxide and 7-ketocholesterol in scrambled egg mix powder stored at ambient temperature for five years. Minor amounts of 25-hydroxycholesterol, 5α-cholestane 3β,5,6β-triol, and the α and β isomers of 7-hydroxycholesterol were also found. Similarily, these cholesterol oxides were identified by Nourooz-Zadeh and Appelqvist (1987) in dried egg powder stored at 4 °C for 2 months.

In the present study, the ratio of cholesterol α-epoxide to cholesterol β-epoxide was 1:3. Tsai and Hudson (1985) reported a ratio of 1:4 in commercially dried egg powders. The relative abundance of cholesterol β-epoxide to the α-isomer is supported by the results of a study by Nourooz-Zadeh and Appelqvist (1987), who

reported a ratio of 1:5 for egg powder and 1:10 for scrambled egg mix. However, Missler et al. (1985) detected a greater concentration of the α-isomer, with the ratio being dependent on drying method. Indirect air heating resulted in a ratio of 10:1, while a ratio of 5:4 was observed in egg powder produced using a direct gas-fired burner. The difference in the ratio of α- and β-epoxide can not be explained by these studies, and warrants further investigation.

The effect of drying method on COP formation

The method of drying influenced the formation of cholesterol oxides in the egg powder during storage (Table 7). A significant difference ($p<0.05$) in the concentration of cholesterol β-epoxide was observed in those powders produced by the direct gas-fired spray drying system (Figure 11). Concentrations of 49 and 29 μg cholesterol β-epoxide / g dried powder were detected in egg powder processed with a high NOx and low NOx dryers, respectively, and stored at 40 °C for four months.

The difference in the concentrations of 7 β-hydroxy-cholesterol, in egg powders produced by the high NOx and low NOx dryers was significant ($p<0.05$), with values of 24 and 16 μg/g dried powder, respectively (Table 7). No significant differences ($p<0.05$) were found in the concentrations of cholesterol 5,6 α-epoxide and 7-keto-cholesterol in the egg powders from the two direct gas-

Table 6. The effect of storage temperature on theconcentration of cholesterol oxides (μg/g powder) in egg powders stored for 4 months.

Mode of Drying	Cholesterol Oxide	Storage Temperature 20 °C	40 °C
Indirect Electric	Cholesterol β-epoxide	16.0	23.7
	Cholesterol α-epoxide	4.3	7.9
	7-Ketocholesterol	3.8	5.1
	7 β-Hydroxycholesterol	8.4	16.2
Direct Low NOx	Cholesterol β-epoxide	12.8	29.0
	Cholesterol α-epoxide	1.7	8.9
	7-Ketocholesterol	1.7	6.3
	7 β-Hydroxycholesterol	5.0	16.2
Direct High NOx	Cholesterol β-epoxide	30.4	49.0
	Cholesterol α-epoxide	11.5	17.0
	7-Ketocholesterol	7.2	8.4
	7 β-Hydroxycholesterol	21.0	24.4

Table 7. The influence of spray drying method on the concentration of cholesterol oxides (μg/g powder) in egg powder stored at 40 °C for 4 months.

Cholesterol oxide	Mode of drying: Indirect electric	Direct low NOx	Direct high NOx
Cholesterol β-epoxide	a 23.7	a 29.0	b 49.0
Cholesterol α-epoxide	a 7.9	a 8.9	b 17.0
7-Keto-cholesterol	a 5.1	a 6.3	a 8.4
7 β-Hydroxy-cholesterol	a 16.2	a 16.2	b 24.4

Values in the same row bearing the same superscript do not differ significantly at $p<0.05$.

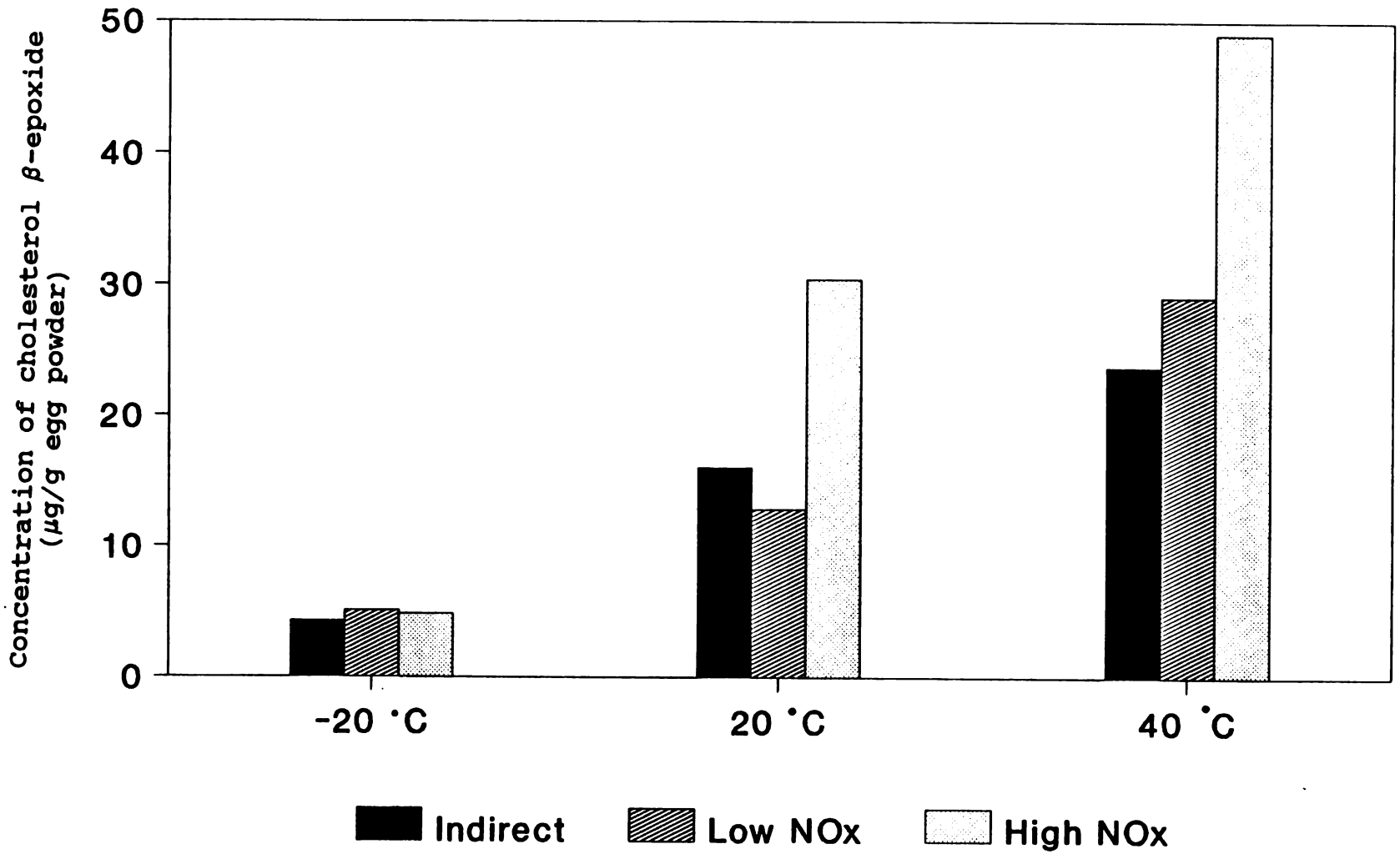


Figure 11. Concentration of cholesterol β-epoxide in egg powder as influenced by drying method.

fired burners. It appeared that the concentrations of these oxides (ranging from 5 to 8 μg/g dried powder) were too small to enable the detection of significant differences between burners.

No significant difference ($p<0.05$) was found in the concentrations of any cholesterol oxide in powders from the indirect (electric dryer) and direct, low NOx dryer (Table 8). Thus, the low NOx burner appears to be an effective alternative to the electrically heated spray dryer in limiting COP formation in dried egg products. The use of direct gas-fired spray dryers has been shown to be more economic than electric spray dryers, and results in substantial savings in energy cost and in capital expenditure (Jansen and Elgersma, 1985).

There are no reports in the literature relative to cholesterol oxide formation in products dried using a direct gas-fired, low NOx burner. Conversely, direct high NOx burners have been compared with indirect, electric spray dryers for the formations of COP's in egg powder (Tsai and Hudson, 1985; Missler et al, 1987). In all cases, the concentrations of cholesterol oxides in egg products dried with a direct, high NOx burner were greater than the quantities detected when an indirect, electric spray dryer was used.

A comparison between the formation of cholesterol epoxides in egg powders produced using indirect (electric) and direct, high NOx driers as reported by

Table 8. A comparison of the concentrations (μg/g powder) of cholesterol epoxides in dried egg products.

	Type of sample dried	Indirect electric	Direct high NOx	Conditions of storage
Missler et al. (1985)	egg mix	23.4	87.4	5 years, ambient temperature, nitrogen flushed cans
Tsai & Hudson (1985)	whole egg	* - 8	* - 236	commercial samples, 1-6 months, 21 °C, plastic bags
Morgan & Armstrong (1985)	egg yolk	6	__	4 months, 20 °C polyethylene bags
MSU	whole egg	20.3	41.9	4 months, 40 °C polyethylene bags
		31.6	66.0	

* not detected
__ not analyzed

Cholesterol epoxides represent the mixture of α and β isomers.

various research groups is shown in Table 8. Although the conditions of storage and type of sample vary (mix, yolk and whole egg), the concentrations of cholesterol epoxides are generally two to four times greater in samples produced using the direct, high NOx burner. Increasing the time or temperature of storage results in greater concentrations of cholesterol epoxides in the egg powder for both the indirect (electric) and direct, high NOx drier.

The differences in cholesterol oxide concentrations in eggs dried with the high NOx and low NOx burners, and the indirect (electric) spray dryer, are indicative of the role that oxides of nitrogen play in the initiation and propagation of cholesterol oxidation. For the high NOx burner, 8 ppm nitrogen oxides was detected in the air at the inlet of the dryer. The concentration of nitrogen oxides was 20.5 ppm at the low NOx burner. This appears contradictory, but it should be pointed out that the large burner concentration of NOx gases (low NOx burner) is diluted by fresh air before reaching the inlet. With this in mind, the level at the inlet would have been considerably lower than that of the high NOx burner. Unfortunately, it was not possible to quantitate the level of nitrogen oxides at the inlet for the low NOx burner.

Morgan and Armstrong (1987) observed the formation of cholesterol epoxides up to 7 μg/g dried yolk when the

exhaust air contained 5 ppm NOx. In comparison, no cholesterol epoxides were detected initially in egg yolk when produced using an electric heater. They postulated that initiators of cholesterol oxidation (ie. NOx) must be present during spray drying to generate significant amounts of cholesterol epoxides. The difference in COP's between generated by the three methods of spray drying used in this study support this theory.

In addition, the initiation of oxidation in model systems of lipids and cholesterol by oxides of nitrogen has been demonstrated (Kamel et al., 1971; Rhoem et al., 1971). Moreover, in vivo studies of rats inhaling 6 - 10 ppm NOx have revealed more lipid oxidation in lung tissue compared to the control groups (Thomas et al., 1968; Roehm et al. 1971; Sevanian et al., 1979). Therefore, it is likely that the level of NOx in spray dryers could effect the formation of COP's in egg powder.

The effect of selected additives on COP formation

A number of food-approved ingredients were added to the liquid whole egg prior to drying in order to evaluate their ability to slow down cholesterol oxidation during storage of the powder. In addition, eggs were collected and spray dried from hens fed a diet of 200 mg α-tocopherol acetate / kg feed. This dietary level produced a corresponding α-tocopherol concentration of 45 μg/g whole egg before drying.

The concentration of cholesterol oxides in powder from the α-tocopherol-supplemented eggs was compared to those in the control eggs, as a result of spray drying using the three methods previously mentioned. Cholesterol oxidation was partially reduced by the presence of α-tocopherol in the eggs (Figure 12, Table 9). Although visual observation of the data indicates this trend, the only significant differences ($p<0.05$) were found between the high NOx and low NOx burners for cholesterol β-epoxide. A 26 - 37 % reduction in cholesterol β-epoxide in egg powder produced using the high NOx burner was observed when the samples were stored at 40 and 20 °C, respectively. Comparing the low NOx and electric spray dryers, the effectiveness of α-tocopherol in reducing the formation of cholesterol oxides varied from 3-48 %; and the concentrations of each COP present were approximately the same for both dryers. Alpha-tocopherol was more effective in reducing the concentrations of the cholesterol oxides most prominant in the samples.

No reports in the literature were found on the effect of α-tocopherol on COP formation in spray dried products. It was demonstrated in this study that α-tocopherol reduces the extent of cholesterol oxidation. However, the dietary feeding of α-tocopherol was not as effective in reducing the formation of COP as was the low NOx burner. In egg powder stored for four months at

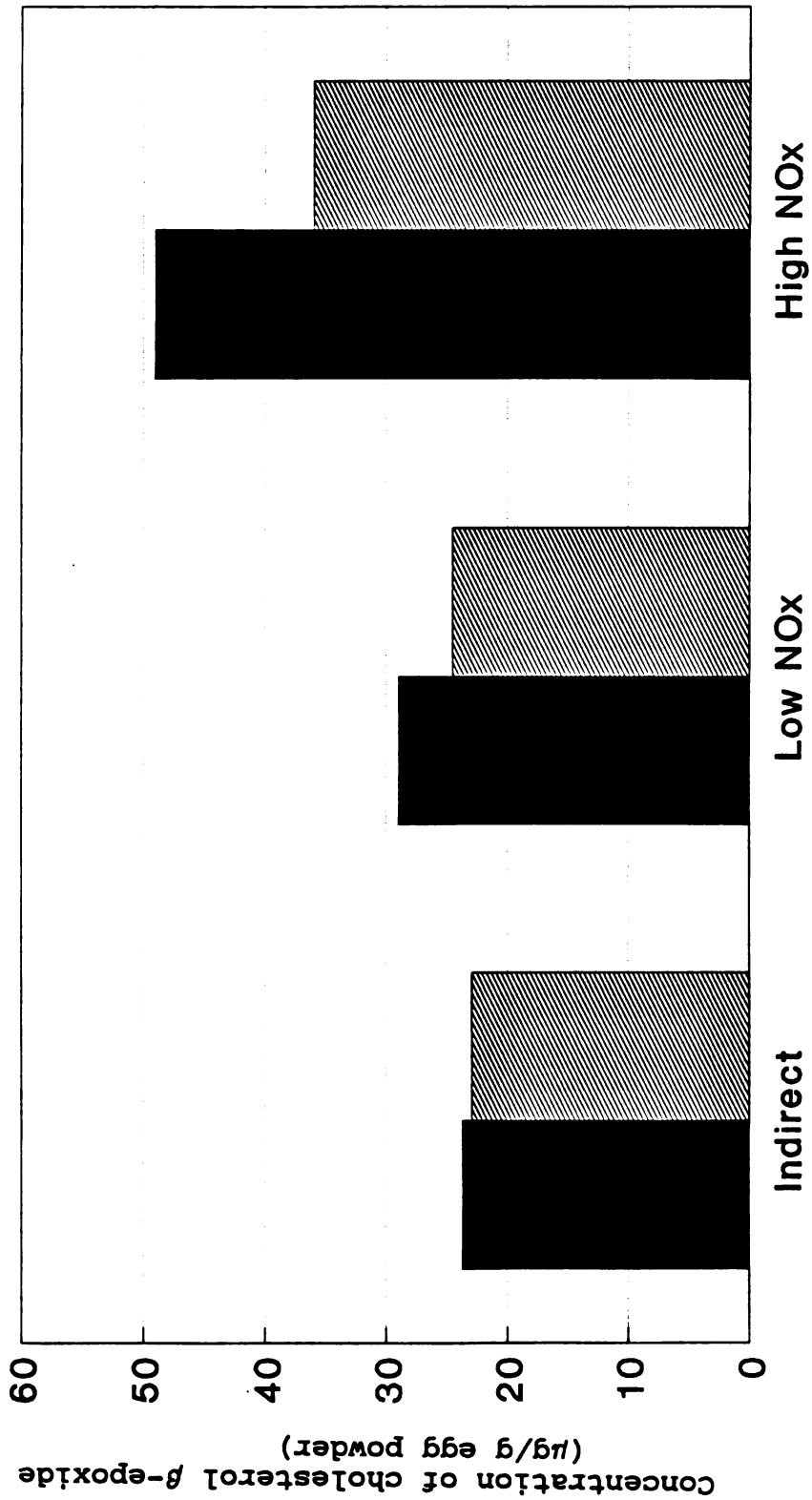


Figure 12. The effect of drying method on the concentrations of cholesterol β-epoxide in control and α-tocopherol supplemented egg powders stored at 40 °C for 4 months.

Table 9. The effect of drying method on the concentrations of cholesterol oxides in control and α-tocopherol-supplemented egg powder stored at 40 °C for 4 months.

	Indirect electric		Direct low NOx		Direct high NOx	
Cholesterol oxide	control	α-tocopherol	control	α-tocopherol	control	α-tocopherol
Cholesterol β-epoxide	a 23.7	a 22.9	b 29.0	c 24.5	d 49.0	e 36.0
Cholesterol α-epoxide	a 7.9	a 5.5	b 8.9	b 8.4	d 15.0	d 12.9
7-Keto-cholesterol	a 5.1	a 5.3	b 6.3	b 5.3	d 8.4	d 7.2
7 β-Hydroxy-cholesterol	a 16.2	b 6.3	b 16.2	b 14.1	d 24.3	d 21.0

Values in the same row bearing the same superscript for each method do not differ significantly at $p<0.05$.

40 °C, a 10 % greater reduction in cholesterol β-epoxide using the low NOx burner in control eggs was observed, in comparison to α-tocopherol-supplemented eggs dried using the high NOx burner.

The effectiveness of butylated hydroxytoluene (BHT) in reducing cholesterol oxidation was also examined. At the concentration of 200 μg/g fat, BHT did not significantly ($p<0.05$) reduce the amount of cholesterol β-epoxide in egg powder (Table 10). It may have been that the BHT was steam volatilized during the drying process. On the other hand, Morgan and Armstrong (1987) reported at 200 μg/g yolk solids, BHT reduced the amount of cholesterol epoxides in egg powder to about one-half the concentration found in the controls. At 67 μg/g yolk solids though, BHT had little effect on cholesterol epoxide concentration.

The other additives investigated were sodium nitrite /perchloric acid and sodium bisulfite. Both additives failed to conclusively produce the predicted results. It was postulated that the addition of the sodium/perchlorate mixture would result in the formation of dinitrogen trioxide. Dinitrogen trioxide would then decompose into the highly reactive oxides of nitrogen (NO and NO_2). Unexpectedly, no difference in COP's was observed between the control and treated eggs dried using the indirect electric burner. Nitric acid might have been formed instead.

Bisulfites exhibit antioxidant properties by acting as reducing agents (Fennema, 1985). In this case, the addition of sodium bisulfite significantly ($p<0.05$) lowered the amount of cholesterol α-epoxide and 7 β-hydroxycholesterol produced in the egg powder, whereas the concentration of 7-ketocholesterol was significantly ($p<0.05$) increased (Table 10). The concentration of cholesterol β-epoxide was not affected by the addition of bisulfite. The observed differences in response between cholesterol oxides may be attributable to the small sample size used in this analysis. It would be expected that the concentration of all cholesterol oxides in egg powder would be reduced by the addition of bisulfite, since the mechanism of reduction would be the same.

The results of this investigation demonstrated that the formation of cholesterol oxides in egg powder is reduced when α-tocopherol is incorporated into the egg through dietary means. BHT was not effective in reducing oxidation, and bisulfite produced inconclusive results. The addition of sodium nitrite/perchloric acid failed to accelerate cholesterol oxidation.

Nitrite analysis of egg powder

Oxides of nitrogen may dissolve in the moist atmosphere of the spray dryer leading to the formation of nitric and nitrous acid. Nitrous acid can be detected as

Table 10. The effect of treatment on the concentration of cholesterol oxides in egg powder for the high NOx burner at 40 °C.

Cholesterol oxide	Treatment (μg/g dried powder)			
	Control	Tocopherol	BHT	Bisulphite
Cholesterol β-epoxide	a 49.01	b 35.96	a 49.28	a 50.07
Cholesterol α-epoxide	a 16.98	a 12.91	a 16.26	b 5.56
7-Keto-cholesterol	a 8.38	a 7.24	a 8.67	b 16.91
7-β Hydroxy-cholesterol	a 24.38	a 20.95	a 23.62	b 10.42

Values in the same row bearing the same superscript do not differ significantly at $p<0.05$.

nitrite in the dried egg powder. Therefore, the concentration of nitrite in the samples is a reflection the level of NOx present in the spray drying chamber (Kelly and Gray, 1986).

Significant differences ($p<0.05$) were found in nitrite content in the egg powder dried using the three burners. The concentration of nitrite using the indirect, electric burner was about 0.06 μg/g egg powder. The level increased in the low NOx burner to approximately 1.00 μg/g egg powder, and to about 2.00 μg/g egg powder in the high NOx burner (Table 11). Using the same burners, Kelly et al. (1989) reported a nitrite concentration in dried milk powder of 0.460 μg/g for the electric, and 0.811 μg/g for the low NOx burner. In a prior study, nitrite content of milk powder ranged from 0.38 to 1.4 μg/g for the high NOx burner (Kelly and Slattery, 1985).

Table 11. Nitrite concentration in control spray dried egg powder.

Spray Dryer	Concentration * (μg/g powder)
Indirect Electric	0.04 a
Direct Low NOx	1.04 b
Direct High NOx	1.98 c

* Mean concentration of three replicates.

Values in the same column bearing the same superscript do not differ significantly at $p<0.05$.

SUMMARY AND CONCLUSIONS

This study focused on the stabilization of egg yolk lipids through dietary supplementation, and on the formation of cholesterol oxides in egg powder as influenced by storage conditions, method of spray drying, and use of selective additives prior to drying.

By increasing the concentration of α-tocopherol in the feed of laying hens, the concentration of α-tocopherol in the egg yolk was subsequently increased. Feeding levels of 10 mg/kg, 100 mg/kg, and 200 mg/kg of α-tocopherol acetate resulted in α-tocopherol yolk concentrations of 80 μg/g, 200 μg/g, and 350 μg/g yolk, respectively.

When subjected to conditions conducive to oxidation, the oxidative stability of egg yolk was enhanced by the higher concentrations of α-tocopherol in the yolk. Under acidic conditions (pH 3), lipid oxidation proceeded at a significantly ($p<0.05$) higher rate in the yolks containing the lowest concentration of α-tocopherol. Concentrations of 200 and 350 μg α-tocopherol / g yolk effectively reduced lipid oxidation in the yolks. At the natural pH (pH 6), egg yolks from all three groups were highly stable against lipid oxidation during the storage

oxidation was greatest in the egg yolk containing the smallest concentration of α-tocopherol, although no significant difference ($p<0.05$) was found between oxidation rates in all three groups.

In spray dryed egg powder, the COP's present in descending order were: cholesterol β-epoxide >> 7 β-hydroxycholesterol > cholesterol α-epoxide and 7-keto-cholesterol. Storage for four months at the higher temperature (40 °C) increased the concentration of COP's in the egg powder.

The method of spray drying also influenced the concentration of COP's in the egg powder. In samples produced by the direct, high NOx system, the concentrations of cholesterol β-epoxide and 7 β-hydroxycholesterol were significantly ($P<0.05$) higher than those in samples dried using an indirect,electric or a direct, low NOx dryer. At 40 °C, the concentrations of these cholesterol oxides in the egg powder processed with the high NOx system were approximately two times greater than the amounts found in samples dried using an electric or low NOx dryer. Thus, it appears that the level of NOx generated in the spray dryer affects the formation of cholesterol oxides in egg powder.

Of the selective additives, only α-tocopherol incorporated into the egg yolks by dietary supplementation was effective in reducing the extent of cholesterol oxidation in egg powder. However, the dietary feeding of α-tocopherol was not as effective in reducing the formation of

COP's as was the low NOx burner. Butylated hydroxytoluene did not signficantly ($p<0.05$) reduce the extent of oxidation in egg powder, and the effect of sodium bisulfite was inconclusive. In addition, sodium nitrite / perchloric acid failed to accelerate the formation of COP's in egg powder.

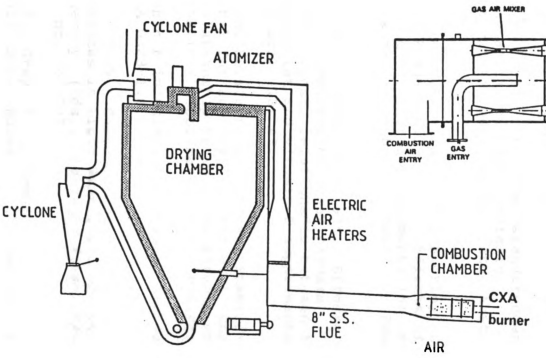
In conclusion, the generation of oxides of nitrogen during the spray drying process results in high concentrations of cholesterol oxides in egg powder. The direct, low NOx dryer is an effective alternative in reducing the formation of cholesterol oxides in egg powder.

APPENDIX

Description of CXA burner:

The low NOx burner is of excess air type, in which gas is burned at its lower level of inflammability. Before entering the combustion chamber, the gas and air are pre-mixed to reduce the formation of air pockets. Flame stabilizers are added and the combustion chamber is refractory lined, in order to further enhance the stability of the flame and ensure complete combustion before mixing with fresh air.

(Kelly et al., 1989)



CXA Burner Design

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