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CHOLESTEROL OXIDATION IN FOOD PRODUCTS

OF ANIMAL ORIGIN

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J. I. Gray

Major professor

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CHOLESTEROL OXIDATION IN FOOD PRODUCTS OF ANIMAL ORIGIN

By

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ABSTRACT

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The presence of cholesterol oxidation products (COP's) was investigated in illuminated butter, heated tallow, fat extracted from commercial french fries, powdered dairy products and spray-dried eggs. Cholesterol oxidation was not evident in the butter although the peroxide value increased slowly with time over 16 days of storage. Addition of β-carotene to the butter resulted in lower peroxide values, while α-tocopherol and TBHQ had no effect. 7-Ketocholesterol, 7β-hydroxycholesterol and cholesterol-α-epoxide were formed in heated tallow after 24 hours, with 7-ketocholesterol dominating. The addition of 0.05% oleoresin rosemary decreased the extent of cholesterol oxidation. Small amounts of cholesterol oxidation products were detected in commercially produced french fries, but the level did not correlate well with the amount of cholesterol present. Powdered whole milk, powdered butter and powdered sour cream all contained 7-ketocholesterol. After incubation for two weeks at 38°C, eggs that had been spray-dried with air heated directly by a natural gas flame contained cholesterol-α-epoxide, while eggs spray-dried with indirectly heated air did not contain any COP's.

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INTRODUCTION

Cholesterol is a major sterol present in food products of animal origin and is known to undergo oxidation under various conditions. Cholesterol oxidation products (COP's) are important because of their possible involvement in atherosclerosis, cancer and other deleterious conditions (Smith and Van Lier, 1970; Taylor et al., 1979; and Peng and Taylor, 1984).

Cholesterol oxidation usually begins with the formation of allylic hydroperoxides (Maerker, 1986) and may occur at three different locations in the cholesterol molecule: 1) on the number 3 carbon; 2) in the B ring at carbons 5 and 6 which are joined by a double bond and also at carbon 7, due to allylic rearrangement; and 3) in the side chain where hydroxyl groups can be formed. The cholesterol oxides most often found in food are those arising from oxidation originating in the B ring.

The oxidation of cholesterol may be initiated by light/radiation, heat, prolonged exposure to air, enzymes and other components present in food (Smith et al., 1981). Recently, there has been interest in the capability of oxides of nitrogen in initiating cholesterol oxidation in powdered foods (Tsai and Hudson, 1985). Oxides of

nitrogen may be present in directly-heated air that is used in spray-drying, originating from the combustion of natural gas used for the flame. Use of antioxidants and/or processing modifications may provide the means for control of cholesterol oxidation in such food products.

The major objective of this study was to determine the extent of cholesterol oxidation in various food products including heated tallow, butter, powdered dairy products and spray-dried eggs. Various physical and chemical analyses and chromatgraphic techniques were employed to assess the oxidative changes. Specific objectives included evaluating the effectiveness of oleoresin rosemary as an antioxidant in heated tallow, and evaluating α-tocopherol, β-carotene, and TBHQ as antioxidants in butter.

In heated tallow, various useage levels of oleoresin rosemary were investigated to find a level that would provide maximum protection. The effects of continuous heating were compared to those of intermittent heating to determine which was more harmful.

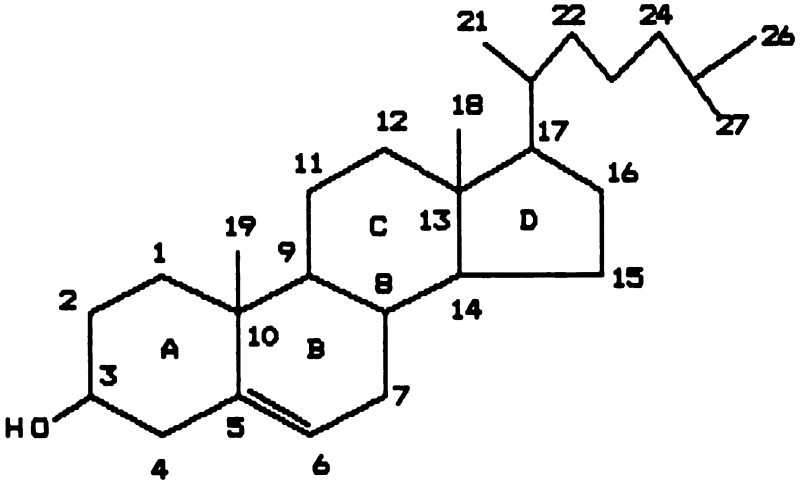
The antioxidants in butter were each evaluated at one level. Various packaging materials were compared for their ability to prevent oxidation of illuminated butter stored at 4°C.

The difference in cholesterol oxidation between eggs spray-dried with indirectly heated air and those spray-dried with directly heated air was evaluated before

and after incubation. The purpose was to determine if oxides of nitrogen in directly heated air may have had an effect on the extent of oxidation.

REVIEW OF LITERATURE

Cholesterol, $C_{27}H_{44}O$, has a cyclopentanophenanthrene ring structure with an eight carbon aliphatic side chain at C_{17}, two angular methyl groups at C_{18} and C_{19}, and a double bond between carbons 5 and 6.



5-cholesten-3β-ol (cholesterol)

This white crystalline compound was first discovered in the latter half of the eighteenth century during studies on the formation of gallstones in the body. In 1816, Chevreul gave it the name "cholesterine", coming from Greek for chole, meaning bile and stereos, meaning solid. The name was changed to cholesterol when it was shown to have an alcohol structure (Berthelot, 1859).

Cholesterol is an essential metabolite as it is the

basis for families of bile acids, hormones and vitamin D, and is a vital component in cell membrane structure (Brun et al., 1985). It affects permeability and osmotic pressure in the lipid bilayer of the cell membrane. Cholesterol-poor red blood cells show increased osmotic fragility (Gibbons et al., 1982).

One to two g/kg of the body weight of adults are made up of cholesterol, 5.5% of which exists in plasma (Gibbons et al., 1982). Table I shows the distribution of cholesterol in the rat, which correlates well with the distribution in other mammals with a few minor exceptions (Gibbons et al., 1982).

Most of the cholesterol in the body is unesterified, except in plasma and skin where a greater portion exists in the ester form. The average daily metabolic requirement for an adult weighing 70 kg is approximately 350mg (Sabine, 1977). Approximately 80mg of this are excreted daily through the skin (Parsons and Goss, 1978).

HEALTH IMPLICATIONS OF CHOLESTEROL OXIDATION PRODUCTS

Cholesterol has, for a long time, been implicated in the development of atherosclerosis in humans. It is now thought of by many as a contributor, but not the primary cause. A new theory is that cholesterol oxidation products (COP's) begin the atherosclerotic process. Because it is now known that cholesterol is readily oxidized in the presence of air over a period of time, it has been suggested by some, including Peng and Taylor (1984), that cholesterol used for dietary experiments by

Table 1. Cholesterol content in organs and tissues of adult rats[a].

Organ or tissue	Percent of total body cholesterol
Plasma	1.7
Small intestine	4.4
Liver	10.2
Kidneys	2.7
Adrenels	0.2
Skin	16.6
Hair	11.2
Adipose	3.9
Skeletal muscle	18.5
Bone marrow	5.4
Red cells	2.4
Nervous tissue (includes brain)	22.7

[a]Adapted from Gibbons et al. (1982).

Anitschkow in 1913 and others following could have contained COP's. Therefore, the atherosclerotic effects originally attributed to cholesterol may actually be due to COP's. It has been suggested that COP's could replace cholesterol in aortic cell membranes, thereby decreasing their functionality and making them abnormally susceptible to the formation of atherosclerotic deposits (Peng and Taylor, 1984).

There is a large body of literature on the deleterious effects of COP's in human health. They can be

regulators of sterol synthesis, immune suppressors, carcinogens, cytotoxins and mutagens (Finocchiaro et al., 1984). Twelve COP's were isolated and identified in atheromata from human aortas (Smith and Van Lier, 1970). The level of cholesterol-α-epoxide in human serum may be related to the severity of atherosclerosis (Gray et al., 1971).

After feeding USP-grade cholesterol containing spontaneous oxidation products to New Zealand White rabbits, Taylor et al. (1979) reported that adverse effects were observed within 24 hours. When a similar dose of purified cholesterol was administered to rabbits, no adverse effects were observed. It was reported by Peng et al. (1984) that, when 25-hydroxycholesterol and 5-cholestan-3β,5,6β-triol were added to cultured aortic smooth muscle cells of rabbits, the activity of 3-hydroxy-3methyl-glutaryl coenzyme A reductase (HMG CoA reductase), a regulatory enzyme in cholesterol biosynthesis, was significantly depressed.

Other health implications of COP's have been suggested. The mutagenicity of air-aged samples of USP or reagent-grade cholesterol toward three strains of *Salmonella typhimurium* was demonstrated by Smith et al. (1979). Cholesterol-α-epoxide, which is carcinogenic in rodents, can cause aberations in chromosomes of human fibroblasts (Parsons and Goss, 1978). Hyperthermia enhanced the formation of aberations induced by cholesterol-α-epoxide and ultraviolet light when used

separately and in combination. Cholesterol-α-epoxide has also been found in human skin that has been irradiated with ultraviolet light (Black and Lo., 1971). Both the α- and β- epimer have been reported in breast fluid and prostatic secretions (Petrakis et al., 1981), common sites of cancer in humans.

Addis et al. (1983), suggested that oxidation products derived from cholesterol appear to have the greatest potential for health impairment of all classes of compounds isolated from rancid foods. Kandutsch (1978) has mentioned that certain sterols formed metabolically from cholesterol can exhibit the same inhibitory effect on sterol synthesis as cholesterol.

OXIDATION OF CHOLESTEROL

General mechanism and resulting oxidation products

Food products containing fat are likely to undergo some lipid oxidation, whether it is induced by radiation, heat, enzymes or free radical initiators such as metals and oxides of nitrogen. Cholesterol oxidation usually begins with the formation of allylic hydroperoxides (Maerker and Unruh, 1986). Portions of the molecule that are especially susceptible to oxidation are the unsaturated B ring, positions allylic to it, and the two tertiary carbons at the side chain. The structures of some COP's commonly found in foods are shown in Figure 1. Table 2 lists the common COP's found in food products.

Allylic hydroperoxides are formed by oxidative attack following hydrogen abstraction (Maerker and Unruh, 1986).

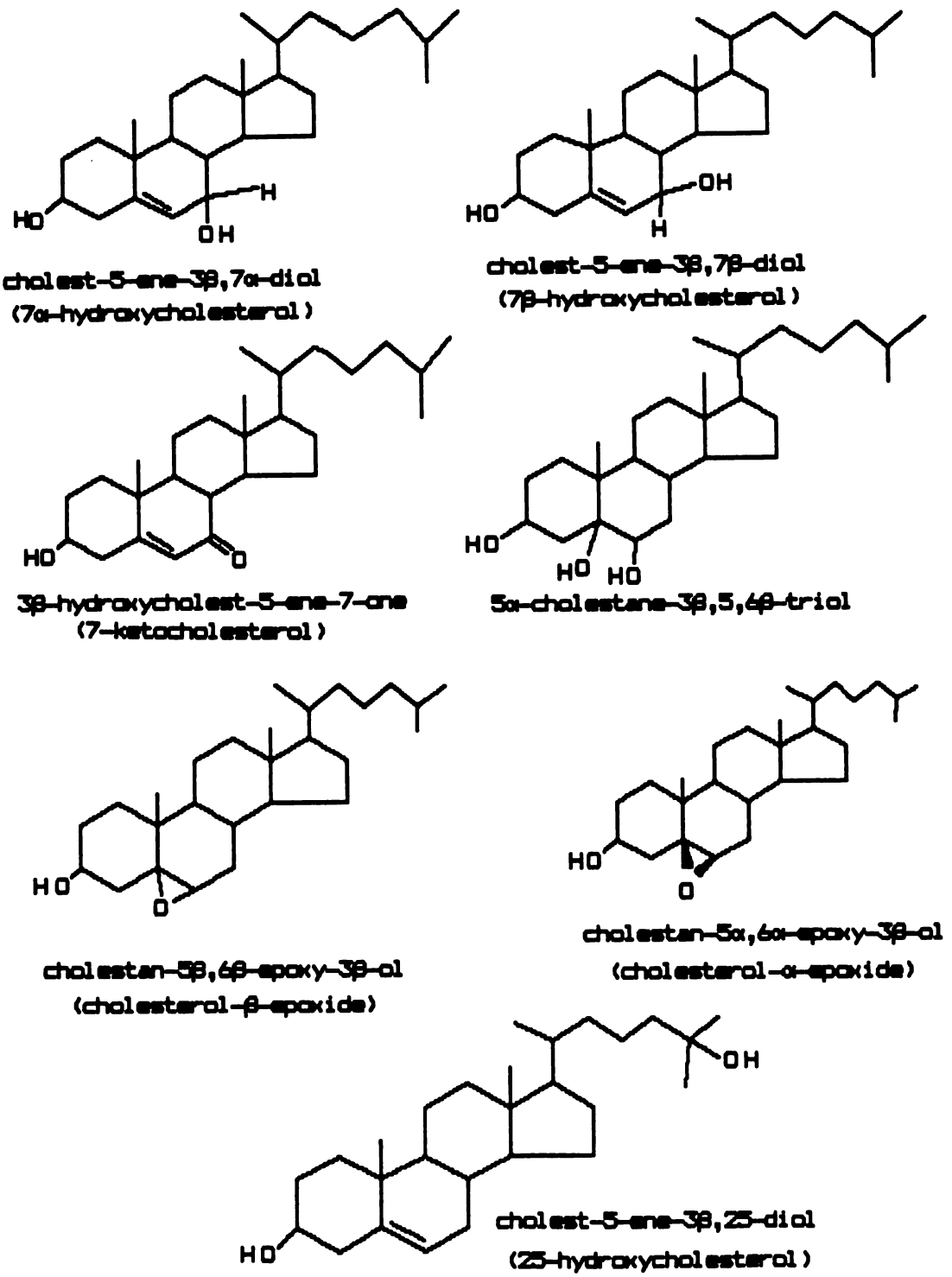


Figure 1. Structures of the cholesterol oxidation products commonly found in food.

Table 2. Cholesterol oxidation products which have been isolated from foods of animal origin.

Cholesterol oxidation product	Food
7α-Hydroxycholesterol	tallow, spray-dried eggs, dry egg nog mix, powdered scrambled egg mix, spray-dried egg yolk, butter
7β-Hydroxycholesterol	same as 7α-hydroxycholesterol
7-Ketocholesterol	tallow, spray-dried egg yolk, powdered scrambled egg mix, nonfat dry milk, anhydrous milk fat
5α-Cholestan-3β,5,6β-triol	tallow, spray-dried egg yolk, powdered scrambled egg mix,
Cholesterol-β-epoxide	tallow, spray-dried egg, spray-dried egg yolk, scrambled egg mix, nonfat dry milk, anhydrous milk fat
Cholesterol-α-epoxide	nonfat dry milk, anhydrous milk fat, tallow, spray-dried egg, powdered scrambled egg mix, commercial dried egg yolk
25-Hydroxycholesterol	tallow, powdered scrambled egg mix

[a] Adapted from Smith et al., 1981.

The hydrogen is abstracted primarily at C_7 because it is an allylic position in the plane of the double bond of the B ring. C_4 is also in the plane of the double bond, but attack does not usually happen there, possibly due to hinderance by the C_3 hydroxyl group and C_5 which is connected to three alkyl groups (Maerker and Unruh, 1986). The major oxidative pathway involves the formation of a 7-peroxycholesterol intermediate which then abstracts a hydrogen from another species, forming the α and β isomers of 7-hydroperoxycholesterol. This pathway is shown in Figure 2. These hydroperoxides can be reduced to 7α- and 7β-hydroxycholesterol, or they can undergo dehydration to yield 7-ketocholesterol. The latter compound can undergo dehydration to form 3,5-cholestadiene-7-one (Smith, 1981).

Cholesterol-epoxides are formed from secondary oxidation reactions in which cholesterol is attacked by hydroperoxides (either hydrogen peroxide or previously formed cholesterol hydroperoxides) yielding cholestan-5β,6β-epoxy-3β-ol (cholesterol-β-epoxide) and cholestan-5α,6α-epoxy-3β-ol (cholesterol-α-epoxide) (Smith and Kulig, 1975). Cholestan-3β,5α,6β-triol is formed by hydration of the α or β-epoxide.

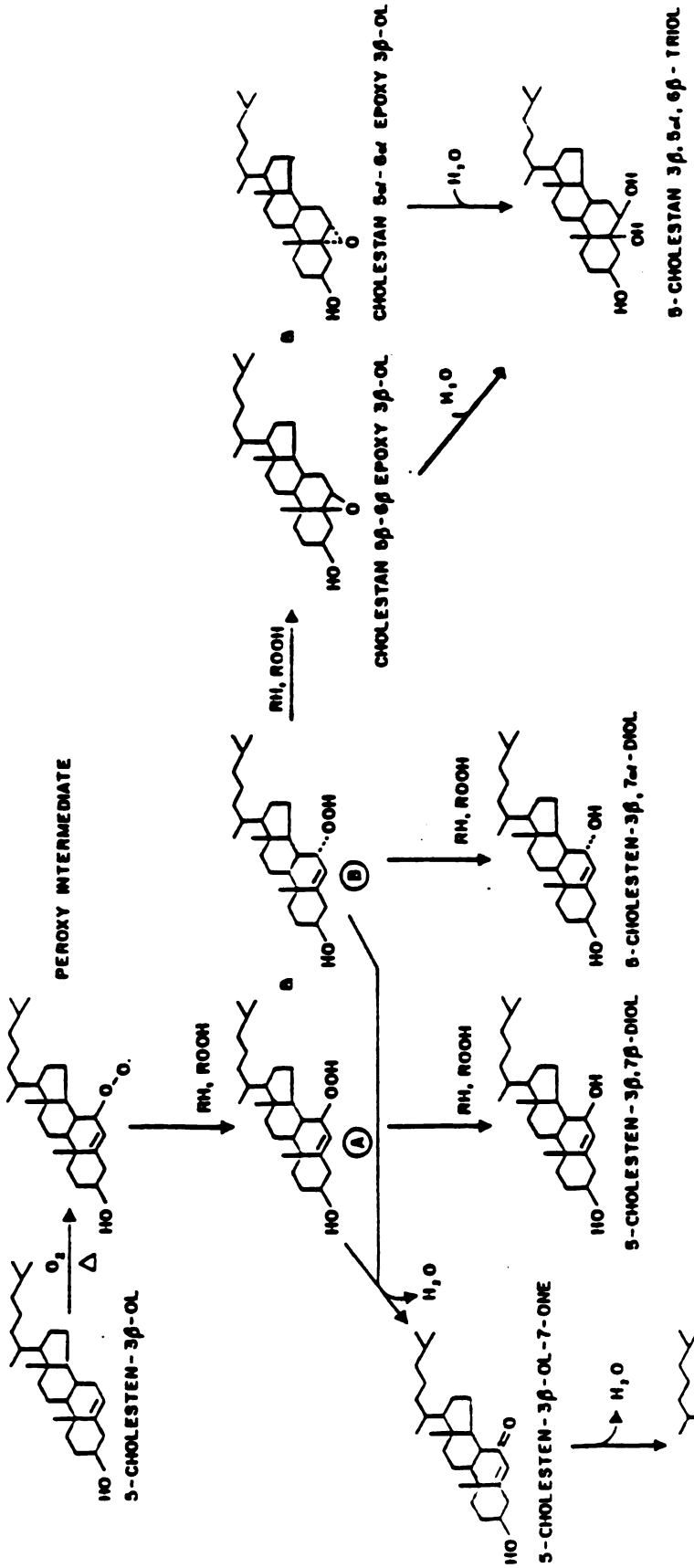


Figure 2. Major pathway for cholesterol oxidation (Adapted from Luby, 1982).

Initiation of cholesterol oxidation

There are several ways that cholesterol oxidation in foods can be initiated but experimental evidence is lacking in this area (Smith, 1981). The types of radiation which are suspected of initiating autoxidation of cholesterol are infrared, visible, ultraviolet, and ionizing radiation (α and γ) (Smith, 1981). Irradiatation of pure cholesterol can produce energized species by increasing the rotational, vibrational or electric bond energy levels. These excited molecules could undergo bond homolysis or ionization and form known cholesterol oxidation products after reaction with molecular oxygen.

Ionization of cholesterol could also be involved in the initiation of autoxidation. Radiation could cause the excited cholesterol molecule to lose or gain an electron, forming a carbocation or carbanion which can react with oxygen as shown in Figure 3. The resultant hydroperoxide anion (CHOLESTEROL-OO^-) is protonated to form a more stable hydroperoxide.

Singlet oxygen

Singlet oxygen oxidation of cholesterol is used for the determination of ^3H distribution at the C_7 position in biosynthesis studies (Smith, 1981). Photosensitized formation of singlet oxygen has been demonstrated in the gas phase but direct proof of its formation in solution is lacking. This is because spectroscopic techniques used in the gas phase cannot be used in solution. Indirect methods such as chemical trapping, kinetic and quenching

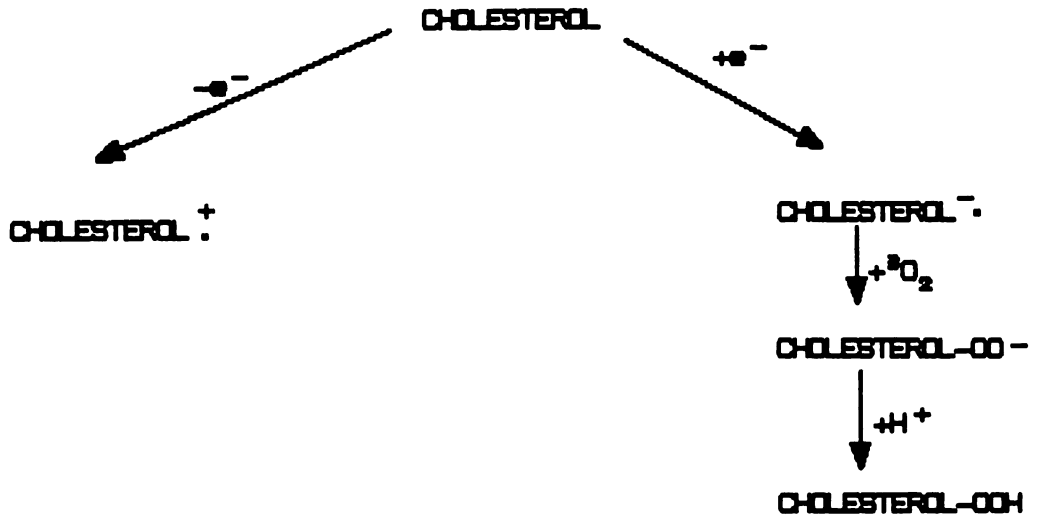


Figure 3. Ionization of cholesterol.

studies indicate the intermediacy of singlet oxygen in photooxidative reactions occurring in solutions.

Photosensitizers

A photosensitizer is a molecule which absorbs light to produce a chemical reaction which would not occur in its absence. It may or may not be chemically changed in this process. Excited nitrogen dioxide can be a photosensitizer in the gas phase (Pryor and Lightsey, 1981). Riboflavin is also a photosensitizer in simple systems (Foote, 1976).

Photosensitizers can exist in two electronically excited states, singlet and triplet. Usually the triplet state is much longer lived, but the singlet state is the initial product of light absorption. Photosensitized oxidations usually proceed by way of the triplet sensitizer. Schenck and Koch (1960) and Livingston (1961)

classified triplet sensitizer reactions as type I and II (Figure 4). Type I reactions occur when the photosensitizer interacts with another molecule directly, to give either a hydrogen atom or electron transfer. The resulting radicals react further with oxygen or other molecules. They can extract hydrogen or an electron from other substrates, initiate free radical chain autoxidation, or initiate a back reaction. Type I sensitizer reactions are much more frequent than type II.

Type II reactions take place when the photosensitizer triplet interacts with oxygen. The most common reaction of this type involves the transfer of excitation from the sensitizer to the oxygen, producing an electronically excited singlet state of oxygen ($^1O_2$). This excited oxygen can then further react with various acceptors in solution. Deactivation collisions between oxygen and most sensitizer triplets result in electron transfer less than one percent of the time. Electron transfer from sensitizer to oxygen can also occur leading to the formation of a superoxide ion ($O_2^{\cdot -}$) and an oxidized form of sensitizer, but this transfer is less efficient.

The singlet oxygen formed from the type II photosensitized reaction causes a stereospecific abstraction of the quasiaxial 7α hydrogen, resulting in a shift of the Δ^5 double bond of cholesterol to the Δ^6 position, subsequent bonding of dioxygen at the 5α position, and hydrogen transfer to yeild 5α-hydroperoxide. Minor oxidation products formed by singlet oxygen are

6-hydroperoxides.

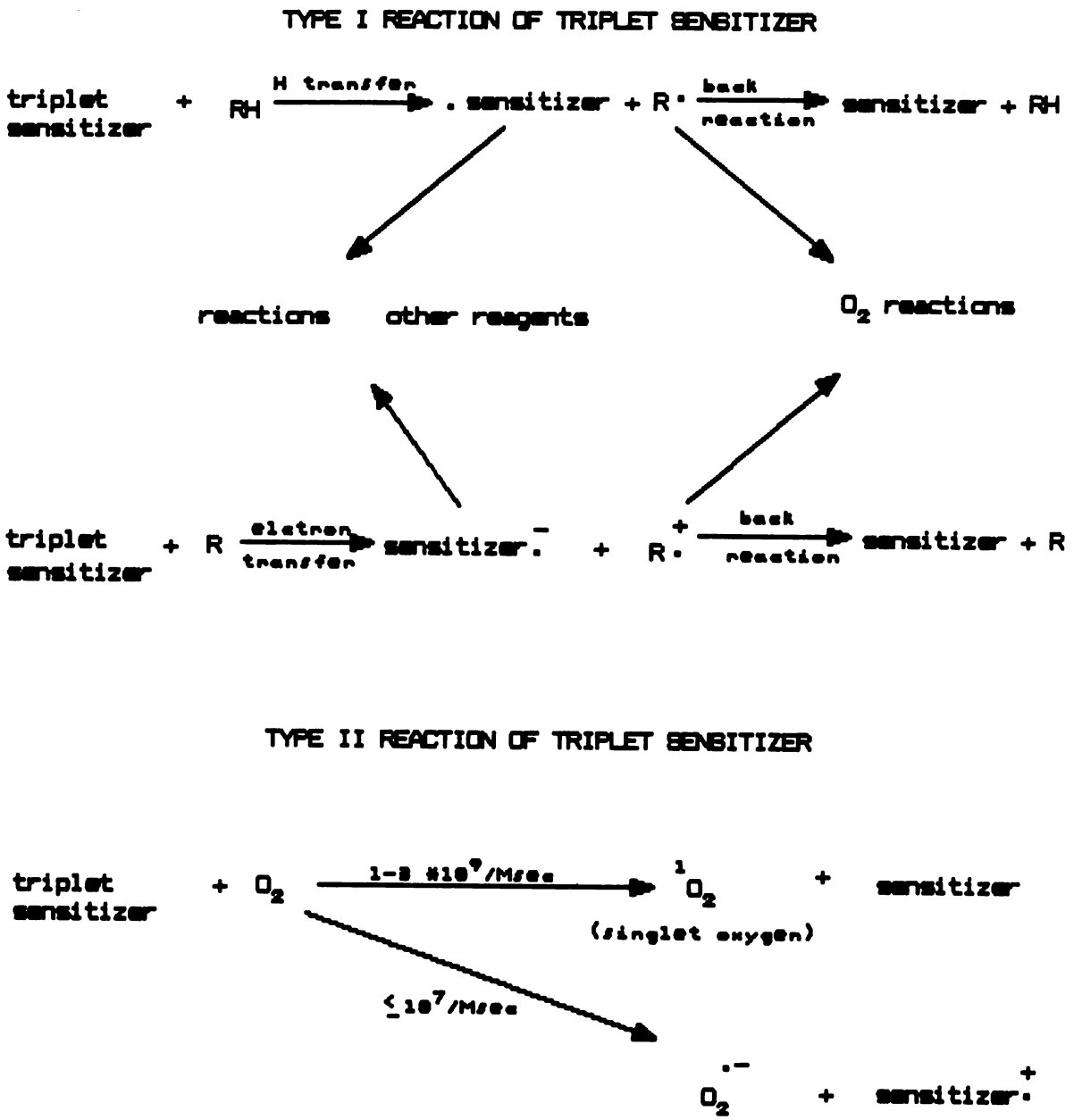
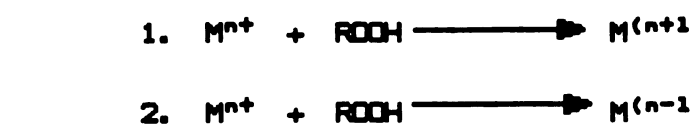


Figure 4. Two types of reactions involving triplet sensitizers.

Transition metals

Copper and iron which exist in two or more valence states are effective free radical oxidation catalysts because they can donate or accept a single electron (Smith, 1981). The following homolytic equations show how initiation of oxidation proceeds.



The resulting radicals could react with cholesterol or other hydrocarbons, extracting H protons. This is a concern in the case of meat and meat products where iron is abundant. Sato and Hegarty (1971) and Love and Pearson (1974) presented data that suggested that non-heme iron is the major prooxidant in cooked meat. This was supported by an investigation of the influence of heme pigments, nitrite, and non-heme iron on the development of warmed-over-flavor in cooked meats (Igene et al., 1979).

Enzymes

Limited experimental evidence is available concerning enzyme-initiated cholesterol oxidation in foods (Smith, 1981). According to Gumulka et al. (1982), ground state dioxygen ($^3O_2$) is involved in specific enzymic hydroxylations and the superoxide radical ($O^{-}\cdot_2$) is generated in some enzymic oxidations.
Cholesterol-α-epoxide and 25-hydroxycholesterol are the only oxidation products that can be found in food which are known to come from enzymic origins (Smith, 1981). It

has also been suggested that a 7β-hydroxylase might give rise to 7β-hydroxycholesterol (Smith, 1981). The formation of 7α- and 7β-hydroperoxides has been demonstrated with soybean lipoxygenase, horseradish peroxidase, and the microsomal NADPH- dependent lipid peroxidation system of rat liver. These enzymes are all dioxygenases, which means that they add $^3O_2$ to their substrate, forming a hydroperoxide (Smith, 1981).

Heat

Heat is known to cause extensive autoxidation of cholesterol (Smith, 1981). For this reason, the methodology used for determining the concentrations of cholesterol oxides in foods is very crucial because hot saponification of lipids can cause artifactual formation of the 7-hydroxycholesterols, the 3β,5α,6β-triol, 7-ketocholesterol and 3,5-cholestadiene-7-one, the thermal degradation product of 7-ketocholesterol.

The formation of high concentrations of cholesterol oxides were reported by Horvath (1966) after heating crystalline cholesterol in the dark at 60°C for 48 days, 65°C for 30 days, 70°C for 28 days, and 100°C for 42 hours. Heating the cholesterol at 105°C for a week resulted in oxidation of approximately one half of the cholesterol. Korahani et al. (1982) indicated that autoxidation of cholesterol heated to 100°C could be due to disintegration of the protective crystalline structure at 100°C, or to the particular molecular arrangement existing at that temperature being favorable to oxidation.

Upon heating recrystallized cholesterol Korahani et al. (1981) determined that 80% of the cholesterol remained unchanged after 30 days in the presence of air at 100°C. Only 6% was detected as oxidation products. They found traces of peroxides, indicating that hydroperoxides were first formed in the autoxidation reaction. These investigators also noted that 25-hydroxycholesterol is highly stable at 100°C and it did not undergo any change when heated for six months in the presence of air.

Both 7α- and 7β-hydroperoxides were detected after cholesterol was stirred in an aqueous colloidal suspension dispersed with sodium stearate. It was exposed to molecular oxygen at 85°C and pH 8 for three hours (Kimura et al., 1979). The instability of these 7-hydroperoxides has been known since the early 1940's (Maerker, 1987), their products being 7-ketocholesterol and both isomers of 7-hydroxycholesterol. Kimura et al. (1979) also observed some of the 5,6-cholesterol-epoxides produced under the same heating conditions.

Processing of foods

Many processed foods containing cholesterol, especially those that are spray-dried, retorted, cured or deep-fat fried may contain some COP's. Other oxidation products formed during cooking and subsequent storage may act as catalysts for further cholesterol oxidation (Pearson et al., 1983). Fieser (1953a,b) reported the presence of 5α-cholestan-3β,5α,6β-triol in powdered egg yolk. Dried eggs are exposed to high temperatures and

forced through air, two conditions favoring oxidation, especially when the large surface area of the atomized egg is considered. Further details relative to the formation of COP's in dried eggs are presented later in this review.

METHODS FOR DETECTION OF CHOLESTEROL OXIDATION PRODUCTS IN FOOD

There is a wide variety of analytical techniques used for the isolation and quantitation of cholesterol oxides in foods, including high performance liquid chromatography (HPLC), gas liquid chromatography (GLC), mass spectrometry (MS), and thin layer chromatography (TLC). One of the major problems in developing adequate techniques for the analysis of cholestrol oxidation products is that sterol oxides can break down during isolation (Higley et al., 1986b). Interfering substances in foods also present a problem during isolation, making it difficult to determine the concentrations and types of oxides present. It has already been mentioned that 7-ketocholesterol is easily degraded, and that cholesterol-α- and β-epoxides are unstable during hot saponification. All of the cholesterol oxides shown in Figure 1 can arise as artifacts from isolation and sample storage (Smith, 1981). Both 7α- and 7β-hydroxycholesterol and 7-ketocholesterol can be produced from cholesterol during hot saponification (Smith, 1981).

Thin layer chromatography

Currently, thin layer chromatography is used most often in combination with HPLC or GLC. R_f values and the

colors of the spots can be used for compound identification. The usual methodology includes developing the plates (silica gel type G) with heptane:ethyl acetate (1:1) and spraying them with 50% sulfuric acid for visualization. After spraying, the plate is heated at 110 to 120°C for approximately ten minutes for color development. Cholesterol will appear magenta, the 7α- and β-hydroxycholesterols and the epoxides are blue, while 5-cholestan 3β,5α,6β-triol is yellow.

TLC has been used for identifying COP's from irradiated spray-dried egg yolk (Chicoye et al., 1968b), butter exposed to fluorescent light (Luby, 1982) and grated cheese and bleached butteroil (Finocchiaro et al., 1984). Recently, it has been used for analyzing dry column saponification fractions, recovering the 5,6-epoxides and 7α- and β-hydroxycholesterols (preparative TLC) (Maerker and Unruh, 1986), and for analysis of reaction mixtures of cholesterol in aqueous dispersion with sodium stearate or Triton surfactants treated under different conditions. Purifying or determining the purity of standards can also be done with TLC (Lee et al., 1985).

There are some disadvantages in using TLC rather than other available chromatography techniques. Alone, it is not a good method for identifying 7-ketocholesterol and cholesterol-α- and β-epoxide because they are not well resolved. Quantitation from TLC can be inaccurate due to the necessary steps of scraping the plates and eluting and

transferring the oxides (Park and Addis, 1985b). TLC is also labor-intensive and time-consuming.

High performance liquid chromatography

High performance liquid chromatography gives higher resolution that TLC. It has been used for the analysis of cholesterol oxides by Shen and Sheppard (1983), Ansari and Smith (1979), Tsai and Hudson (1981), and Maerker and Unruh (1986), among others. Cholesterol does not have a strong absorption peak but it does have an unsaturated center and functional group, so it absorbs in the 203 to 214nm range (McCleur, 1976). Normal phase adsorption HPLC systems where the stationary liquid phase is polar and the mobile phase is nonpolar, have given the best separation although reverse phase systems (polar mobile phase, nonpolar stationary phase) have been used (Maerker, 1987). In the normal phase, the polar cholesterol oxides are retained in the stationary phase, so that more polar substances have a longer retention time. With reverse phase HPLC, the oxides favor the mobile phase.

Semipreparative HPLC can be used instead of using saponification to separate cholesterol and cholesterol oxides from other lipids (Maerker and Unruh, 1986). Maerker and Unruh (1986) eliminated the saponification step by subjecting a cholesterol-in-triolein solution to HPLC enrichment (normal phase) and then analyzing the oxide fraction by GLC. A higher amount of the heat-labile 7-ketocholesterol was found than when the solution was initially saponified with heat. A difficulty with this

procedure is that some cholesterol oxides which are less polar than cholesterol (such as 3,5-cholestadiene-7-one) are lost because they are eluted before cholesterol. Butylated hydroxytoluene (BHT) and other phenolic antioxidants added for protection against artifactual COP formation could also be lost.

Hexane:ethyl acetate (100:5) was reported by Tsai and Hudson (1981) to be a good mobile phase for compounds that elute earlier than cholesterol, which includes reduced or dehydroxylated products of cholesterol. It was unable to resolve compounds which differed in only 1 or 2 unsaturated carbons which was also a problem for 2-propanol. These compounds include 5α-cholestane (often used as an internal standard), 3,5-cholestadiene, ketones at the 3 positon and some other COP's not important for food products.

A reverse phase C_{18} column was used by Shen and Sheppard (1983) with methanol or methanol:water (9:1 v/v) to separate cholesterol oxides that differ in their additional functional group and also the isomers of the 5,6-epoxide. They resolved cholesterol and C_7 oxide products isocratically. They found an adsorption liquid chromatographic system to be the most effective. Using a μPorasil column with hexane:isopropanol (96:4) as the solvent system, they got baseline resolution of the 5,6-epoxides and the 7-hydroxycholesterols. They were also able to resolve side chain hydroxycholesterols that differ only in the position of the hydroxyl groups. Based

on their results and the results of others such as Ansari and Smith (1979) and Tsai et al. (1980), Shen and Sheppard claimed that HPLC was an effective method for separation of a complex mixture of cholestrol oxides, particularly because it is operated at room temperature and uses nondestructive detection. Most sterol oxides can be detected by monitoring the effluent adsorption at 212nm (Ansari and Smith, 1979).

Gas liquid chromatography

Gas liquid chromatography offers better separation and quantitation capabilities (especially with capillary columns) than either TLC or HPLC. The detection level is easily in the parts per million range (Krull et al., 1984; Missler et al., 1985). GLC is usually carried out with direct on-column capillary injection on a nonpolar column with a flame ionization detector. Mass spectometry can also be used as a detection method for derivatized samples (Maerker and Bunick, 1986). Samples containing cholesterol and cholesterol oxidation products are derivatized with a silylating reagent for improved thermal stability prior to GLC analysis.

Packed columns were first used for analysis of cholesterol oxidation products. Teng et al. (1973) developed adequate packed column methodology for separation of various hydroperoxides including the epimeric 7-hydroperoxides. However, these compounds decomposed partially to the 7-hydroxycholesterols and other minor components. The hydroperoxides also tended to

epimerize to a small degree. Flanagan et al. (1975) used packed column GLC (3% OV-225) along with mass spectrometry to separate and identify 3,5-cholestadiene-7-one in anhydrous milk fat. The packed column did not give good resolution but using mass spectometry they could still identify the compound. Taylor et al. (1979) were able to quantify several cholesterol oxidation products from USP-grade cholesterol using a packed column with 3% SE-30 or 3% OV-1. They reported 25-hydroxycholesterol, 7-ketocholesterol, the α- and β- isomers of 7-hydroxycholesterol and the triol.

Tsai et al. (1980) used packed column GLC and HPLC for the analysis of egg and egg products. They reported the lowest detectable concentration of cholesterol-α-epoxide to be 50ng/μl.

A packed column with 3% SP-2100 was utilized by Luby et al. (1986) for analysis of the nonsaponifiable lipid fraction from butter exposed to fluorescent light. They observed that as the size of the peaks that eluted before cholesterol decreased, the actual number of peaks also decreased. There was a possible increase in the number of peaks eluting after cholesterol with increase in exposure time. The difficulties with the analysis were that peaks were poorly resolved and the relative quantity of oxides compared to the concentration of cholesterol was small.

Capillary columns greatly improve the results obtainable with gas chromatography. The α- and β- epimers of 7-hydroxycholesterol can be separated (Teng et al.,

1973), and Gumulka et al. (1982) reported resolution of the epimeric 5,6-epoxides using a capillary column. Missler et al. (1985) completely resolved four common oxides in egg products along with cholesterol using on-column injection onto a bonded phase fused silica capillary column. A cold technique was used for the injection so that 7α-hydroxycholesterol was not dehydrated to 7-dehydrocholesterol.

An extensive study of the application of gas chromatography for the analysis of cholesterol oxides in foods was conducted by Park and Addis (1985a). They used 3 different types of columns and experimented with some 11 different sterols including cholesterol, 5α-cholestane and the common cholesterol oxides found in food products. They used derivatized and nonderivatized forms, and mass spectometry for confirmation of the identities of the compounds. They concluded that derivatization was necessary to avoid thermal degradation of the diols. With a DB-1 column, resolution was complete. When a column more polar than DB-1 was used (DB-5), 7-ketocholesterol and 25-hydroxycholesterol eluted as one peak. Using the same liquid phase on a packed column, the compounds did not separate well.

There are many more examples of cholesterol/cholesterol oxidation research that have been with gas chromatography. Discussing all of them is beyond the scope of this literature review.

There are some disadvantages to GLC. The sample

concentration is more limited (Smith, 1981), especially with capillary columns. Thermal instability of some of the compounds can lead to loss and/or artifactual formation. Some of the oxides have close structural similarity making their complete separation difficult (Teng, 1973), and GLC/FID is not suitable for isolation of individual compounds (Shen and Sheppard, 1983).

Cholesterol oxidation products in tallow were analyzed by GLC and good resolution was obtained, but the investigators thought that TLC-FID gave better estimates for quantitation (Bascoul et al., 1986). GLC appeared to underestimate the level of oxides, which could have been a result of incomplete derivatization, irreversible absorption of highly polar compounds on the column and/or thermal degradation.

Mass spectrometry

Mass spectrometry (MS) has been used as a confirmation tool for HPLC or GLC in the analysis of cholesterol and cholesterol oxidation products. Van Lier and Smith (1970) utilized MS in a study of the thermal composition of cholesterol hydroperoxides. Flanagan et al. (1975) used MS with packed column GLC in their analysis of milk products and components. MS was also carried out by Korahani et al. (1981) following capillary column GLC for confirmation of cholesterol derivatives.

Eggs/egg products have been analyzed using MS (Tsai et al., 1984; Missler et al., 1985; Sugino et al., 1986; and Nourooz-zadeh and Appleqvist, 1987). Finocchiaro et

al. (1984) analyzed cholesterol oxides in grated cheese and bleached butteroil using MS with HPLC. Park and Addis (1985a,b, 1986a,b, 1987) have used MS to identify cholesterol oxidation products in tallow and other food products.

CHOLESTEROL OXIDATION PRODUCTS IN FOODS

The presence of cholesterol oxides have been reported in a variety of foods including dairy products, meats, eggs and egg products, french fries and potato chips.

Dairy products

To date, cholesterol oxidation products have been reported in butter (Luby et al., 1986), bleached butteroil and grated cheeses (Finocchairo et al., 1984), and dried milk products (Flanagan et al., 1975).

The presence of 4-cholesten-3-one and 3,5-cholestadiene-7-one in anhydrous milk fat (AMF) and nonfat dry milk (NFDM) were reported by Flanagan et al. (1975). They did not determine the concentration of either oxide.

The isomeric 5,6-cholesterol-epoxides, 7β-hydroxycholesterol, 7α-hydroxycholesterol and the triol in stored bleached butteroils and grated cheeses were measured by Finocchiaro et al. (1984). 7-Ketocholesterol and 3,5-cholestadiene-7-one were tenatively identified but not quantified. The bleaching of the butteroil was done by benzoyl peroxide, a widely used free radical-producing agent which is used in the United States to bleach milk for Blue, Swiss and Italian cheeses. The results of their study are summarized in Table 3.

Table 3. Sterol contents of bleached butteroils and aged Italian cheese samples[a].

Product	Cholesterol	µg/g oil 5,6-Epoxide	7α-OH	7β-OH
Bleached butteroil				
Nitrogen[b]				
90 days at 15°C	3400 ±350	ND	20 ±3	10 ± 3
One year at -20°C (after 90 days at 15°C)	3300 ±190	20 ±3	30 ± 6	20 ± 6
Air[c]				
90 days at 15°C	3400±250	ND	30 ± 2	30 ± 2
One year at -20°C (after 90 days at 15°C)	3300± 300	30±5	90 ± 5	60 ±5
Cheese samples				
Parmesan				
brand A clear glass bottle	4100 ± 510	110 ± 10	20 ± 2	20 ±2
brand A carboard shaker	4100 ±340	30 ± 4	10 ± 4	10 ± 4
brand D carboard shaker	4400 ± 440	20 ± 4	ND	ND
Romano				
brand A clear glass bottle	4300 ± 570	50±5	10 ±2	10±2

[a]Adapted from Finocchairo et al. (1984)
[b]Bleached with benzoyl peroxide, stored in a nitrogen atmosphere.
[c]Bleached with benzoyl peroxide, exposed to air.

Luby et al. (1986) investigated the oxidative stability of cholesterol in butter exposed to fluorescent light. COP's were detected after eight days of exposure to 1500 lux at the butter surface. The oxides were identified as 7α- and 7β-hydroxycholesterol using TLC.

Tallow and meat products

More information is available on the presence of cholesterol oxidation products in tallow and meat products. Ryan et al. (1981) investigated the loss of cholesterol and the formation of cholesterol oxidation products in tallow that was heated at 180°C for eight hours/day for a total of 300 hours. The cholesterol concentration decreased with time, and cholesterol oxides (3,5-cholesta- diene-7-one, 7α- and 7β-hydroxycholesterol) were detected after heating. However, Park and Addis (1986b) stated that 3,5-cholestadiene-7-one is rarley reported as an oxidation product of cholesterol, but may be a degradation product of 7-ketocholesterol.

Bascoul et al. (1986) obtained samples from deep-fat fryers while they were in use and analyzed them for cholesterol and COP's. The heating time reported by the users ranged from 56 to 70 hours. Approximately 25% of the original cholesterol was destroyed during cooking as shown in Table 4. It was partially transformed into the triol, 7α- and 7β-hydroxycholesterol, 7-ketocholesterol, 3,5-cholestadiene-7-one and both isomers of 5,6-cholesterol epoxide. The triol was the oxide formed in greatest concentration, followed by 7α- and

7β-hydroxycholesterol, 3,5-cholestadiene-7-one, cholesterol-β-epoxide and some α-epoxide, and 7-ketocholesterol. Two other compounds, 20- and 25-hydroxycholesterol, were observed only after preliminary isolation with prepatative TLC.

Park and Addis (1986b) heated refined edible beef fat continuously at 155°C and 190°C. Four oxides were present in the tallow heated at 155°C; 7α- and 7β-hydroxycholesterol, cholesterol-α-epoxide, and 7-ketocholesterol.

Table 4. Cholesterol loss in tallow from deep-fat fryers and the intensity of cholesterol oxidation products on TLC plates*.

Sample	Temperature (°C)	Cholesterol (mg/kg)	Average Cholesterol loss (%)	intensity of COP's on TLC plate
1a	—	1705	24	low
1b	142	1300		
2a	—	1918	15	low
2b	171	1635		
3a	—	1874	31	high
3b	173	2300		
4a	—	1336	32	high
4b	146	910		
5a	—	1306	10	medium
5b	184	1175		

*Adapted from Bascoul et al., 1986.

After approximately 300 hours at 155°C and 200 hours at 190°C, the cholesterol concentration in the tallow was approximately 40- 45% of the initial content. At 155°C, the oxides were first detected after 36 hours.

7β-Hydroxycholesterol and 7-ketocholesterol appeared first. The 7β-hydroxycholesterol concentration remained at 1-2% of the original cholesterol content for the duration of the heating. Cholesterol-α-epoxide was formed and leveled off at about 4% of the initial cholesterol content.

The tallow heated at 190°C contained lower levels of COP's that were sporadically formed compared to the tallow heated at 155°C. Formation of 7-ketocholesterol was not proportional to heating time at 190°C and the 7-hydroxy-cholesterols were present at approximately 1%. It was suggested that there may be less chance for oxidation to occur at an elevated temperature, or the oxides may break down soon after formation.

Park and Addis (1987) also quantified the oxidation products (COP's) in meat purchased at local stores. Pork Longissimus dorsi muscle was freeze-dried and kept at 22°C for three years. It was exposed to uncontrolled conditions of relative humidity and laboratory fluorescent light. It contained 7α- and 7β-hydroxycholesterol, 7-ketocholesterol, both isomers of cholesterol-epoxide and the triol (Table 5). The total cholesterol oxidation products were almost half the cholesterol content remaining in the freeze-dried sample of pork. Park and Addis (1987) also analyzed beef which was cooked overnight in a Cryovac vacuumized bag and then sliced, wrapped in PVC film and stored at 4°C. It was analyzed every other day for 12 days and contained no detectable amounts of

cholesterol oxidation products. The investigators reported almost zero thiobarbituric acid (TBA) values in the beef samples, suggesting that rancidity development was not noticable throughout the storage.

Cooked ground turkey and extra lean ground beef (less than 10% fat), wrapped in PVC, and analyzed at 0, 3 and 8 days of storage at 4°C did contain COP's. 7-ketocholesterol accounted for almost half of the total COP's formed, followed by 7β- and 7α-hydroxycholesterol and both of the cholesterol epoxides

C_7- oxides were not detected in fried chicken meat or cooked hamburger obtained from fast food restaurants (Park

Table 5. Cholesterol oxidation products in three year-old freeze-dried pork[a].

Sterol	Concentration (mg/kg)	Percent of original cholesterol
Cholesterol	969.8± 108.1	
7α-OH	90.9± 11.8	9.4
7β-OH	68.4± 5.8	7.1
7-Keto	259.8± 44.1	26.8
α-Epoxide	12.5± 2.2	1.3
Triol	28.4± 4.9	2.9

[a]Park and Addis (1987).

and Addis, 1987). Rare and well done broiled beef were also compared to raw meat. All steaks showed some small

peaks after cholesterol on a GC chromatogram. The cooked steaks appeared to contain 7β-hydroycholesterol and 7-ketocholesterol. Two general conclusions were drawn from this study. Firstly, beef, whether fresh, cooked or pre-cooked and refrigerated for several days, represents a very minor contribution of cholesterol oxides to the human diet. Secondly, COP's in cooked turkey were also low but somewhat higher than beef.

Higley et al. (1986b) used HPLC to analyze ten meat samples for cholesterol oxides and found no detectable amounts in the majority of the samples (Table 6). The triol content of one duplicate determination of cooked bratwurst and raw hamburger was high compared to the other duplicate of the same sample. This was attributed to noncholesterol compounds that absorb at the 240nm absorption maximum for triol.

Eggs and egg products

Dehydrated yolk products are used in the processing of bakery products, salad dressings, baby foods, cake mixes and military rations (Tsai and Hudson, 1984). In eggs and egg products, there is a considerable potential for the formation of measurable quantities of cholesterol oxides because the cholesterol content is high. Lipids comprise 60% of the total solids in egg yolk, 4% of which is cholesterol (Tsai and Hudson, 1984).

Fresh eggs have been reported to be free of COP's (Chicoye et al., 1968b; Tsai and Hudson, 1984; Nourooz-Zadeh and Appelqvist, 1987). However, small amounts of

Table 6. Cholesterol oxide content of selected meat samples[a].

Oxide	Food	mg/kg in the sample
5-cholesten-3β,25-diol	Cooked bratwurst	tr
	Beef franks	tr
	Chicken roll	tr
	Cooked hamburger	tr
	Cooked lean bacon	tr
5-Cholesten-3β-ol-7-one	Bratwurst	tr
	Chicken roll	tr
	Raw hamburger	tr+
	Cooked hamburger	tr, tr
	Cooked lean bacon	tr
5-Cholesten-3β,7β-diol	Bratwurst	tr
	Cooked bratwurst	tr
	Beef franks	34
	Chicken roll	tr
	Raw hamburger	3.4
5-Cholesten-3β,7α-diol	Cooked bratwurst	1640, tr
	Beef franks	34, 83
	Chicken roll	tr ,tr
	Raw hambyrger	tr +
	Cooked hamburger	72
	Cooked bacon	tr
5-Cholesten-3β,5α,6β-triol	Cooked bratwurst	1333
	Beef franks	tr
	Turkey bologna	86
	Chicken roll	tr
	Sliced beef loaf	tr
	Raw hamburger	1298
	Cooked lean beef	tr

[a]Adapted from Higley et al. (1986b).

25-hydroxycholesterol, 7-hydroxycholesterol and 7-ketocholesterol were reported in fresh egg yolk by Naber and Biggert (1983). These were possibly artifacts formed by the hot saponification method used in the analysis.

The conditions that exist during the spray-drying of eggs are favorable for the induction of lipid oxidation (Tsai and Hudson, 1984; Missler et al., 1985). The eggs are atomized, which increases the surface area, and then exposed to hot air. Eggs dried by a direct gas-fired system have an even greater chance of undergoing oxidation than those dried by other methods (such as steam coils) because the eggs may come into contact with oxides of nitrogen (NO_x) formed during the combustion of natural gas (Table 7) (Tsai and Hudson, 1985).

Some reactions between oxides of nitrogen and some free radicals are shown in Figure 5 (adapted from Foote, 1976).

Nitrogen dioxide initiates lipid peroxidation by reacting with both alkanes and alkenes by free-radical mechanisms (Pryor and Lightsey, 1981).

The concentration of NO_x in the gas for large utility boilers can be several hundred parts per million. The air that is passed through the gas flame can pick up these stable free radicals which could initiate cholesterol oxidation. Sevanian et al. (1979) demonstrated induction of cholesterol oxide formation in the lung tissue of rats breathing 3-6.5mg/kg NO_x.

Table 7. Effects of air heating methods on cholesterol oxide formation in dehydrated egg yolks[a].

Direct Gas-Fired				Indirect Steam-Heated			
	Temp, °C		mg/kg		Temp, °C		mg/kg
Sample #	Inlet	Outlet	Oxides	Sample #	Inlet	Outlet	Oxides
2	na*	na	311	10	166	64	10
3	na	na	22	11	169	72	———
5	188	64	13	12	169	70	———
6	185	77	12	13	164	72	6
8	138	74	18	15	157	68	———
18	122	69	18	16	157	68	———
19	167	77	41	23	143	64	7
24	169	77	120	30	135	68	———
25	na	na	8	35	116	49	———
28	143	67	29				

[a]Adapted from Tsai and Hudson (1985).

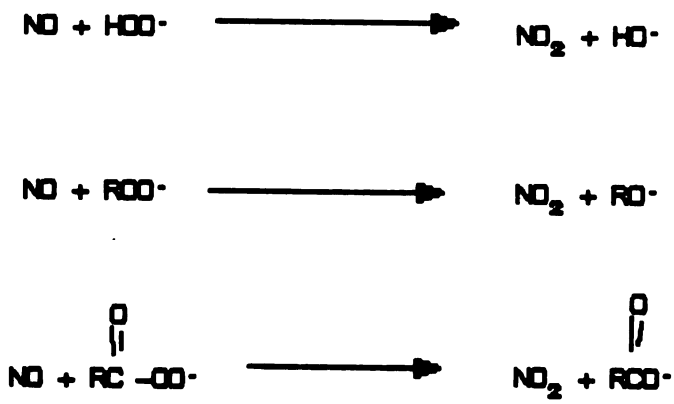


Figure 5. Reactions between oxides of nitrogen and free radicals.

Chicoye et al. (1968b) investigated the photooxidation of spray-dried egg yolk using TLC, GLC and infra-red spectroscopy. Several cholesterol oxidation products including 7-ketocholesterol, 7α- and 7β-hydroxycholesterol, cholesterol-β-epoxide and minor

amounts of the triol were identified. The apparent absence of cholesterol-α-epoxide may have been due to difficulty in separating the α and β isomers Mostly 7α- and some 7β-hydroxycholesterol were also reported in a dry egg nog mix exposed to fluorescent light at room temperature after three weeks of exposure (Herian and Lee, 1985). The levels decreased after 80 days. Other cholesterol oxidation products were not investigated in the egg nog mix. In both of the above cases significant amounts of COP's were not detected in the unirradiated product.

Cholesterol-α- and β-epoxides are often reported in spray-dried egg products. They were successfully separated with HPLC by Tsai et al. (1980). In 1984 they isolated both cholesterol-α- and β-epoxide from freshly dehydrated commercial whole eggs and yolk powders. The range of cholesterol-α-epoxide was from undetectable to 62mg/kg. After incubation at 50°C for 70 days, cholesterol-α-epoxide (approximately 0.1% of cholesterol) and cholesterol-β-epoxide (approximately 0.2% of cholesterol) were reported in commercial spray-dried eggs (Sugino et al., 1986). The epoxides were the dominate COP's in spray dried egg yolk stored for 12 and 18 months at 4°C in closed plastic bags (Nourooz-Zadeh and Appelqvist, 1987).

Powdered scrambled egg mix was analyzed by Missler et al. (1985). It was reported to contain 50mg/kg cholesterol-α-epoxide, 37.4mg/kg cholesterol-β-epoxide, terol, and 13.0mg/kg of the triol (Table 8).

Prolonged storage of dried egg products causes an increase in cholesterol oxidation products (Tsai and Hudson, 1985, Sugino et al., 1986; Nourooz-Zadeh and Appelqvist, 1987). This is of concern since egg yolk powders are stored by the manufacturer at ambient temperatures for up to a year (Nourooz-Zadeh and Appelqvist, 1987). Sugino et al. (1986) monitored cholesterol-α- and β-epoxide in commercial spray-dried egg that was incubated at 50°C and reported that they increased with length of storage. Ater prolonged storage (12- 18 months) spray dried egg yolk lipid extracts contained variable levels of 7α- and 7β-hydroxycholesterol, cholesterol-α-epoxide, 7-ketocholesterol and traces of

Table 8. Quantification of cholesterol oxidation products in scrambled egg mix[a].

Sterol	Indirect Heat Source µg/g	Direct Heat Source µg/g
7α-Hydroxycholesterol	7.0	1.8
7β-Hydroxycholesterol	18.5	1.5
Cholesterol-α-epoxide	50.0	21.5
Cholesterol-β-epoxide	37.4	1.9
25-Hydroxycholesterol	5.1	1.4
7-Ketocholesterol	37.0	2.0
5α-Cholestane-3β,5,6β-triol	13.0	11.6

[a]Adapted from Missler et al. (1985).

the triol and 25-hydroxycholesterol in some of the samples stored for the longest periods.

DEEP-FAT FRYING

Deep-fat frying of foods is a well-liked cookery method. The fat acts as a heat transfer medium and heat penetration is rapid. If prepared correctly, a deep fried food is crisp on the outside and moist and tender on the inside or crisp throughout in the case of most deep fried snack foods. The fat used for frying reacts with the protein and carbohydrates in the food for flavor and color development (Weiss, 1983).

There are many changes that occur during deep-fat frying (Fritsch, 1981). Aeration of the fat takes place when food is dropped into the oil, exposing the frying medium to oxygen and subsequent hydroperoxide formation. The hydroperoxides can undergo fission, dehydration or form free radicals, leading to alcohols and aldehydes, ketones, dimers, trimers, epoxides, and hydrocarbons. Solubilization of the food into the frying medium adds colored compounds and food lipids to the frying medium. Free fatty acids, di-glycerides, and glycerol can be formed when steam from the food causes hydrolysis (Fritsch, 1981).

Since any fat, even under ideal conditions, will deteriorate with use, either the producer of the fat or the user has to try to slow down deterioration of the fat during storage and use (McGill, 1980). The degree of unsaturation and the position of the double bonds determine the susceptibility of a fat to oxidation and hydrolysis. According to McGill (1980), a properly

refined frying fat will have a storage life of at least 12 weeks under reasonable storage conditions.

Nine restaurants were surveyed by Smith et al. (1986) over a three month period in order to evaluate changes that occur in shortenings used for commercial deep fat frying in fast service restaurants. Frying periods varied from 0 to 300 hours, but in most cases used shortening was discarded by the restaurant before 100 hours of frying time. They found that the greatest change in fatty acid profiles occurred in trans-C_{18} monomers which decreased from over 40% to as low as 13%.

It was reported by Gemert and Hoekman (1986) that homemakers did not find any significant differences in acceptability of meat croquettes fried in new oil versus those fried in old oils. In a potato chip sensory test, the homemakers preferred chips cooked in used oil. The length of use of the oil was 3 to 8 days, depending on the type of oil.

Desirable characteristics of fresh frying fats were summarized by Baeuerlen et al. (1968). They should have (a) a light color and a surface free of foam and smoke, (b) a clean, clear appearance free of burnt particles and (c), most importantly, a blandness that enhances the eating quality of the product to be fried.

Some of the common parameters used to determine oil quality are moisture content, peroxide value, smoke point, iodine value, melting point, free fatty acids (Baeuerlen et al., 1968) and dielectric constant (Fritsch, 1979).

There is no one test which is superior for analyzing frying fat deterioration because fats do not always deteriorate in the same way. In one operation, the rate of hydrolysis may be greater than the rate of oxidation, but in another operation the opposite situation could exist.

McDonalds' Corporation and Burger King continue to fry french fries in a tallow and vegetable blend rather than all vegetable oil. According to Haumann (1987), fast food chains that are interested in converting to an all vegetable frying medium have a big problem finding one that will produce the flavor that consumers want. Wendy's Research has shown that consumers prefer french fries cooked in tallow over those cooked in vegetable shortening. Fast food operators may have to pay more for a less appealing taste if they convert to vegetable oils.

Stabilization of frying oils

A synthetic antioxidant such as butylated hydroxyanisole (BHA) or butylated hydroxytoluene (BHT) is usually added to animal fats to improve their stability, since they do not contain the natural tocopherols found in vegetable oils (Weiss, 1983). Many consumers tend to reject synthetic antioxidants because of safety concerns (Chang et al., 1977). Some countries have restricted the use of BHA and BHT because of the undesirable effect they seem to have on certain enzymes of the liver and lung (Inatani et al., 1983).

In the United States, the FDA has expressed concern

about enzyme-inducing effects of BHT on the liver and/or extrahepatic organs, such as the lungs and gastrointestinal tract mucosa. They have also investigated the effect of BHT on the conversion of other ingested materials into toxic substances or carcinogens by the increase of microsomal enzymes (Chang et al., 1977). In feeding tests, BHA, BHT and tertiary butylhydroxyquinone (TBHQ) at high levels caused significant enlargement of the liver. BHT also increased the liver microsomal enzyme activity (Waldrop, 1980).

Besides the safety concerns regarding synthetic antioxidants, there are other instances where they are inadequate. BHT and BHA are both volatile and easily decomposed at high temperatures, thus making them unsatisfactory for the processing of foods such as potato chips and french fries. They also are not effective in vegetable oils or in preventing the development of initial off-flavors such as reversion flavor (Chang et al., 1977). Foods containing TBHQ absorb less oxygen than those without an antioxidant, but the development of objectionable flavors is not retarded (Chang et al., 1977).

These concerns over the safety and effectiveness of synthetic antioxidants have resulted in research interest in natural antioxidants. In 1938, Maverty patented the use of certain spice fractions for the prevention of rancidity in edible fats. Chipault et al. (1952) surveyed the antioxidant effectiveness of 32 spices and found

rosemary to be particularly potent. Synergistic effects were obtained when it was used in lard in combination with citric acid. These investigators concluded that the major antioxidant compounds in rosemary were phenols.

Since it has been known that rosemary has antioxidant activity, various extraction procedures have been developed to isolate and elucidate the chemical structures of the active compounds. Berner and Jacobson (1973) patented the extraction of rosemary with an edible oil. Another patent was received by Chang et al. (1977), when they divided the rosemary extract into fractions containing active antioxidants.

An extraction technique involving micronization of rosemary leaves in an edible oil followed by molecular distillation was developed by Bracco et al., (1981). This technique was modified in order to elucidate the chemical structures of the active components in rosemary. The extract was fractionated by repeated column chromatography with silicic acid using stepwise gradient elution (Wu et al., 1982).

The active components that have been elucidated are carnosol, rosmanol, carnosic acid, rosmaridiphenol and rosmariquinone. Carnosol and rosmariquinone have been reported to be more effective than BHA in lard (Inatani et al., 1983; Houlihan et al.,1984).

There have been many studies comparing the effectiveness of oleoresin rosemary to that of synthetic antioxidants, utilizing various methods of measuring

oxidation. Chang et al. (1977) reported that oleoresin rosemary extract when used at the 0.02% level was more effective than Tenox VI (a mixture of BHA, BHT, propyl gallate and citric acid). Other researchers (Chang et al., 1977; Wu et al., 1982; Inatani et al., 1983; Houlihan et al., 1984) reported rosemary extract to be at least as active as BHT. Inatani et al. (1983) reported that rosmanol was four times more active than BHA and BHT in fresh lard held at 98°C.

Rosemary extract has also been shown to be an effective antioxidant for poultry products. MacNeil et al. (1973) reported it to be as effective as the combination of BHA + citric acid at 0.05% in mechanically deboned poultry meat. Chang et al. (1977) and Bracco et al. (1981) both reported that it retarded oxidation in chicken fat. Rosemary oleoresin at 20mg/kg in turkey breakfast sausages was comparable to a commercial blend of BHA/BHT/citric acid at 200mg/kg in suppressing lipid autoxidation (Barbut et al., 1985).

MATERIALS AND METHODS

MATERIALS

Tallow- Two 35lb tubs of deodorized tallow were received from Anderson Clayton Foods/Humks Products, Inc. (Kraft Inc.), Memphis, TN. They were stored at 4°C for the duration of the study.

Oleoresin rosemary- (Herbalox seasoning type O) was donated by Kalsec, Inc. (Kalamazoo, MI) and stored at 4°C.

Electric fryers- Six Fry Daddy deep fat fryers (National Presta Industries, Inc., Eau Claire, WI) were purchased for the tallow experiment. The temperatures were regulated with rheostats.

Powdered dairy products- Powdered sour cream, butter and cheddar cheese were donated by Beatrice Food Ingredients, Inc., Beloit, WI. Full cream powdered milk (KLIM) manufactured by the Borden Co. was obtained from Ireland.

Egg powders- Whole eggs obtained from a commercial laying operation near Cork, Ireland were spray-dried at the dairy processing facility located at the Agricultural Institute, Moorepark Research Centre, Fermoy, Ireland. Samples were prepared with electrically heated air and direct gas-fired heated air spray-drying operations.

Butter- One 36 lb block of sweet, unsalted butter was purchased from the Kalamazoo Creamery Co. (Kalamazoo, MI).

α-Tocopherol and β-carotene were purchased from Sigma Company, St. Louis, MO.

Tertiary butyl hydroquinone (TBHQ) was donated by Eastman Kodak Company, Kingsport, TN.

Packaging materials- Reynolds 624 heavy duty foil (1.2± 0.1mill), polyethylene film from Crown Zellenback and margarine wrap from Badger Paper Mills, Inc. were used in the packaging study with butter.

Cholesterol and standard cholesterol oxidation products- 5-cholesten-3β-ol, 5-cholesten-3β,7α-diol, 5-cholesten-3β,7β-diol, 5-cholesten-3β-ol-7-one, 3,5-cholestadien-7-one, 5-cholesten-3β,25-diol, cholestan-5α,6α-epoxy-3β-ol, cholestan-3β,5α,6β-triol, and 5α-cholestane- were purchased from Steraloids Inc., Wilton, N.H.

Standard laboratory chemicals and solutions- All were reagent grade and were purchased from various companies. Pyridine and BSTFA- Silylation grade pryridine and N, O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane were purchased from Pierce Chemical Company (Rockford, IL) for derivatization of cholesterol and cholesterol oxides.

METHODS

OXIDATION OF CHOLESTEROL IN HEATED TALLOW

Effects of heating and antioxidant addition on cholesterol oxidation in tallow

Tallow was heated in deep fat fryers to observe the effectiveness of oleoresin rosemary in preventing cholesterol oxidation. Each fryer contained 800± 1g of tallow. Oleoresin rosemary (Herbalox seasoning) was stirred into the melted tallow to obtain concentrations of 0.01%, 0.05% and 0.1%. The fryers were controlled with rheostats to give temperatures of 180± 3°C or 135± 3°C. They were placed without covers under a laboratory hood, and exposed to laboratory light during the day.

In the initial study at 180°C, only a control and tallow samples containing oleoresin rosemary concentrations of 0.01% and 0.05% were used. Tallow samples were analyzed after 0, 48, 96, 144, 192 and 240 hours of continuous and intermittent heating. Some of the tallow samples were heated for 12 hours followed by 12 hours without heat. Samples (15g) were kept frozen in whirl-pack bags until saponification and extraction. They were analyzed by thin layer chromatography and capillary column gas liquid chromatography.

In the second study the tallow was heated at 135°C. Two replications were done and two levels of oleoresin rosemary (0.01 and 0.05%) were evaluated under both continuous and intermittent heating conditions. Samples were taken every 24 hours and analyzed by thin layer

chromatography, as well as by packed and capillary column gas liquid chromatography.

In the last tallow/oleoresin rosemary study, two replications were performed to evaluate the addition of oleoresin rosemary (0.05 and 0.1%) to tallow heated at 135± 3°C. The samples were analyzed by thin layer chromatography, and by packed and capillary column gas liquid chromatography.

Recovery:

An internal standard (5α-cholestane) was added to some of the samples prior to saponification upon removal from the fryers to determine recovery from the beginning of analysis. The recovery was at least 80%. However, due to the expense of 5α-cholestane and the amount required when added prior to saponification, it was not practical to check the saponification/extraction recovery for each sample. Injection recovery was determined for each sample.

Saponification of tallow:

Duplicate samples (2.5± 0.05g) of tallow were weighed for each treatment at each sampling time into 125ml Erlenmeyer flasks. The tallow was dissolved in 50ml of methanol using a hot water bath. The flask was stoppered and shaken vigorously for approximately 60 seconds until the droplets of melted fat were dispersed into the methanol. For saponification, 50ml of 2N potassium hydroxide (KOH) in methanol were added and the flask shaken until the solution was transparent. The samples were kept overnight

in the dark at room temperature during which time clumps of white soapy/waxy material developed at the bottom of the flask.

Extraction of the nonsaponifiable fraction of the tallow:

Extraction of the nonsaponifiable lipids (including cholesterol and cholesterol oxidation products) was carried out in 500ml separatory funnels. Distilled water (100ml) and diethyl ether (100ml) were added to the sample in the separatory funnel, rinsing the sample out of the Erlenmyer flask for the first extraction. The samples were extracted two more times with diethyl ether (100ml aliquots), collecting and combining the ether extracts in another 500ml separatory funnel. The ether fraction containing the nonsaponifiable lipids was then washed twice with 50ml aliquots of 0.5N KOH in distilled water, followed by three washings with 50ml aliquots of distilled water. The ether portion was then passed through anhydrous sodium sulfate in a funnel with Whatman #1 filter paper into a 500ml flat bottom flask.

Evaporation of solvent and preparation for analysis: A Rotavapor-R rotary evaporator from Brinkman Instruments (Westburg, N.Y.) with a water bath temperature of 28°C was used for evaporation of ether from the nonsaponifiable fraction of tallow. Samples were redissolved in acetone (5ml), from which 1ml was removed and placed in a screw-cap vial for capillary column GLC analysis. The remainder of the sample was transferred to a capped vial

for TLC and packed column GLC analyses, rinsing with acetone to assure complete removal of the nonsaponifiable tallow fraction into the second vial.

Capillary GLC analysis:

Sample preparation: Capillary column GLC analysis was used to separate and quantify specific cholesterol oxidation products. The solvent from a 1 ml portion of nonsaponifiable lipids (removed after the sample was redissolved in acetone) was evaporated under nitrogen after the addition of 0.1ml of 5α-cholestane (0.4mg/ml, in ethyl acetate) as the internal standard. Pyridine (100µl) and N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) (50µl) were added for derivatization. The sample was stored in the dark for 30 minutes for the derivatization to be complete. Derivatized samples were stored at 4°C for not more than 12 hours prior to GLC analysis to avoid sample degradation. Samples that could not be analyzed immediately after extraction were stored in chloroform at 4°C.

GLC system: A Hewlett-Packard GC model 5890 with a flame ionization detector (FID) at 300°C was used. It was equipped with a 15M polydimethyl siloxane glass capillary column from Supelco (ID= 0.25mm). The carrier gas was helium. The operating conditions were as follows: The initial oven temperature was 180°C, with an initial time of 0.0. Two rates were utilized, the first one being: 10°/min to 230°C (final time: 0.0 min). The second rate was 0.2°/min to a final temperature of 240°C (final time

2: 0.0min). The total time for each sample run was 55 minutes. The column head pressure was maintained at 60KPa and the detector flow rates were as follows: carrier + He (make-up): 30ml/min, air: 300ml/min, hydrogen: 30ml/min. A split ratio of 40 was used. The recorder was a Hewlett-Packard model 3392A with chart speed: 0.3cm/min, threshold: 4, attn 2": 3, peak width: 0.04, area of rejection until 10 minutes: 999999999, and the area of rejection at 10 minutes was 2000.

TLC analysis:

Sample preparation: TLC was used for initial identification of cholesterol oxidation products. Samples were stored in acetone at 4°C after ether extraction for up to 24 hours. The solvent was evaporated under nitrogen to a volume of 0.5ml. Each sample and its duplicate (20µl) were spotted on the TLC plates.

TLC system: Silica gel type G plates were prepared and activated by placement in an oven at 110°C for 1 hour. After spotting, the plates were developed twice in a heptane:ethyl acetate (1:1) solvent system, sprayed with 50% H_2SO_4 and put in an oven (110-120°C) for approximately 10 minutes for color development.

Packed column GLC analysis:

Sample preparation: Packed column GLC was used to quantitate cholesterol loss. After 20µl were taken from the samples in 0.5ml acetone for TLC, the solvent was evaporated under a nitrogen stream and the samples were redissolved in 3ml of N,N dimethylformamide for analysis

with packed-column GLC. The sample (4µl) was injected with 5α-cholestane (0.2mg/ml in ethyl acetate) to calculate injection recovery.

GLC system: The chromatograph used was a Hewlett-Packard model 5840 with a flame ionization detector. Nitrogen was the carrier gas. It was equipped with a glass column (ID= 2mm, length= 2M), packed with 1% SE-30 from Anspec, Ann Arbor Michigan. Operating conditions were as follows: injection temperature: 275°C, initial oven temperature: 200°C, initial time: 1 minute, rate: 5°/min, final temperature: 230°C, final time: 14 minutes, and the detector temperature was 300°C.

Cholesterol oxidation produced in commercial french fries

French fries were purchased from two fast food franchise restaurants every day at approximately 11:30^{AM} for 7 successive days (Thurs through Wed). The fries were wrapped in PVC and foil and frozen overnight (if necessary) prior to analysis.

Lipid extraction:

The french fries (95± 1g) were blended in a Waring explosion-proof laboratory blender with approximately 325ml hexane for 90 seconds. The blender contents were then filtered through Whatman #1 filter paper in a Buchner funnel. The blender and funnel filter were rinsed with hexane, collecting all the hexane and transferring it to a 500ml flat bottom flask. The hexane was evaporated in a Buchi Rotavapor-R at 60°C. The warm fat (2.5± 0.01g) was transferred (using a disposable pipette) into a 150ml

Erlenmeyer flask in duplicate for saponification. The remaining fat was transferred to a vial for color comparison between french fry fat samples.

Saponification:

Methanol (50ml) was added to the flask and heated in a 60°C water bath to disperse the fat. For saponification, 50ml of 2N potassium hydroxide in methanol were added to the flask. It was stoppered and shaken to disperse all the fat droplets. The samples were kept in the dark at room temperature overnight.

Extraction of nonsaponifiable fraction and analysis by TLC and GLC:

The extraction of the nonsaponifiable fraction and subsequent analysis was carried out as described previously.

OXIDATION OF CHOLESTEROL IN DAIRY PRODUCTS

Effect of fluorescent light on cholesterol oxidation in butter

Experimental design:

One 36 lb block of sweet unsalted butter was purchased from Kalamazoo Creamery Co., Kalamazoo, MI. After powdered (extra fine) popcorn salt (2%) was added by hand to the butter, three different antioxidants were added to separate portions of the butter. The butter was softened to achieve uniform distribution of the salt and antioxidants which were mixed in by hand. The antioxidants investigated were β-carotene (0.06%), α-tocopherol (0.06%) and TBHQ (0.02%). A portion of the butter without any antioxidant was used as a control. Each butter treatment

was formed into blocks (125mm * 65mm * 5mm) and packaged in three different packaging materials- aluminum foil (1.2± 0.1mill), polyethylene film and margarine wrap.

The wrapped butter was placed in a 4°C walk-in cooler and was illuminated with approximately 150 foot candles (1600 lux) of light from Lithonia® fluorescent light fixtures approximately 17cm above the surface of the butter. Samples were taken for analysis every 4 days for 16 days. The experiment was carried out in triplicate, and the butter stored at -20°C until required for analysis.

Peroxide values:

The peroxide values of the butter samples were determined following the AOCS Official Method Cd8-53.

Analysis of cholesterol oxidation products:

Lipid extraction: Butter (10± 0.05mg) was weighed into a dry centrifuge tube (12- 15ml). Hexane (1ml), distilled water (1ml) and 5α-cholestane (50μg) as the internal standard were added. The samples were centrifuged for 3 minutes at speed 4 in the clinical centrifuge that was also used for the peroxide values. The hexane layer containing the lipids was transferred into another centrifuge tube and the remaining water layer was extracted two more times with 1ml of hexane, centrifuging between extractions. The combined hexane was rinsed with distilled water (1ml) and centrifuged as above. The hexane was transferred into a third centrifuge tube. Anhydrous granular sodium sulfate was added to the hexane

to remove any residual water. The hexane containing the lipids was transferred to a screw-cap vial, rinsing the Na_2SO_4 with extra hexane.

Saponification: The hexane was completely evaporated under nitrogen. Potassium hydroxide in methanol (2ml, 2N) was added and the sample was placed in the dark for 20 hours.

Extraction of the nonsaponifiable butter fraction: The saponified sample was returned to a glass centrifuge tube to which distilled water (1ml) and isopropyl ether (2ml) were added. It was mixed gently using a Vortex Genie from Fisher Scientific, followed by centrifugation (3min., speed 4). This was repeated two more times with 2ml aliquots of ether, collecting the top ether layer in another centrifuge tube after each centrifugation. The combined ether extracts were washed with distilled water (1ml) and centrifuged. The ether was removed and dried with anhydrous Na_2SO_4 and then transferred to a 4ml screw-cap vial. The solvent was evaporated under a nitrogen stream and stored in chloroform until GLC analysis.

GLC analysis:

The chloroform in the vials was evaporated under nitrogen. Pyridine (50µl) and BSTFA silylating reagent (50µl) were added and the sample was placed in the dark at room temperature for 30 minutes for derivitization. The samples were analyzed immediately by GLC or stored at 4°C for up to 24 hours.

Survey of powdered dairy products for cholesterol oxidation products

Lipid extraction:

Dry column lipid extraction (Marmer and Maxwell, 1981) was used because of its simplicity compared to the Folch or Bligh and Dyer methanol/chloroform extraction methods. Sample size was determined by fat content, estimated by the values cited by Hansen et al. (1979). Powdered whole milk (5± 0.1g), powdered sour cream (1.5± 0.1g), butter powder (1.5± 0.1g) and powdered cheddar cheese (1.5± 0.1g) were analyzed.

Saponification and extraction:

Saponification of the lipids was carried out in a 500ml flask, according to the method described previously. The extraction of the nonsaponifiable fraction from the powdered dairy products was also completed by the previously described method.

Analysis of cholesterol oxidation products:

TLC and capillary column GLC were utilized as in the tallow analysis. Packed column GLC was not used because identifying and quantifying cholesterol oxidation products was the major concern rather than measuring cholesterol loss.

OXIDATION OF CHOLESTEROL IN EGG POWDER

Samples were prepared at the Agricultural Institute, Moorepark Research Centre, Fermoy, Ireland. The spray drier used was a pilot-scale Anhydro LAB 3 with a pneumatic nozzle atomizer. It was a single-stage drier

with the conventional conical- based tower design. The exhaust air fan maintained an air flow rate of 190M^3/hr. The dryer was equipped with electrical heating elements for the indirect heating, and was converted by the fitting of a standard gas burner (Radiant Superjet model GX 25) to the extended air inlet duct for direct gas fired heating. The Irish Gas Board supplied natural gas from the Kinsale gas field. During drying the air inlet and outlet temperatures were 190-200°C and 88-95°C, respectively.

Oxides of nitrogen content:

Oxides of nitrogen (NO_x) were measured in the heated drying air at the electrical coils of the indirect spray drying system and at the inlet of the burner throat for the direct heat system. A CSI Chemiluminescence Analyzer model 2200 with multi-range selector: 0-0.5, 0-1.0, 0-2.0, 0-5.0ppm NO_x was used. The precision was 0.01ppm on the 0-0.5ppm scale.

Sample treatment:

The powdered egg samples were incubated in the dark for two weeks at 38°C in glass beakers covered with polyvinylchloride film.

Sample analysis

Sample preparation and analysis of the powdered eggs were carried out as for the powdered dairy products, using only 0.2± 0.01g for the initial dry column lipid extraction.

STATISTICS

Tallow:

The statistical model for this experiment was a randomized

block design, the blocks being the heating times. The F ratio determined from the analysis of the variance was used to establish significant differences between oleoresin rosemary levels and time. The F ratios from the analysis of the variance were also used to compare heating methods.

A difference in cholesterol loss between pure tallow and tallow containing 0.05% oleoresin rosemary was detected by Tukey's multiple comparison of the means test.

For the commercial french fry study, significant differences among restaurants and days were determined using the F ratio from a two factor analysis of the variance and Duncan's multiple range test. These tests were done for cholesterol loss and COP formation.

Butter:

Two factor analysis of the variances were done to determine the significant differences between antioxidants and between packaging materials over storage time. Tukey's multiple comparison of the means test was used to compare antioxidants and packaging materials.

Eggs:

The F ratio from the analysis of the variance was utilized to determine the significance of the drying method and the incubation.

RESULTS AND DISCUSSION

OXIDATION OF CHOLESTEROL IN HEATED TALLOW

The effects of oleoresin rosemary addition and heating at 180°C on the stability of cholesterol in tallow

Cholesterol oxidation was measured in heated tallow that contained added oleoresin rosemary. In unheated tallow, two compounds less polar than cholesterol were observed on the TLC plates. The R_f values of these compounds ranged from 0.88 to 0.90, while that of cholesterol was 0.82. The spots were pink-purple in color. These compounds were observed on TLC plates after 240 hours of continuous and intermittent heating of the tallow, but they were not identified as any of the seven standard cholesterol oxidation products (7α-hydroxy-cholesterol, 7β-hydroxycholesterol, 7-ketocholesterol, 3,5-cholestadiene-7-one, 25-hydroxycholes- terol, cholesterol-5,6-α-epoxide, and 5-cholestan-3β,5α,6β- triol). None of the seven cholesterol oxidation products were detected by TLC or GLC capillary column analysis (detection limit 0.2 mg/kg) in any of the tallow samples, even after 240 hours of heating.

When the tallow extracts were analyzed by capillary column gas chromatography, some small peaks were eluted after cholesterol in the samples that had been continuously and intermittently heated for 48 hours, but none of these peaks corresponded to any of the seven standard cholesterol oxidation products. The peaks did not increase in size with increased heating time, and the addition of oleoresin rosemary did not affect their formation.

The results of heating tallow to 180°C were not unexpected considering the results of earlier studies by Park and Addis (1986a,b). They found only small amounts of cholesterol oxidation products (COP's) in tallow heated at 180°C. When they investigated the effect of a short heating time at an elevated temperature (6 hours at 200 ± 2.5°C), no COP's were detected by gas liquid chromatography. Sporadically-formed COP's were found in tallow heated at 190°C. Park and Addis (1986b) concluded that at elevated temperatures (>150°C), there may be either less chance for cholesterol oxidation to occur or a greater probability for COP's to break down quickly after formation.

The variation in the results of the present study from those reported by Ryan (1982), who cited the presence of 7-ketocholesterol and 3,5-cholestadien-7-one in tallow heated at 180°C, may be explained in part by the fact that the fryers used in the two experiments were different. Ryan (1982) used large commercial fryers that had heating elements in direct contact with the fat. The fryers used in this case were of the home-type and produced no turbulence, and had a smaller tallow surface exposed to air. In addition, the heating elements were contained within the walls of the fryers, resulting in a more gradual melting of the tallow. Thus the temperature of the fat remained more constant throughout the fryers.

<u>The effect of oleoresin rosemary addition and heating at 135°C on the stability of cholesterol in tallow</u>

Two separate heating methods- intermittent and

continuous; and three levels of oleoresin rosemary (0.01%, 0.05%, and 0.1%) were examined.

Cholesterol loss

Cholesterol loss was monitored to determinine the amount of cholesterol broken down or oxidized during heating. Less cholesterol loss was observed in the tallow containing 0.05% oleoresin rosemary than in either the tallow containg 0.01% olesoresin rosemary or the control. Preliminary tests revealed that 0.1% oleoresin rosemary did not provide any more stability than the 0.05% (Figures 6 and 7). However no statistical analyses were performed on the 0.1% oleoresin samples due to limited data collection.

Oleoresin rosemary did not completely inhibit the formation of COP's in heated tallow, but appeared to act more consistently than an ascorbyl palmitate/α-tocopherol combination used by Park and Addis (1986). The level of COP's in tallow containing 0.05% oleoresin rosemary was lower than that of the control throughout the heating. These results suggest that oleoresin rosemary, which contains several compounds exhibiting antioxidant activity, is more stable at 135°C that ascorbyl palmitate/α-tocopherol. Tallow with added ascorbyl palmitate/α-tocopherol heated at 135°C for up to 70 hours did not contain any of the standard COP's (Park and Addis, 1986a). However, after 70 hours, the inhibitory effect of the antioxidant was no longer observed. At heating times of 96, 144 and 216 hours they reported similar levels of COP's in the control tallow and tallow treated with ascorbyl palmitate/α-tocopherol. They suggested

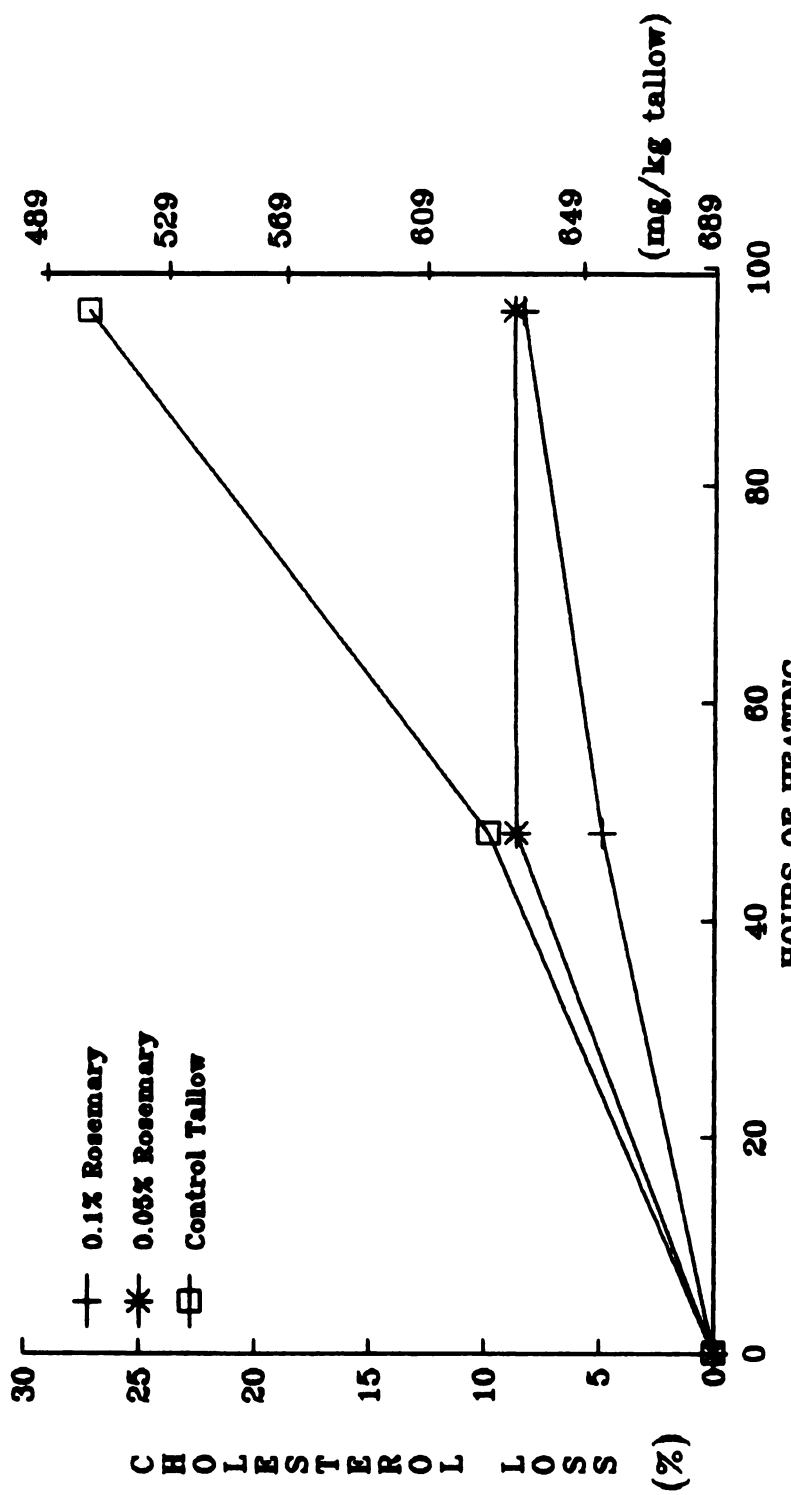


Figure 6. Effect of oleoresin rosemary on cholesterol loss in heated tallow.

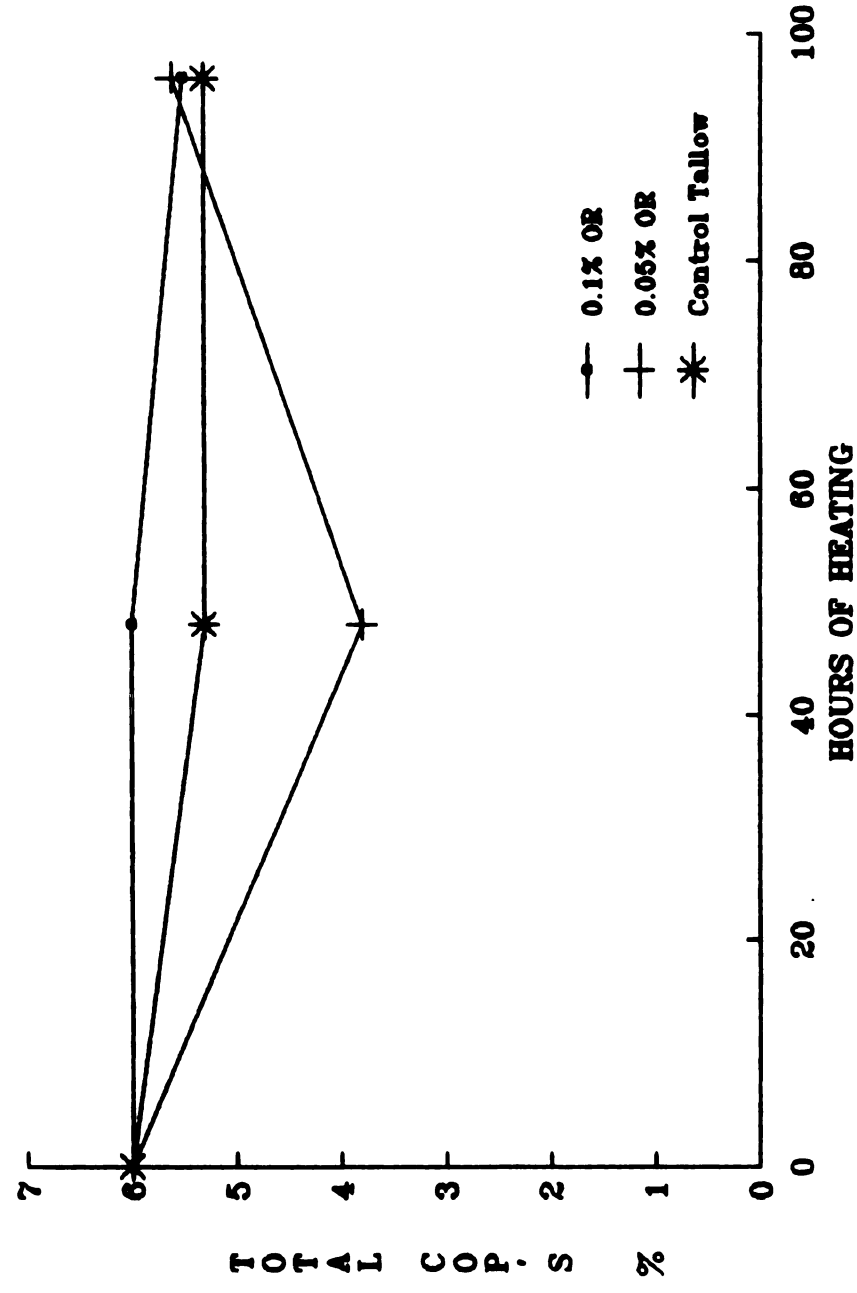


Figure 7. Effect of oleoresin rosemary on the formation of cholesterol oxidation products (COP's) in tallow heated at 135°C.

that at high temperature ascorbyl palmitate and α-tocopherol either do not survive sufficiently long enough to have an impact, or are consumed by the excessive quantity of free radicals formed by extended heating.

The equation describing the statistical model for the levels of oleoresin rosemary as a treatment over time for the continuous heating was $Y = \mu + a_i + b_j + \epsilon$, while that for the intermittent heating was $Y = \mu + a_i + b_j + c_{ij} + \epsilon$. The term c_{ij} represents interaction between time and the oleoresin rosemary level that existed in the intermittent system but did not exist in the continuous system ($p < 0.01$, Appendix I). Oleoresin rosemary (0.05%) was an effective antioxidant for both the continuously and intermittently heated tallow (Figures 8 and 9).

Tables 9 and 10 show the cholesterol loss in heated tallow. A comparison was made between continuous and intermittent heating.

Continuous heating caused more cholesterol loss than intermittent heating (Figure 10), except in tallow containing 0.01% oleoresin rosemary, where there was no significant difference between the two heating methods Appendix II, part B). This was not expected since repeated heating and cooling of fats is a more detrimental treatment than maintaining a constant temperature (Ryan et al., 1981). Ryan (1982) reported that intermittent heating caused more cholesterol oxidation in tallow. In this study the time periods of heating (12 hours) for the intermittently heated tallow may have been too long. The time period of heating used by Ryan

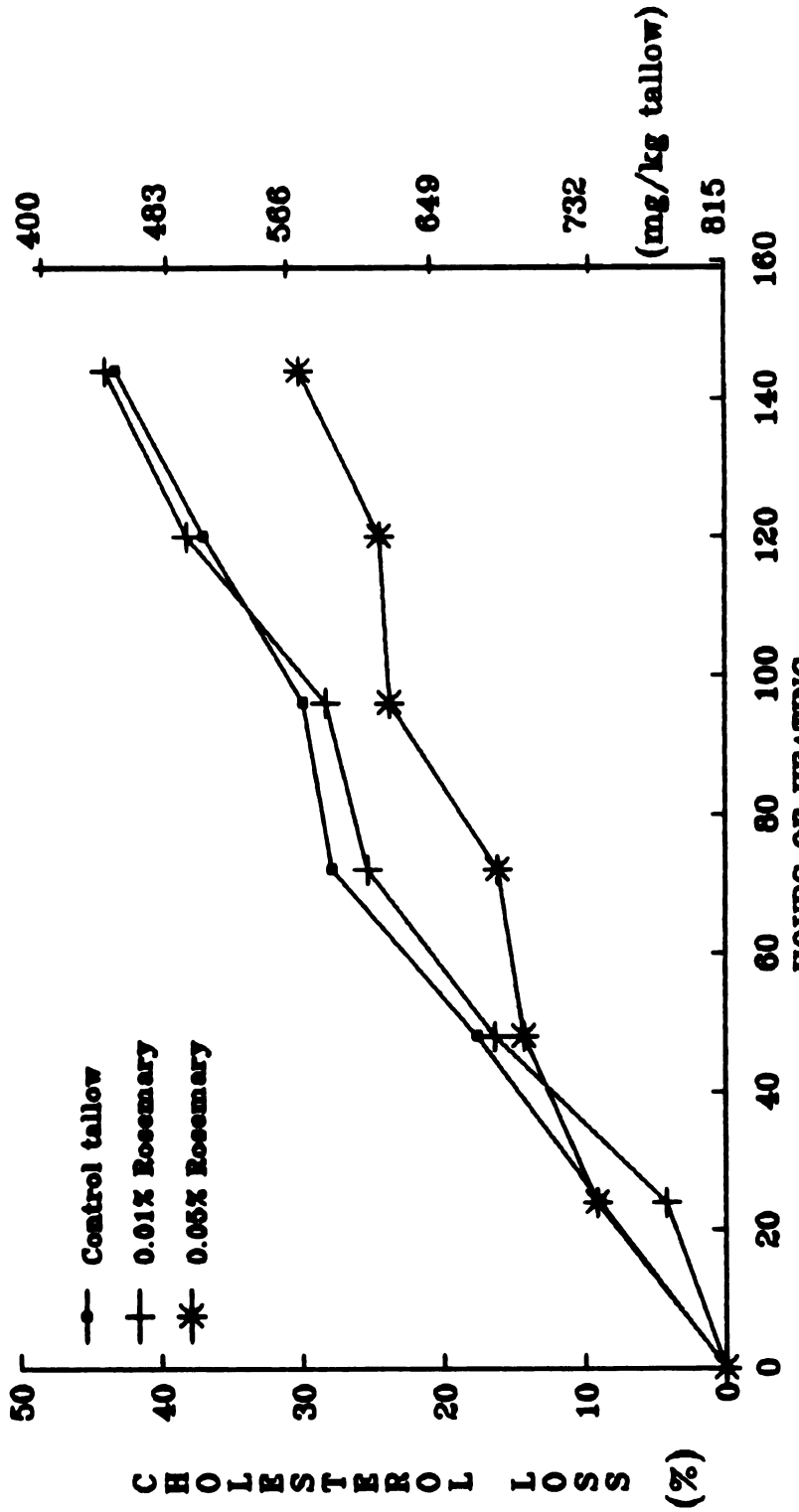


Figure 8. Effect of continuous heating at 135°C on cholesterol loss in tallow containing oleoresin rosemary.

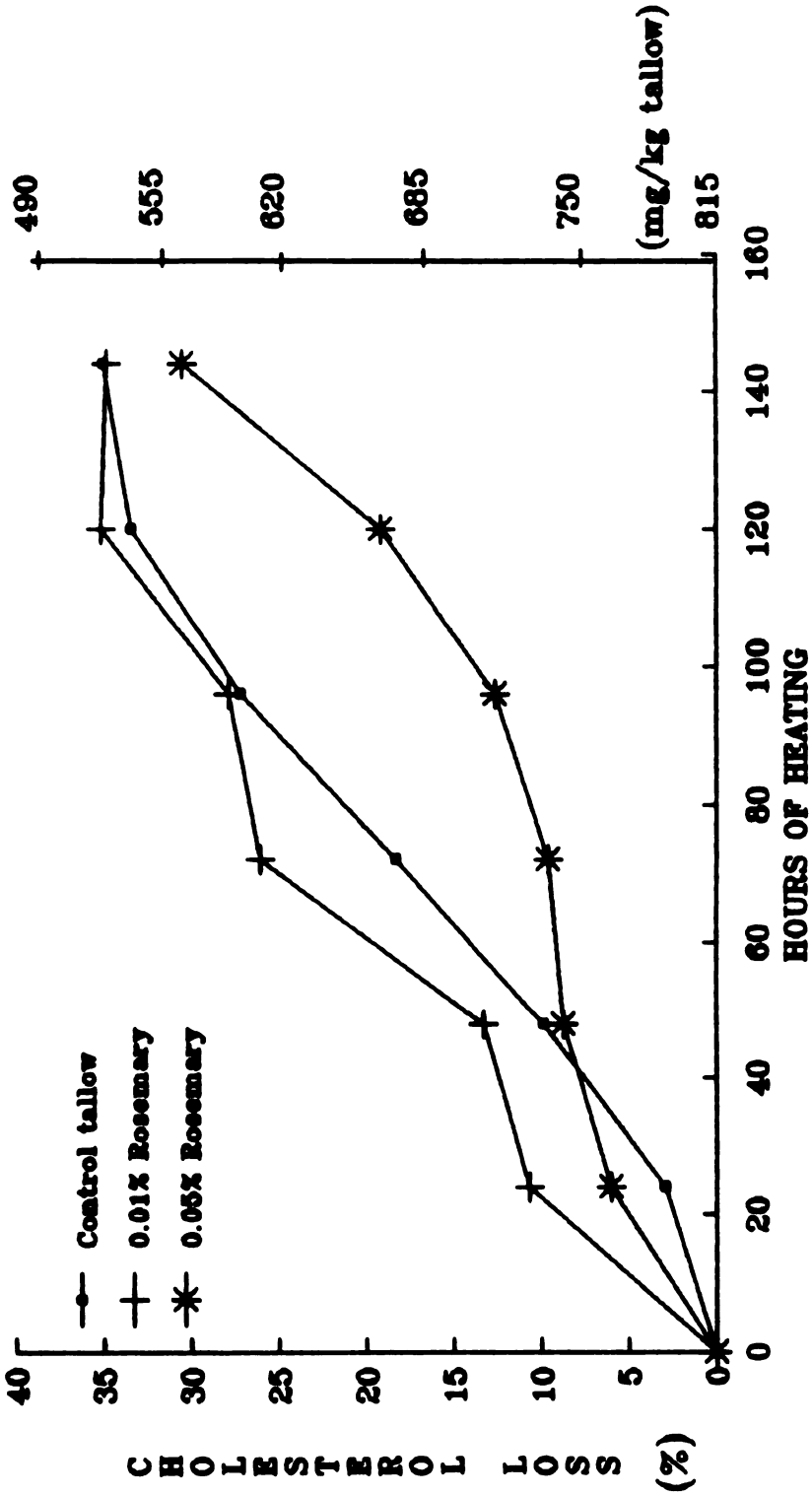


Figure 9. Effect of intermittent heating at 135°C on cholesterol loss in tallow containing oleoresin rosemary.

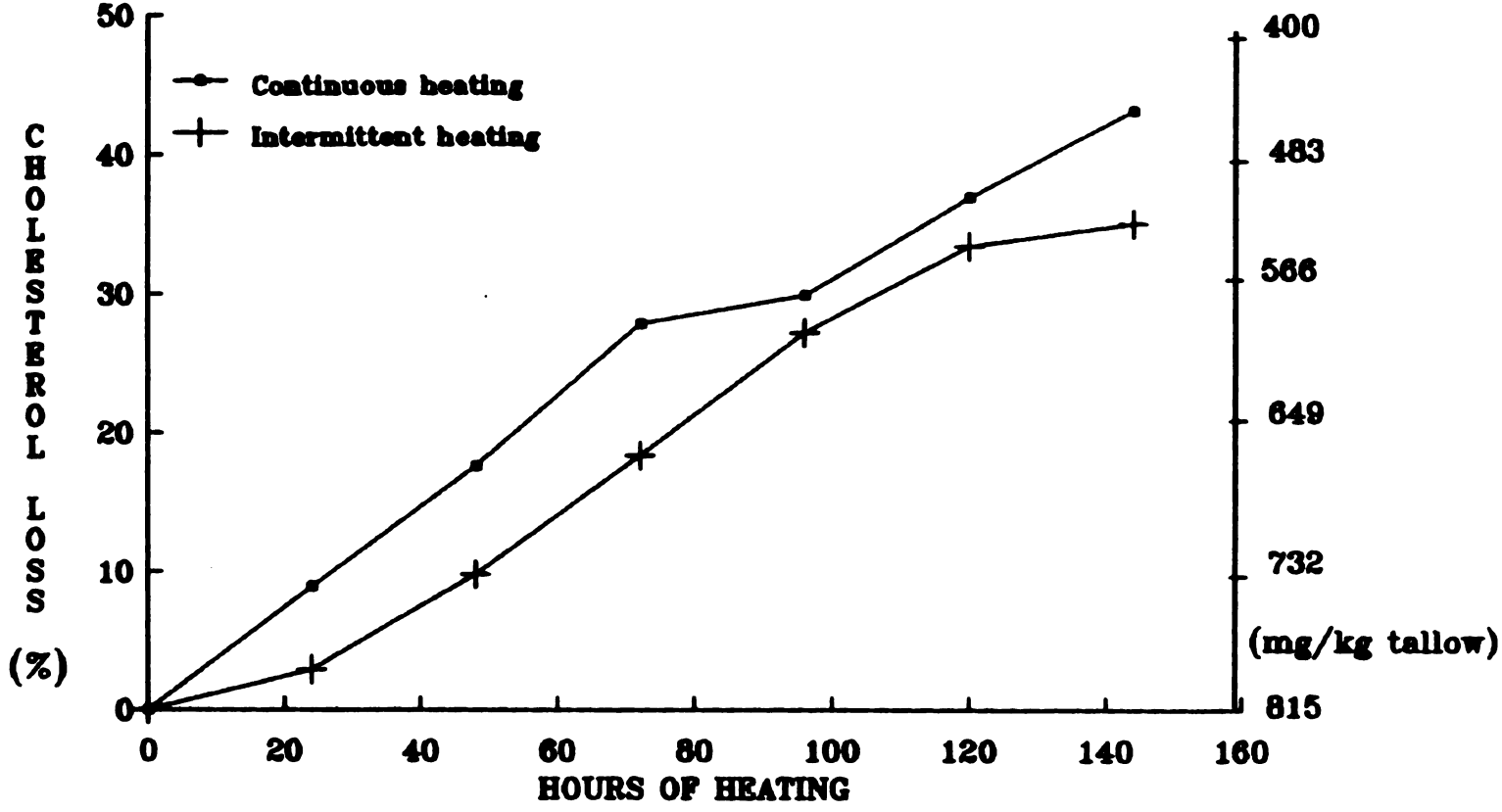


Figure 10. Effects of continuous versus intermittent heating at 135°C on cholesterol loss in tallow.

Table 9. The effect of oleoresin rosemary on cholesterol loss in continuously heated tallow.

Heating Time (hr)	Oleoresin Rosemary (%)	Cholesterol Loss (%)	Heating Time (hr)	Oleoresin Rosemary (%)	Cholesterol Loss (%)
24	0.0	8.9	96	0.0	29.8
	0.01	4.2		0.01	28.2
	0.05	9.5		0.05	23.7
48	0.0	17.6	120	0.0	41.7
	0.01	16.3		0.01	38.0
	0.05	14.3		0.05	24.4
72	0.0	27.8	144	0.0	43.0
	0.01	25.3		0.01	43.0
	0.05	16.1		0.05	29.6

Table 10. The effect of loeoresin rosemary on cholesterol loss in intermittently heated tallow.

Heating Time (hr)	Oleoresin Rosemary (%)	Cholesterol Loss (%)	Heating Time (hr)	Oleoresin Rosemary (%)	Cholesterol Loss (%)
24	0.0	2.9	96	0.0	27.1
	0.01	10.7		0.01	27.8
	0.05	6.0		0.05	12.6
48	0.0	9.8	120	0.0	33.3
	0.01	13.3		0.01	35.0
	0.05	8.7		0.05	19.1
72	0.0	18.3	144	0.0	34.9
	0.01	26.0		0.01	34.7
	0.05	9.6		0.05	30.4

was eight hours. If the fryers had been turned on and off more frequently, the tallow may have undergone more extensive oxidation.

Comparing treatment means using the Tukey multiple comparison procedure revealed that the largest difference in cholesterol loss was between the tallow containing 0.01% oleoresin rosemary heated intermittently and the tallow containing 0.05% oleoresin rosemary heated intermittently after 120 hours. The difference was 82.9 ± 3.4 mg/kg cholesterol (Appendix IV). The addition of 0.01% oleoresin rosemary to the tallow did not stabilize the cholesterol and may have acted as a prooxidant, since the control tallow had a smaller cholesterol loss.

Formation of cholesterol oxidation products

The effects of continuous and intermittent heating on the formation of COP's in tallow are shown in Figures 11 and 12. Formation of COP's was very random which indicated that compounds may have formed and then reacted further to form other products. In the continuously heated tallow, the addition of 0.05% oleoresin rosemary lowered the formation of COP's throughout the heating period. In the intermittently heated tallow, after 72 hours there was a substantial formation of cholesterol oxidation products in the sample containing 0.01% oleoresin rosemary. The total COP's amounted to 7.3% of the original cholesterol, while the COP's in the control tallow amounted to 1.7% of the original cholesterol.

The most prevelant COP in the heated tallow system was

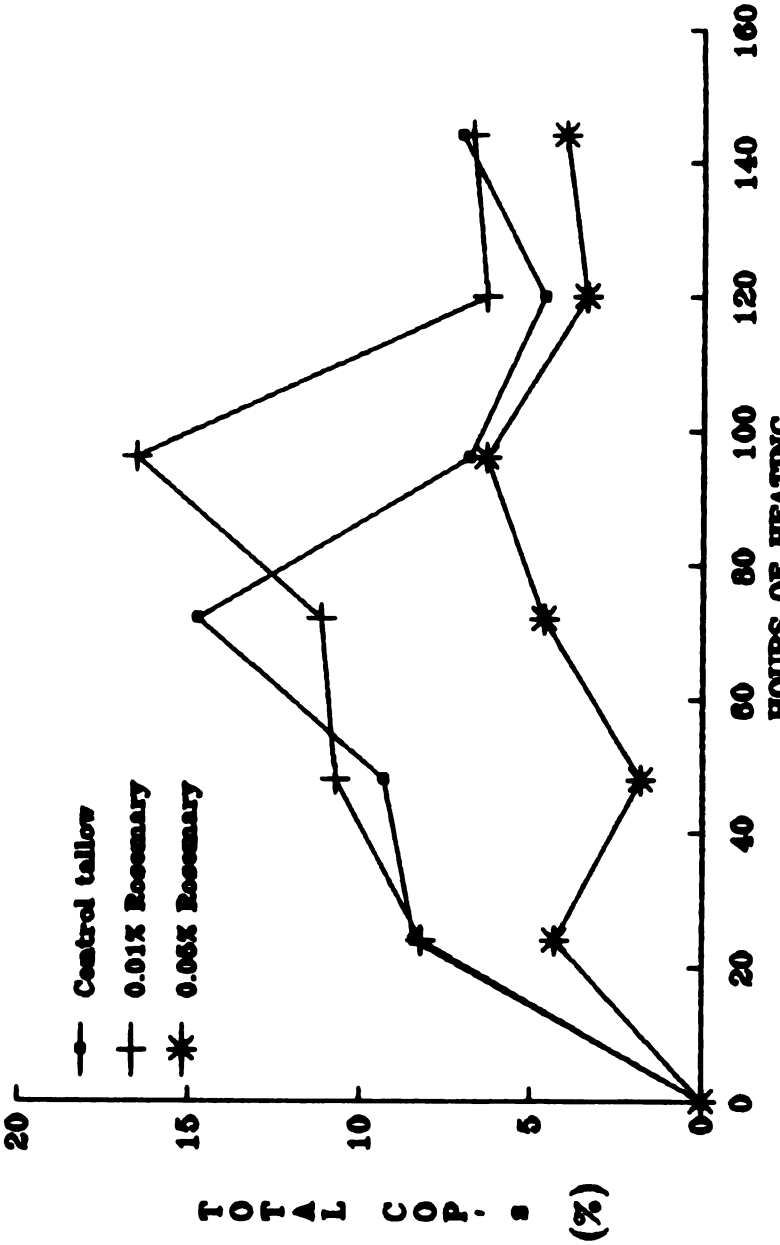


Figure 11. Effect of continuous heating at 135°C on the formation of cholesterol oxidation products in tallow.

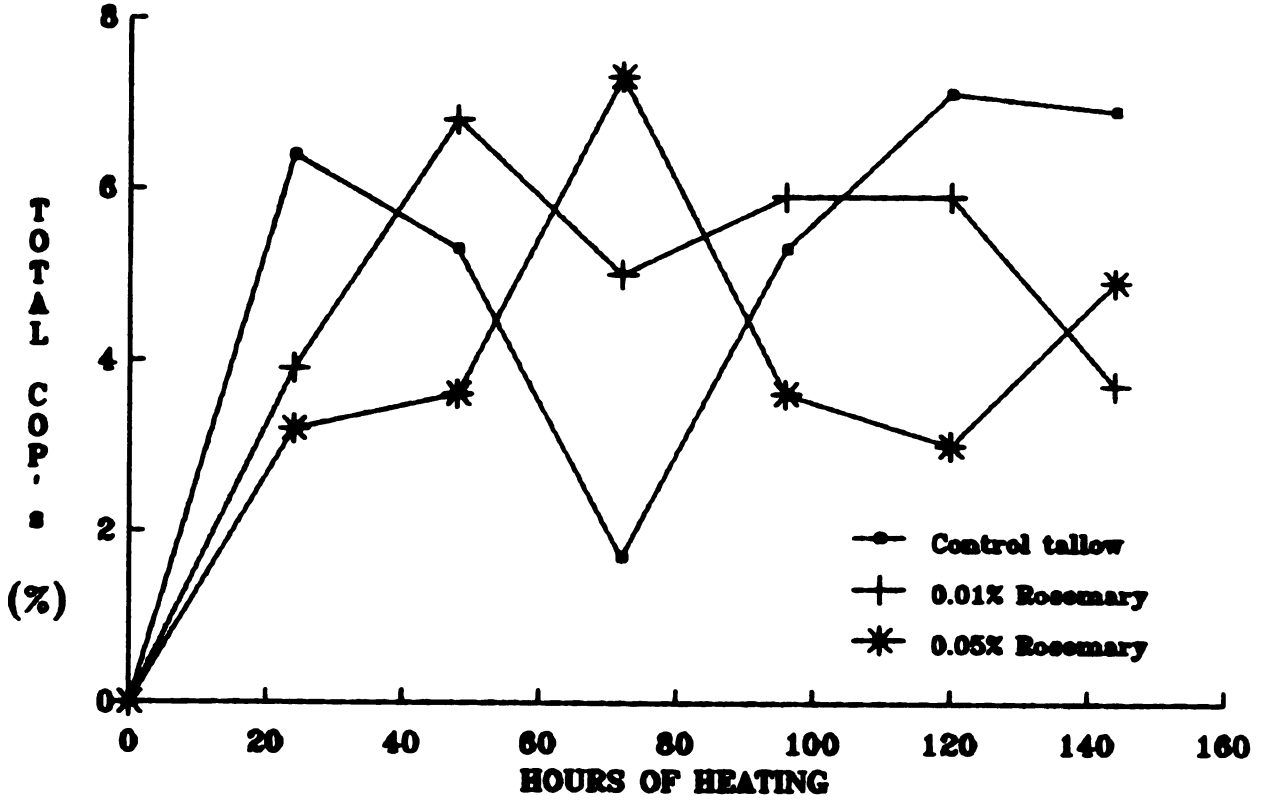


Figure 12. Effect of intermittent heating at 135°C on the formation of cholesterol oxidation products (COP's) in tallow.

tentatively identified as 7-ketocholesterol (Table 11). It was identified by TLC and capillary column gas liquid chromatography by comparison of retention times with a standard. The standard COP was also added to some of the samples to observe the change in peak size of the suspected COP. Tallow contained an average of 3.2% 7-ketocholesterol before heating (six samples were analyzed). After heating, the concentration range was 1.3% - 9.7%, with the exception of one sample of the tallow containing 0.01% oleoresin rosemary that had been heated continuously for 96 hours and which contained 19% 7-ketocholesterol. Park and Addis (1986) reported that the formation of 7-ketocholesterol was almost linear with heating time, and after 376 hours of heating at 155°C, its concentration was approximately 10% of the initial cholesterol content. They also reported concentrations of 4.6% 7-ketocholesterol in tallow which was heated at 135°C for 216 hours.

The results shown in Table 11 suggest that due to the cycling nature of lipid oxidation reactions, new compounds are being formed and broken down continuously. Thus COP concentration does not increase over time but fluctuates. Even though the COP concentration was random, the highest concentration in the tallow containing 0.05% oleoresin rosemary was always lower than that of the control and tallow containing 0.01% oleoresin rosemary.

In the majority of tallow samples, 7β-hydroxycholesterol was observed after 48 hours of heating (Table 11). It was identified in the same manner as the 7-ketocholesterol. The

highest level of 7β-hydroxycholesterol (8%) was found in the same sample that contained 19% 7-ketocholesterol (heated continuously for 96 hours, containing 0.01% oleoresin rosemary). Excluding this sample, the range of 7β-hydroxycholesterol concentration for all samples heated intermittently and continuously was 0 - 6.2% of the original cholesterol.

Table 11. The maximum concentrations of 7-ketocholesterol, 7β-hydroxycholesterol and cholesterol-α-epoxide in heated tallow samples containing oleoresin rosemary.

Continuous Heating			Intermittent Heating		
OR (%)	Heating Time (hr)	7-Keto (%)	OR (%)	Heating Time (hr)	7-Keto (%)
0.0	24	9.7	0.0	24	5.6
0.01	96	19	0.01	48	8.6
0.05	72	6.1	0.05	96	4.5
		7β-OH (%)			7β-OH (%)
0.0	96	3.4	0.0	120	2.6
0.01	96	8.0	0.01	72	3.2
0.05	96	2.9	0.05	148	2.3
		α-Epox (%)			α-Epox (%)
0.0	72	16.2	0.0	120	1.1
0.01	72	7.3	0.01	48	6.0
0.05	72	1.1	0.05	148	1.3

The highest level of 7β-hydroxycholesterol detected in the continuously heated sample containing 0.05% oleoresin roseemary was 2.9%, after 96 hours of heating. For the intermittently heated tallow containing 0.05% oleresin rosemary, the highest level of 7β-hydroxycholesterol detected was 2.3%, and occurred after 148 hours of heating. The control tallow contained a maximum of 3.4% and 2.6% 7β-hydroxycholesterol after 96 hours of continuous heating and 120 hours of intermittent heating. The continuously heated tallow containing 0.01% oleoresin rosemary contained more 7β-hydroxycholesterol than its intermittently heated counterpart, with 3.2% after 72 hours of intermittent heating, and 8.0% after 72 hours of continuous heating. Since there was less cholesterol loss in the intermittently heated tallow, it follows that fewer cholesterol oxidation products would be formed.

In most of the samples that contained 7β-hydroxycholesterol, 7α-hydroxycholesterol was also tentatively identified by TLC. Both 7α- and 7β-hydroxycholesterol were reported by Park and Addis (1986) in the 1 - 2% range throughout the heating of tallow at 155°C. It could not be quantified in this study with capillary gas chromatography due to incomplete resolution with cholesterol. When sample size was decreased in attempt to resolve the peaks, the concentration of 7α-hydroxycholesterol was too low for detection.

Cholesterol-α-epoxide was also observed in approximately 25% of the tallow samples, usually at the beginning and reappearing again toward the end of the heating. The highest

amount detected was 16.2%, in the control sample heated continuously for 72 hours. Excluding this sample, the range of cholesterol-α-epoxide was 0 - 7.3%. The continuously heated sample that contained 19% 7-ketocholesterol and 8% 7β-hydroxycholesterol contained no cholesterol-α-epoxide. In both the continuously and intermittently heated samples containing 0.05% oleoresin rosemary, only the samples heated for 148 hours contained any cholesterol-α-epoxide. The oleoresin rosemary may have prevented the oxidation of 7β-hydroxycholesterol to the epoxide. The intermittently heated sample contained 1.3% and the continuous sample contained 1.1%. The maximum level of cholesterol-α-epoxide (1.1%) in the control tallow heated intermittently occurred after 120 hours of heating. After 48 hours, 6.0% cholesterol-α-epoxide was detected in the 0.01% oleoresin rosemary sample heated intermittently, which was the highest level detected in that sample. The tallow containing 0.01% oleoresin rosemary and continuously heated contained a maximum of 7.3% cholesterol-α-epoxide after 72 hours.

The results obtained in this study are generally consistent with those of Park and Addis (1986a) in that the same four COP's (7α-hydroxycholesterol, 7β-hydroxycholesterol, 7-ketocholesterol and cholesterol-α-epoxide) were present and the 7-ketocholesterol was present in the greatest amount. However, unlike Park and Addis (1986a), linearity was not obtained for the formation of any of the COP's with time. This could be due to several factors. The initial tallow may have been dissimilar from that used by Park and

Addis (1986a). The cholesterol content of tallow has been reported to be as low as 705± 12 mg/kg (Park and Addis, 1986) and as high as 1620± 236 mg/kg (Bascoul et al., 1986). Rosemary may interact with the free radicals differently depending on the temperature stages, possibly even acting as a prooxidant at some times. This may be the case with the low level (0.01%) of rosemary, since the control tallow appeared to be slightly more stable when heated intemittently.

Cholesterol oxidation in commercial french fries

Frying fat darkens with increased use. The french fries that yielded the darkest fat also had higher fat contents. This indicates that used fat is absorbed by the french fries more readily than fresh fat. Variations in the color of the fat may be attributed to the addition of fresh fat to the fryers, changing of the fat in the fryers and length of use. The fat content of the french fries (measured from day 2 through day 7) ranged from 14.7 to 16.8%. This range is lower than that reported by Ryan (1982) who found 16.4 to 20.9%, but higher than that reported by Slover et al. (1980), which was 11.94 to 13.76%.

Figure 13 shows the cholesterol content of the fat extracted from the french fries. A comparison of the F-ratios from the analysis of the variance indicated a significant difference ($p < 0.01$) in the cholesterol content of the frying media from the two restaurants (Appendix V). It is possible that they have different tallow suppliers, since there was always less cholesterol present in the fat

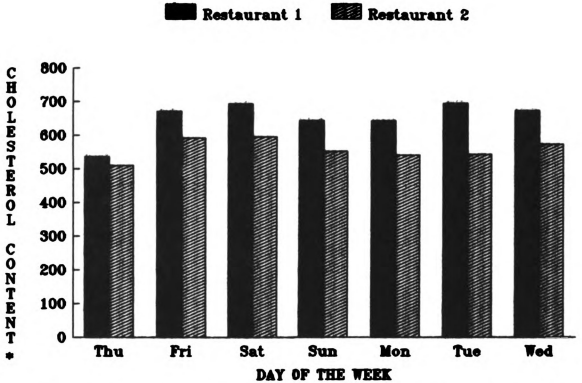


Figure 13. Cholesterol content in fat extracted from commercial french fries collected over a one week period. *Expressed as mg/kg fat.

samples from restaurant 2. There was a significant difference ($p < 0.05$) in cholesterol levels between at least two of the seven sampling days for each restaurant (Appendix V). Using Duncan's multiple range test for restaurant 1 it was determined that there was no significant difference between the cholesterol content in the fats extracted from the fries on days 2, 3 and 7 ($p < 0.05$). There was also no significant difference between days 4 and 5. This indicates that the condition of the frying media was similar on days 2, 3 and 7 and also on days 4 and 5. The days in between similar days possibly represent a replacement of the fat or addition of new fat. For restaurant 2 there was no significant difference between days 2, 3 and 7 and also between days 4, 5 and 6.

Figure 14 shows the total cholesterol oxidation products found in the fat extracted from the french fries. The F-ratio from the analysis of the variance showed that there was no significant difference ($p < 0.01$) in total COP's between restaurants 1 and 2 (Appendix VI). There was a significant difference in the content of cholesterol oxidation products between at least two of the seven sampling days for both restaurants ($p < 0.01$, Appendix VI). The Duncan multiple range test was used to determine which days were not significantly different from each other. The concentration of cholesterol oxidation products tended to be similar during the middle days and during the last two sampling days. This indicates that there was a lag time in formation of cholesterol oxidation products, such that the lipids and

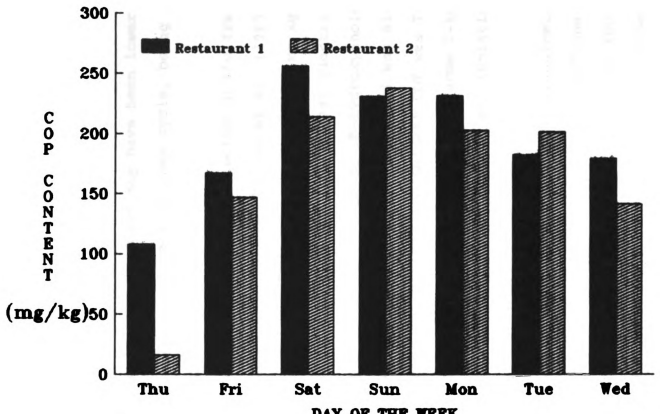


Figure 14. Total cholesterol oxidation products (COP's) in fat extracted from commercial french fries collected over a one week period.

cholesterol had to break down to a certain point before cholesterol oxidation products were formed. It is also possible that there was total replacement of the frying medium soon after the fat reached the stage where COP's were formed. When the frying media was replaced the total cholesterol oxidation products may have been lower than at other points during use because they cycle, being broken down and formed continuously.

The actual level of COP's detected in the french fries was higher than those reported by Lee et al. (1985) and Park and Addis (1985). Lee et al. found up to 81 mg/kg 7β-hydroxycholesterol (the COP in greatest quantity) and Park and Addis reported up to 58.8 mg/kg 7β-hydroxycholesterol. In this investigation 7β-hydroxycholesterol was also found in some of the samples, but the predominant COP was 7-ketocholesterol (Table 12). It is possible that some 7-ketocholesterol was formed during saponification and isolation, even though cold saponification was used.

Ryan et al. (1982) detected 7β-hydroxycholesterol, 3β-hydroxycholesterol, and 3,5-cholestadiene-7-one in the lipids extracted from french fries from a fast food franchise. The 3,5-cholestadiene-7-one could have been a degradation product of 7-ketocholesterol.

Table 12. Cholesterol oxidation products in fat extracted from commerciallt produced french fries collected over a period of one week.

	Restaurant 1			Restaurant 2		
	7-keto.	7β-OH	7-keto + 7β-OH	7-keto.	7β-OH	7-keto + 7β-OH
Day	(mg/kg)		(% of chol.)	(mg/kg)		(% of chol.)
1	108	0	20.2	138	16	30.3
2	167	17	27.4	147	15	27.4
3	256	0	36.9	214	0	35.8
4	226	5	35.9	238	0	43.2
5	228	10	37.0	170	0	31.5
6	176	7	30.8	202	2	37.6
7	170	10	26.7	142	0	24.7

OXIDATION OF CHOLESTEROL IN DAIRY PRODUCTS

The effects of various antioxidants and packaging materials on the oxidation of lipids and cholesterol in butter stored under fluorescent light

Lipid oxidation in butter

The effects of various packaging materials and antioxidants on the oxidative stability of butter stored under fluorescent lights were investigated.

Comparison of packaging materials

Light in the range of 300- 700 nm has been reported to cause oxidation in butter (Sattar and deMan, 1975), however no measurements of light intensity or wavelength distribution were made in this study. The effect of packaging materials on the stability of butter with added salt stored under fluorescent light was investigated. The results are shown in

Figure 15. As expected, aluminum foil prevented light exposure of the butter and in three replications there was no evidence of lipid oxidation in any of the foil-wrapped samples after 16 days of illumination. Similar results for samples wrapped in aluminum foil have been reported by Wilster (1957), Downey and Murphy (1968), Emmons et al. (1981) and Luby et al. (1986).

The peroxide values of the butter samples wrapped in margarine wrap were similar to those of the butter wrapped in polyethylene. At a wavelength of 300 nm, the percent light transmission for polyethylene is 88%, while that for margarine wrap is only 48% (Luby et al., 1986). However, at 400 nm, their light transmission is equal and at wavelengths above 400 nm margarine wrap has a slightly higher transmission than polyethylene. Therefore, it was not unexpected that the samples in margarine wrap and polyethylene would undergo similar levels of oxidation. Statistical analysis (Appendices VII and VIII) indicate that there was no significant difference between the overall means of the peroxide values of the butter in polyethylene and margarine wrap. A factor that could have influenced the rate of oxidation in the margarine wrap sample is water permeability, although this is unlikely since the samples were stored at 4°C.

The difference between the overall means of the peroxide values for foil and margarine wrap was 12.8± 3.2 while that for foil and polyethylene was 9.8± 3.2, ($p < 0.05$) (Appendix VIII). Therefore, the peroxide values of the butter samples

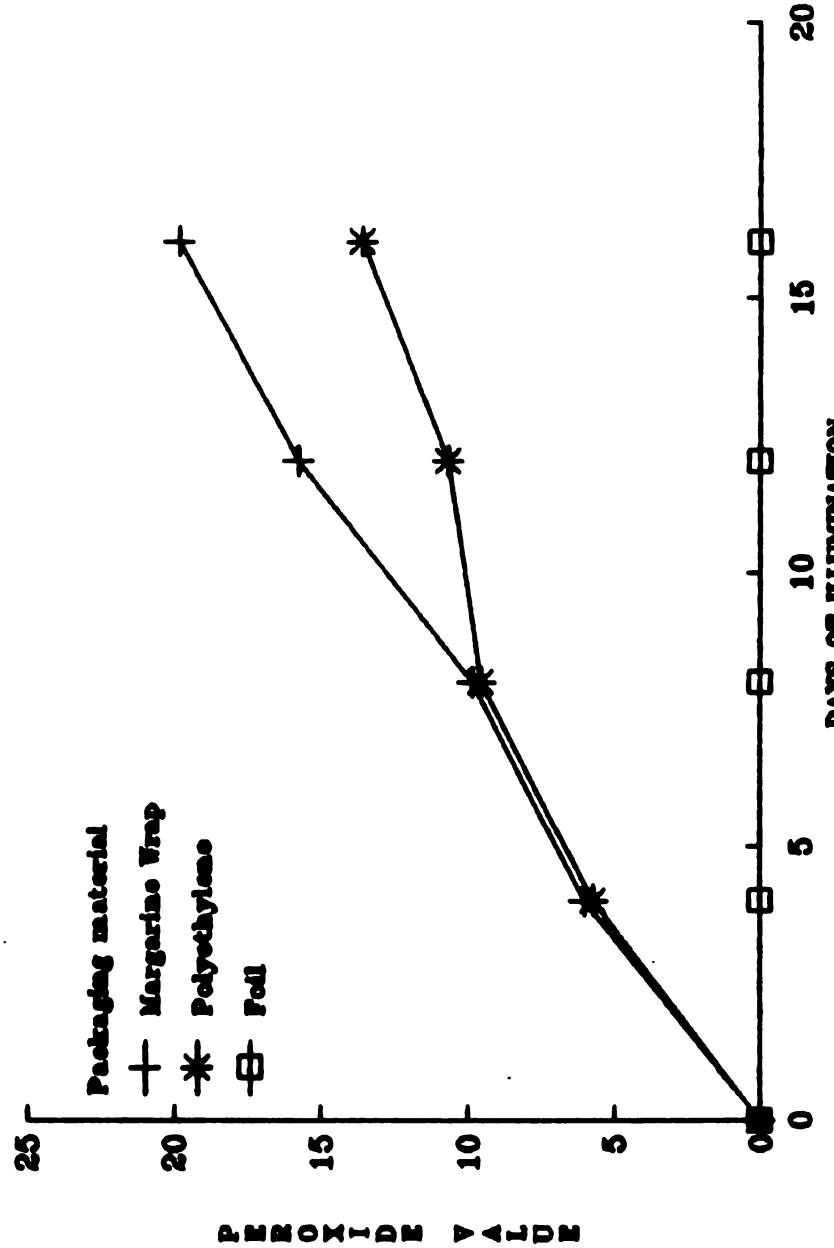


Figure 15. Effect of illumination on the peroxide value (meq/kg) of butter packaged in various materials.

in foil were significantly lower than those in both the margarine wrap and the polyethylene wrapped samples. Unpackaged butter stored under fluorescent lights was analyzed by Luby et al. (1986), and was reported to contain peroxides and cholesterol oxidation products.

Effect of antioxidants on lipid oxidation in butter

The effects of antioxidant addition on the stability of lipids was determined in butter which was packaged in polyethylene and exposed to fluorescent light (Figure 16). Two natural antioxidants (α-tocopherol and β-carotene) were compared to TBHQ (tertiary butylhydroxyquinone), a syntheic antioxidant. β-carotene significantly lowered the peroxide value, while α-tocopherol and tertiary butlyhydroquinone (TBHQ) did not (Figure 16, Appendix VIII).

TBHQ and α-tocopherol are phenols which function as free radical chain-breaking antioxidants. In photooxidation, β-carotene (Figure 17) is expected to be a more effective antioxidant than the free radical chain-breaking phenols because of its quenching effect on singlet oxygen (Trappel, 1980). It has been demonstrated that β-carotene minimizes the oxidation of soybean oil stored under light by quenching singlet oxygen (Lee and Min, 1988).

Cholesterol oxidation products in butter

Butter was analyzed for the presence of cholesterol oxidation products using thin layer chromatography and a capillary gas chromatography system with a detection limit of 0.2 mg/kg. However, no COP's were present in the butter samples. Several small peaks appeared on the GLC

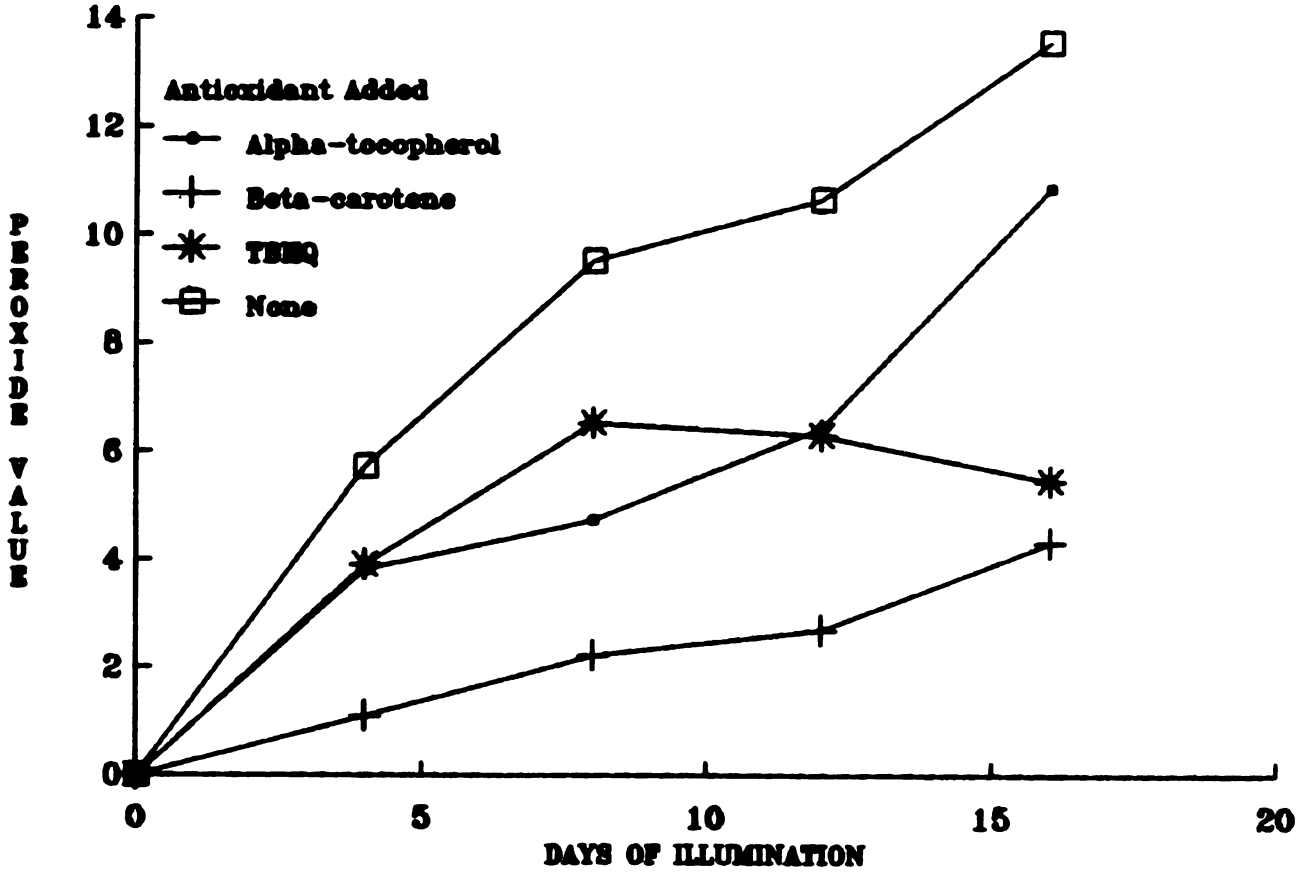


Figure 16. Effect of illumination on the peroxide value (meq/kg) of butter containing antioxidants.

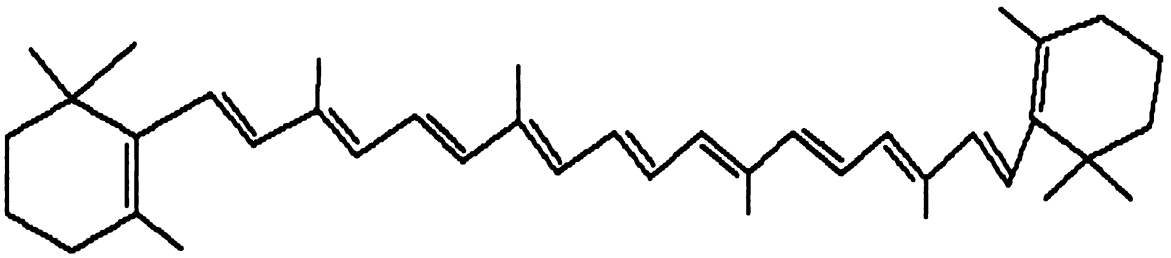


Figure 17. Structure of β-carotene.

chromatograms near the cholesterol peak in most of the samples, but did not have retention times corresponding to those of known COP standards.

Contrary to these results, Luby et al. (1986) detected 7α- and 7β-hydroxycholesterol in unpackaged butter after eight days of exposure to light. Two weeks of exposure to fluorescent light produced very low concentrations of compounds in some packaged butter samples that were tentatively identified as 7α- and 7β-hydroxycholesterol (Luby et al., 1986). The samples were packaged in polyethylene, margarine wrap, opaque parchment and wet strength dry wax paper. A possible explanation for the difference in the results of the present study and the results obtained by Luby et al. (1986) is that less oxidation of the lipids in the butter occurred in this study. Peroxides are the main initial products of lipid oxidation. The peroxide values of the butter when cholesterol oxidation products were first detected by Luby et al. ranged from 18.2 to 49.7 meq/kg after 10 to 15 days of exposure to fluorescent light. The peroxide

values in this study were between 4.2 and 19.7 meq/kg, after the same storage period. Of the ten samples that Luby et al. (1986) reported to contain COP's, only two of them had peroxide values below 20 when the COP's were first detected. Five of them had peroxide values greater than 36.

Lower peroxide values in this study indicated that conditions were not as favorable for oxidation. It would be expected that if peroxide formation was low, the rate of cholesterol oxidation would also be low. As the overall rate of lipid oxidation was lower in this case, there would be less free radicals formed that could initiate cholesterol oxidation.

Oxidation of cholesterol in powdered dairy products

Four commercial powdered dairy products (sour cream, cheddar cheese, butter and whole milk) of unknown history were analyzed for cholesterol oxidation products. The sour cream, butter and whole milk were all found to contain 7-ketocholesterol. The results are shown in Table 13.

Since powdered milk contains less cholesterol than powdered sour cream and butter, it would be expected to also contain fewer cholesterol oxidation products. The fact that the powdered cheddar cheese did not contain any COP's may have been due to the addition of an antioxidant by the processor.

These levels of 7-ketocholesterol have not been previously reported in powdered dairy products, but 3,5-cholestadien-7-one, a thermal degradation product of 7-ketocholesterol, was reported by Flanagan et al. (1975) in

Table 13. 7-Ketocholesterol content in powdered dairy products.

Product	7-Ketocholesterol (mg/kg)
Powdered Sour Cream	45
Powdered Cheddar Cheese	*ND
Powdered Butter	43
Powdered Milk	16

*ND- not detected

anhydrous milkfat. Cholesterol-α-epoxide, 7α- and 7β-hydroxycholesterol and the triol were reported by Finocchairo et al. (1984) in bleached butteroils and aged Italian cheese samples (Table 3).

It is possible that the oxidative conditions during the drying of the dairy products in this study were such that the initially formed 7α- and 7β-hydroperoxides were directly hydrated to form 7-ketocholesterol, rather than being reduced to the diols (Figure 2).

OXIDATION OF CHOLESTEROL IN EGG POWDER

Powdered egg samples prepared in Fermoy, Ireland were analyzed for cholesterol loss and the formation of cholesterol oxidation products during storage. A direct heating method of the drying air was used for half of the samples, while an indirect method was used for the other half. The directly heated air contained 7-8 mg/kg oxides of

nitrogen at the dryer inlet, while the indirectly-heated air contained less than 1 mg/kg. It is important to note that the indirectly-heated air was measured closer to the heating source than was the directly heated air which was measured away from the flame. Thus, the concentration of the oxides of nitrogen in the drying chamber would have been much lower for the indirectly heated system.

Cholesterol loss

There was a significant difference in cholesterol content ($p < 0.05$, analyzed in duplicate) between the sample dried with indirectly heated air and the sample dried with directly heated air (Table 14, Appendix X). Commercial spray-dried eggs have previously been reported to contain 1570± 130mg/kg cholesterol (Sugino et al., 1986). This higher level may have been due to a difference in moisture of the product, a difference in the biological make-up of the eggs used in this experiment, or a difference in methodology. After incubation at 50°C for 40 days, those investigators reported 1590± 140mg/kg cholesterol indicating experimental error or error in calculations.

The sample dried with directly heated air may have come into contact with oxides of nitrogen which are formed during the combustion of natural gas (Tsai and Hudson, 1985). Figure 5 shows some reactions that can occur between oxides of nitrogen and free radicals. The lower cholesterol content of the egg sample dried with indirect heat indicates that more cholesterol oxidation occurred in the sample dried with directly heated air.

Table 14. Cholesterol loss in spray-dried eggs after incubation for two weeks at 38°C.

Air heating method	Cholesterol content (mg/kg)	
	Before incubation	After incubation
Direct	8123	7575
Indirect	8874	8365

In this study, cholesterol was oxidized by the oxides of nitrogen in the directly heated air. In an investigation of dehydrated egg yolk, Tsai and Hudson (1985) reported concentrations of 8 to 311ppm total COP's in samples dried with directly heated air, while those dried with indirectly heated air contained 10ppm and less. After incubation the drop in cholesterol content was slightly greater in the sample dried with directly heated air than in the sample dried by indirectly heated air (6.7% versus 5.7%).

Cholesterol oxidation products

Several cholesterol oxidation products have been reported in spray-dried eggs and egg products. These include 7α- and 7β-hydroxycholesterol, 25-hydroxycholesterol, cholesterol-α-epoxide, cholesterol-β-epoxide, the triol and 7-ketocholesterol (Chicoye et al., 1968; Tsai and Hudson, 1984; Herian and Lee, 1985; Missler et al., 1985; Naber and Biggert, 1985; and Nourooz-Zadeh and Appelqvist, 1987). In the present study, no cholesterol oxidation products were detected before incubation of the spray-dried eggs, and

cholesterol-α-epoxide (187 mg/kg) was the only COP detected after two weeks of incubation at 38°C in the egg sample spray-dried with air heated directly. The egg sample spray-dried with indirectly heated air did not contain any COP's.

The concentration of cholesterol-α-epoxide was high, especially considering that none of the other common COP's were present on TLC plates or detected with GLC. Several investigators have reported the presence of cholesterol-α-epoxide in freshly spray-dried eggs. In freshly dehydrated commercial whole eggs and yolk powders Tsai et al. (1980) reported the range of cholesterol-α-epoxide to be from undetectable to 62 mg/kg. A concentration of 17.4± 1.9 mg/kg cholesterol-α-epoxide was reported by Sugino et al. (1986), 50 and 21.5mg/kg for eggs dried with directly and indirectly heated air respectively was reported by Missler et al. (1985) and a range of 1- 30 mg/kg was reported by Tsai and Hudson (1985). After storage for nine months Tsai and Hudson (1985) reported 33 mg/kg cholesterol-α-epoxide. After strorage for five months at ambiant temperature (22.5°C) Nourooz-Zadeh and Appelqvist (1987) reported 2.4 mg/kg cholesterol-α-epoxide in dehydrated yolk.

The particular conditions in this study may have been favorable for the formation of cholesterol-α-epoxides, while in most cases several different cholesterol oxidation products are formed. After incubation for 70 days at 50°C, Sugino et al. (1986) reported 31.9± 3.3 mg/kg cholesterol-α-epoxide in the spray-dried egg which previously contained

17.4± 1.9 mg/kg. They also detected cholesterol-β-epoxide, but their HPLC methodology was not specific for other oxidation products that were possibly present. The presence of cholesterol oxidation products prior to incubation may have altered the effect of the incubation compared to the present study, where cholesterol oxidation products were not detectable prior to incubation. The temperature used in the present study (38°C) was lower than that used by Sugino et al. (1986), and more likely to be similar to temperatures encountered in warehouse storage. Higher temperature may cause oxidation reactions to occur more randomly, producing a wider range of products. The specific conditions that existed during the spray-drying of the samples used by Sugino et al. were also unknown. Variations from the conditions used for this study may have caused oxidation to proceed differently.

Spray-dried egg yolk stored in closed plastic bags at 4°C for 12 months contained 2.5 mg/kg cholesterol-α-epoxide, as reported by Nourooz-Zadeh and Appelqvist (1987). Powdered scrambled egg mix contained 50 mg/kg cholesterol-α-epoxide (Missler et al., 1985).

None of the other investigators mentioned above reported the high levels of cholesterol-α-epoxide found in this study, however the total cholesterol oxidation products reported were often in the range of the 187 mg/kg cholesterol-α-epoxide reported here. Several samples of dehydrated yolk contained between 150 and 188 mg/kg total cholesterol oxidation products as reported by Tsai and Hudson (1985), and

one sample contained 311 mg/kg cholesterol oxidation products.

There was a small peak that eluted after cholesterol in the sample containing cholesterol-α-epoxide wich did not correspond to any of the standards available for this study. It was possibly 20α-hydroxycholesterol which Nourooz-Zadeh and Appelqvist (1987) reported in dehydrated egg yolk, or an artifactual cholesterol degradation product.

SUMMARY AND CONCLUSIONS

The oxidation of cholesterol in heated tallow, several dairy products, and spray-dried eggs was investigated. The effectiveness of oleoresin rosemary as an antioxidant in tallow was evaluated, as well as the efficacy of β-carotene, TBHQ and α-tocopherol as antioxidants in butter. Several packaging materials were tested for their ability to prevent oxidation caused by illumination of lipids and cholesterol in butter. A survey of powdered dairy products was also completed to identify COP's present and an indirect heating system was compared to direct air-heating methods for spray-drying eggs.

Analysis by TLC and GLC showed that no COP's were present in tallow and tallow containing oleoresin rosemary which was heated at 180°C for up to 240 hours. Tallow heated at 135°C did contain COP's and it was determined that the cholesterol content decreased with heating. Less cholesterol loss was observed in tallow containing 0.05% oleoresin rosemary than in the control tallow or tallow containing 0.01% oleoresin rosemary. The addition of 0.1% oleoresin rosemary did not provide any greater protection against cholesterol loss than the 0.05% level.

Continuous heating of the tallow caused more

cholesterol loss than intermittent heating. This observation was attributed to the duration of the heating times employed in the intermittent heating study. Several COP's were formed randomly in the tallow which were identifiable after 24 hours of heating. These COP's were identified by TLC and GLC as 7-ketocholesterol, 7α- and 7β-hydroxycholesterol, and cholesterol-α-epoxide. The predominant COP was 7-ketocholesterol.

Tallow containing 0.05% oleoresin rosemary contained less total COP's than the control tallow or tallow containing 0.01% oleoresin rosemary (analyzed every 24 hours) during the total 144 hours of continuous heating. In the intermittent heating experiment, there were generally less total COP's in the 0.05% oleoresin rosemary sample than in the control tallow samples.

Commercial french fries were obtained daily from two fast food restaurants on seven consecutive days to investigate the cholesterol and COP concentrations in the extracted fat extracted from them. There was a significant difference in the cholesterol contents of the extracted fats obtained from the two restaurants. This indicates that the two restaurants may not have had the same tallow suppliers, since the cholesterol content of the fat from restaurant 2 was always lower than that of the fat extracted from the french fries obtained from restaurant 1 on all seven days. The cholesterol content of the extracted fat also varied from day to day. There was a significant difference between at least two of the

seven sampling days for each restaurant.

The predominant COP in the fat extracted from the french fries was 7-ketocholesterol, indicating that initially formed 7α- and 7β-hydroperoxides were directly hydrated to form 7-ketocholesterol. Some samples also contained 7β-hydroxycholesterol. There was no significant difference in total COP's between the two restaurants.

The effects of various antioxidants and packaging materials on the oxidation of lipids, including cholesterol, in butter stored under fluorescent light at 4°C for 16 days were investigated. Analysis with TLC and GLC showed that none of the butter samples contained any COP's. Data obtained from peroxide values every four days revealed that aluminum foil effectively protected the butter from oxidation. Substantial oxidation occurred in butter packaged in margarine wrap and polyethylene.

β-carotene significantly lowered the peroxide value of butter wrapped in polyethylene exposed to fluorescent light, while α-tocopherol and TBHQ did not. In photooxidation, β-carotene has a quenching effect on singlet oxygen and is expected to be a more effective antioxidant than free radical chain-breaking phenols.

Four commercial powdered diary products (sour cream, cheddar cheese, butter and whole milk) of unknown history were analyzed for COP's. The powdered sour cream, butter and whole milk all contained 7-ketocholesterol. The powdered cheddar cheese did not contained any COP's

Cholesterol loss and formation of COP's were

investigated in two powdered egg samples prepared in Fermoy, Ireland that were incubated at 38°C for two weeks. One sample was spray-dried with indirectly heated air, while the other was spray-dried using directly heated air. After spray-drying, the direct-heat sample initially contained less cholesterol than the sample dried with indirectly heated air, possibly indicating that there was greater cholesterol oxidation in the direct-heat sample. However, this could be due to variability in the original eggs. There were no COP's in either sample prior to incubation. Cholesterol-α-epoxide was identified by TLC and GLC in the direct-heat sample after the incubation period.

Conclusions derived from this study are summarized as follows:

1) At extremely high temperatures, COP's are not formed in tallow or are broken down too quickly after formation to be detected.

2) With moderate heating, 7-ketocholesterol, 7α- and 7β-hydroxycholesterol and cholesterol-α-epoxide will form in tallow, 7-ketocholesterol being predominant.

3) Oleoresin rosemary (0.05%) is an effective antioxidant for reducing cholesterol oxidation and COP formation in heated tallow.

4) Commercial french fries may contain 7-ketocholesterol and 7β-hydroxycholesterol, 7-ketocholesterol being predominant.

5) The concentration of COP's in french fries cannot

be correlated to the concentration of cholesterol.

6) Packaging materials that prevent light transmission effectively reduces the extent of lipid oxidation occurring in butter. β-carotene also slows down the oxidation process.

7) Cholesterol oxidation due to illumination does not easily occur in butter and would not be expected under normal conditions of production, distribution and storage of butter.

8) Direct heating of air for spray-drying eggs is more likely to cause cholesterol oxidation than indirect heat. Cholesterol-α-epoxide, which was present in eggs dried with directly heated air after incubation, has been implicated as having carcinogenic properties.

PROPOSALS FOR FURTHER RESEARCH

Since this study was first initiated, several questions have been raised that may be worthy of further research.

1) An investigation of the relationship between the extent of lipid oxidation in butter measured by peroxide value and the extent of cholesterol oxidation.

2) The analysis of the effectiveness of β-carotene as an antioxidant in products other than butter which are exposed to illumination.

3) Acceptance tests of the sensory qualities of foods fried in tallow containing oleoresin rosemary.

4) A concentrated study of the effects of continuous versus intermittent heating of frying media.

5) Cholesterol oxidation products appeared to be formed erratically in french fry oil. An investigation of frying temperature, frying time, and fry load may provide insight to the cause.

6) Further work needs to be done to establish components of cheddar cheese and other dairy products which may initiate or retard cholesterol oxidation.

7) Cholesterol oxidation could be monitored in spray-dried eggs under various storage and packaging since literature currently offers conflicting observations

8) Continuing medical research must attempt to define the relationship between cholesterol, diet, exercise, cardiovascular disease and cholesterol oxidation products.

APPENDICES

Appendix I

Analysis of the variance for the effect of oleoresin rosemary (OR) on cholesterol loss in heated tallow.

A. Continuously heated tallow

Source	Sums of squares	d.f.	Mean square	F ratio
OR	27234.4	2	13617.2	18.8**
Time	255246.5	5	51049.3	70.5**
Interaction	19011.8	10	1901.2	2.6*
Residual	13040.0	18	724.4	

B. Intermittenly heated tallow

Source	Sums of squares	d.f.	Mean square	F ratio
OR	42592.0	2	21296.0	46.7**
Time	215810.1	5	43162.0	94.6**
Interaction	22396.0	10	2239.6	4.9**
Residual	8210.5	18	456.1	

Critical F values:

	$\alpha = 0.05$	$\alpha = 0.01$
(2, 18)	3.6	6.0
(5, 18)	2.8	4.3
(10, 18)	2.4	3.5

*Indicates significance at $\alpha = 0.05$

**Indicates significance at $\alpha = 0.01$

Appendix II

Analysis of the variance for the effect of continuous versus intermittent heating on cholesterol loss in tallow.

A. Pure tallow

Source	Sum of squares	d.f.	Mean square	F ratio
Heating method	15453.4	1	15453.4	23.2**
Residual	800.8	12	667.3	

B. Tallow containing 0.01% oleoresin rosemary

Source	Sum of squares	d.f.	Mean square	F ratio
Heating method	570.4	1	570.4	.94
Residual	7244.5	12	603.7	

C. Tallow containing 0.05% oleoresin rosemary

Source	Sum of squares	d.f.	Mean square	Fratio
Heating method	10375.0	1	10375.0	20.8**
Residual	5998.5	12	499.9	

Critical F values:

	$\alpha = 0.05$	$\alpha = 0.01$
(1, 12)	4.8	9.3

Appendix III

Tukey multiple comparison of the means for cholesterol loss in continuous versus intermittently heated tallow.

A. Pure tallow

$1 - \alpha = 0.90$	$1 - \alpha = 0.95$
50.7± 37.2 mg/kg	50.7± 41.7 mg/kg

B. Tallow containing 0.05% oleoresin rosemary

$1 - \alpha = 0.90$	$1 - \alpha = 0.95$
41.5± 32.1 mg/kg	41.5± 36.1 mg/kg

Appendix IV

Tukey multiple comparison of the means for cholesterol loss in heated tallow.

A. Intermittently heated tallow

	$1 - \alpha = 0.90$	$1 - \alpha = 0.95$
0.01% OR: control:	28.5± 3.4 mg/kg	28.5± 3.7 mg/kg
0.05% OR: control:	82.9± 3.4 mg/kg	82.4± 3.7 mg/kg
0.05% OR: 0.01% OR	54.4± 3.4 mg/kg	54.4± 3.7 mg/kg

B. Continuously heated tallow

	$1 - \alpha = 0.90$	$1 - \alpha = 0.95$
0.01% OR: control:	12.4± 3.4 mg/kg	12.4± 3.7 mg/kg
0.05% OR: control:	63.6± 3.4 mg/kg	63.6± 3.7 mg/kg
0.05% OR: 0.01% OR	51.2± 3.4 mg/kg	51.2± 3.7 mg/kg

Appendix V

Analysis of the variance for the cholesterol content of oil extracted from french fries obtained from two fast food restaurants over seven consecutive days.

Source	Sum of squares	d.f.	Mean square	F ratio
Restaurant	21607.1	1	21607.1	51.0**
Day	21506.4	6	3584.4	8.5*
Residual	2543.9	6	424.0	

Critical F values

	$\alpha = 0.05$	$\alpha = 0.01$
(1, 13)	6.0	13.7
(6, 13)	4.3	8.5

Duncan's Multiple Range Test*

Restaurant 1	Restaurant 2
Day	Day
1c	1c
2a	2a
3a	3a
4b	4b
5b	5b
6d	6b
7a	7a

*Days with the same letter were not significantly different from each other.

Appendix VI

Analysis of the variance for the total cholesterol oxidation products in oil extracted from french fries obtained from two fast food restaurants over seven consecutive days.

Source	Sum of squares	d.f.	Mean square	F ratio
Restaurant	2716.1	1	2716.1	4.1
Day	44743.4	6	7457.2	11.3**
Residual	3945.4	6	657.6	

Critical F values

	$\alpha = 0.05$	$\alpha = 0.01$
(1, 13)	6.0	13.7
(6, 13)	4.3	8.5

Duncan's Multiple Range Test*

Restaurant 1	Restaurant 2
1c	1d
2a	2a
3b	3b,c
4b	4b
5b	5c
6a	6c
7a	7a

*Days with the same letter were not significantly different from each other.

Appendix VII

Analysis of the variance for the effect of antioxidants and packaging materials on the peroxide values of butter stored under fluorescent light.

A. Antioxidants

Source	Sum of squares	d.f.	Mean square	F ratio
Time	155.0	3	51.7	12.3**
Antioxidants	365.8	3	121.9	29.0**
Interaction	186.2	9	20.7	4.9**
Residual	133.0	32	4.2	

Critical F values

	$\alpha = 0.1$	$\alpha = 0.05$
(3, 32)	2.28	2.92
(9, 32)	1.84	2.20

B. Packaging materials

Source	Sum of squares	d.f.	Mean square	F ratio
Time	337.3	3	112.4	21.6**
Packaging	1075.6	2	537.8	103.4**
Interaction	171.8	6	28.6	5.5**
Residual	124.3	24	5.2	

Critical F values

	$\alpha = 0.1$	$\alpha = 0.05$
(3, 24)	2.3	3.0
(2, 24)	2.5	3.4
(6, 24)	2.0	2.5

**Indicates significance at $\alpha = 0.1$ and $\alpha = 0.05$.

Appendix VIII

Tukey multiple comparison of means of the peroxide values of butter with various antioxidants and packaging materials.

A. Antioxidants

	$1 - \alpha = 0.90$	$1 - \alpha = 0.95$
α-tocopherol: β-carotene	3.9± 3.2 meq/kg	3.9± 3.4 meq/kg
α-tocopherol: TBHQ	0.9± 3.2 meq/kg	0.9± 3.4 meq/kg
α-tocopherol: salt only	2.8± 3.2 meq/kg	2.8± 3.4 meq/kg
β-carotene: TBHQ	3.0± 3.2 meq/kg	3.0± 3.4 meq/kg
β-carotene: salt only	6.7± 3.2 meq/kg	6.7± 3.4 meq/kg
TBHQ: salt only	3.7± 3.2 meq/kg	3.7± 3.4 meq/kg

B. Packaging materials

	$1 - \alpha = 0.90$	$1 - \alpha = 0.95$
Foil: margarine wrap	12.8± 2.9	12.8± 3.2
Foil: polyethylene	9.8± 2.9	9.8± 3.2
Margarine wrap: polyethylene	3.0± 2.9	3.0± 3.2

Appendix IX

Equations for Tukey's multiple comparison of treatment means.

$$D \pm Ts(D) \qquad s = \sqrt{\frac{2MSE}{n}}$$

$$D = \overline{Yi'j'} - Yi'j' \qquad T = \frac{1}{\sqrt{2}} q[1 - \alpha; ab, (n - 1)ab]$$

i = level of factor A

j = level of factor B

a = number of levels of factor A

b = number of levels of factor B

ab = total number of possible treatments

n = number of units receiving a given treatment (all being equal)

Appendix X

Analysis of the variance for the effects on cholesterol content of eggs spray-dried with directly or indirectly heated air, and after two weeks of incubation at 38°C.

Source	Sum of squares	d.f.	Mean square	F ratio
Drying method	593670.3	1	573670.3	1561.3*
Incubation	279312.3	1	279312.3	734.6*
Residual	380.3	1	380.3	

Critical F values

	$\alpha = 0.05$	$\alpha = 0.01$
(1, 3)	161.0	4052

REFERENCES

Addis, P.B. 1983. Some lipid oxidation products as xenobiotics. ACS Symposium Series 254. J.W. Finley, Ed.

Addis, P.B. 1986. Occurence of lipid oxidation products in foods. Fd. Chem. Toxic. 24:1021.

Ansari, G.A.S. and Smith, L.L. 1979. High performance liquid chromatography of autoxidation products. J. Chromatog. 175:307

Baeuerlen, R., Brody, H. and Erickson, D. 1968. Frying fats and their uses. Bakers Digest 12:51

Barbut, S., Josephson, D. and Maurer, A.J. 1985. Antioxidant properties of rosemary oleoresin in turkey sausage. J. Food Sci. 50:1356.

Bascoul, J., Domerque, N. and Crastes de Paulet, A. 1983. Preparation of autoxidized cholesterol mixtures from (4-^{14}C) cholesterol and cholesteryl fatty acid esters. J. Steroid Biochem. 19:1779.

Bascoul, J., Domerque, N., Olle, M. and Crastes de Paulet, A. 1986. Autoxidation of cholesterol in tallows heated under deep frying conditions: evaluation of oxysterols by GLC and TLC-FID. Lipids 21:383.

Berner, D.L. and Jacobson, G.A. 1973 U.S. patent 3,732,111.

Black, H.S. and Lo, W.-B. 1971. Formation of a carcinogen in human skin irradiated with ultraviolet light. Nature 234:306.

Bracco, U., Loliger, J. and Viret, J.L. 1981. Production and uses of natural antioxidants. J. Amer. Oil Chem. Soc. 58:686.

Brun, J.P., Burt, R. and Nicols, B.W. 1985. "Role of Fats in Human Nutrition", F.B. Padley and J. Podmore, Eds. Ellis Horwood Ltd. Chichester, England.

Burton, G.W. and Ingold, K.U. 1984. β-Carotene: An unusual type of lipid andtioxidant. Science 224:569.

Chang, S.S., Biserka, O.M., Hsieh, O.A.L., Huang, C.L. 1977. Natural antioxidants from rosemary and sage. J. Food Sci. 42:1102.

Chicoye, E., Powrie, W.D. and Fennema, O. 1968a. Isolation and characterization of cholesterol-5β,6β-oxide from an aerated aqueous dispersion of cholesterol. Lipids 3:335.

Chicoye, E., Powrie, W.D. and Fennema, O. 1968b. Photoxidation of cholesterol in spray-dried egg yolk upon irradiation. J. Food Sci. 33:581.

Chipault, J.R., Mizuno, G.R., Hawkins, J.M. and Lundberg, W.O. 1952. The antioxidant properties of natural spices. Food Res. 17:46.

Csiky, I. 1982. Trace enrichment and separation of cholesterol oxidation products by adsorption high performance liquid chromatography. J. Chromatog. 241:381.

DeFouw, C., Zabik, M.E. and Gray, J.I. 1981. Fractionated beef tallow as a deep fat frying medium for french fries. J. Food Sci. 46:452.

Draper, H.H. 1980. Nutrient interrelationships. In "Vitamin E- A Comprehensive Treatise", Part 5D. L.J. Machlin, Ed. In: "Basic and Clinical Nutrition", Marcel Dekker, Inc, New York, Vol. 1, pp. 272-288.

Fieser, L.F. 1953a. Cholesterol and companions III. Cholestanol, lathosterol and ketone 104. J. Am. Chem. Soc. 75:4395.

Fieser, L.F. 1953b. Cholesterol and companions IV. Steroid dibromides. J. Am. Chem. Soc. 75:5422.

Finocchiaro, E.T., Lee, K. and Richardson, T. 1984. Identification and quantitation of cholesterol oxides in grated cheese and bleached butteroil. J. Am. Oil Chem. Soc. 61(5):877.

Finocchiaro, E.T. and Richardson, T. 1983. Sterol oxides in foodstuffs: a review. J. Food Prot. 46(10):917.

Flanagan, V.P., Ferretti, A., Schwartz, D.P. and Ruth, J.M. 1975. Characterization of 2 steroidal ketones and 2 isoprenoid alcohols in dairy products. J. Lipid Res. 16:97.

Foote, C.S. 1976. Photosensitized oxidation and singlet oxygen: consequences in bioloical systems. Ch. 3. In "Free Radicals in Biology", Vol. II, W.A. Pryor, Ed. Academic Press, New York.

Fritsch, C.W. 1981. Measurements of frying fat deterioration: A brief review. J. Am. Oil Chem. Soc. 58(3):272.

Fritsch, C.W., Egberg, D.C. and Magnuson, J.S. 1979. Changes in dielectic constant a measure of frying oil deterioration. J. Am. Oil Chem. Soc. 56(8):746.

Gemert, L.J. van, Hoekman, H. 1986. Effects of type and duration of use of frying fat on the sensory quality of croquettes and french fried potatos. Voedingsmiddelentechnologie 19(1):28.

Gibbons, G.F., Mitropoulos, K.A. and Myant, N.B. 1982. "Biochemistry of Cholesterol". Elsevier Biomedical Press, New York.

Gray, J.I. 1978. Measurement of lipid oxidation: A review. J. Am. Oil Chem. Soc. 55:539.

Gray, M.F., Lawrie, T.D.V. and Brooks, C.F.W. 1971. Isolation and identification of cholesterol-α-epoxide and other minor sterols in human serum. Lipids 6:836

Gumulka, J., St. Pyrek, J. and Smith, L.L. 1982. Interception of discrete oxygen species in aqueous media by cholesterol: Formation of cholesterol epoxides and secosterols. Lipids 17(3):197.

Haumann, B.F. 1987. Fast foods: trends in frying fat usage. J. Am. Oil Chem. Soc. 64(6):789.

Herian, A.M. and Lee, K. 1985. 7α- and 7β-hydroxycholesterols formed in a dry egg nog mix exposed to fluorescent light. J. Food Sci. 50:276.

Higley, N.A. and Taylor, S.L. 1986a. Cholesterol oxides in processed meats. Meat Sci. 16:175.

Higley, N.A., Beery, J.T., Taylor, S.L., Porter, J.W., Dziuba, J.A. and Lalich, J.J. 1986b. Comparative atherogenic effects of cholesterol and cholesterol oxides. In "Atherosclerosis". Elsevier Scientific Publishers, Ireland, Ltd. pp. 91-104.

Horvath, C. 1966. Quantitative determination of cholesterol in autoxidation mixtures by thin-layer chromatography. J. Chromatog. 22:52.

Houlihan, C.M., Ho, C.-T. and Chang, S.S. 1984. Elucidation of the chemical structure of a novel antioxidant, rosmaridiphenol, isolated from rosemary. J. Am. Oil. Chem. Soc. 61(6):1036.

Houlihan, C.M., Ho, C.T. and Chang, S.S. 1985. The structure of rosemariquinone- a new antioxidant- isolated from Rosmarinus officinalis L. J. Am Oil Chem. Soc. 62(1):96.

Hurst, W.J., Aleo, M.D. and Marir, R.A., Jr. High performance liquid chromatographic analysis of cholesterol in milk. J. Dairy Sci. 66:2192.

Igene, J.O., King, J.A., Pearson, A.M. and Gray, J.I. 1979. Influence of heme pigments, nitrite, and non-heme iron on development of warmed-over flavor (WOF) in cooked meat. J. Agric. Food Chem. 27(4):838.

Imai, H., Wertheson, N.T., Subramanyam, V., LeQuesne, P.W., Soloway, A. and Kanisawa, M. 1980. Angiotoxicity of oxygenated sterols and possible precursors. Science 207(2):651.

Inatani, R., Nakatani, N. and Fuwa, H. 1983. Antioxidant effect of the constituents of rosemary (Rosmarinus officinalis L.) and their derivatives. Agric. Biol. Chem. 47(3):521.

Kandutsch, A., Chen, H.W. and Heiniger, H.J. 1978. Biological activity of some oxygenated sterols. Science 201:498.

Kimura, M., Jin, Y. and Sawaya, T. 1979. Autoxidation of cholesterol and behavior of its hydroperoxide in aqueous medium. Chem. Pharm. Bull. (Tokyo) 27:710.

Korahani, V. Bascoul, J. and Crastes de Paulet, A. 1981. Capillary column gas-liquid chromatographic analysis of cholesterol derivatives. Application to the autoxidation products of cholesterol. 1981. J. Chromatog. 211:392.

Korahani, V., Bascoul, J. and Crastes de Paulet, A. 1982. Autoxidation of fatty acid esters in solid state and aqueous dispersion. Lipids 17:703.

Kou, I.L. and Holmes, R.P. 1985. The analysis of 25-hydroxycholesterol in plasma and cholesterol containing foods by high performance liquid chromatography. J. Chromatog. 330:339.

Krull, U.J., Thompson, M. and Arya, A. 1984. Chromatographic analysis of phospholipid and cholesterol oxidation. Talanta 31:489.

Lee, E.C. and Min, D.B. 1988. Quenching mechanism of β-carotene on the chlorophyll sensitized photooxidation of soybean oil. J. Food Sci. 53(6):1894.

Lee, K., Herian, A.M. and Higley, N.A. 1985. Sterol oxidaiton products in french fries and stored potato chips. J. Food Prot. 48(2):158.

Livingston, R. 1961. Photochemical autoxidation. In: "Autoxidation and Antioxidants", W.O. Lundberg, Ed., Interscience Publishers, John Wiley and Sons, New York, Vol. 1, pp. 249-298.

Love, J.D. and Pearson, A.M. Netmyoglobin and nonheme iron as prooxidants in egg-yolk phospholipid dispersions and cooked meat. J. Agric. Food Chem. 24:494.

Luby, J.M. 1982. Photooxidation of cholesterol in butter. M.S. Thesis, Michigan State University, East Lansing, MI.

Luby, J.M., Gray, J.I., Harte, B.R. and Ryan, T.C. 1986. Effects of packaging and light source on the oxidative stability of cholesterol in butter. J. Food Sci. 51:904.

MacNeil, J.H., Dimick, P.S. and Mast, M.G. 1973. Use of chemical compounds and a rosemary spice extract in quality maintenance of deboned poultry meat. J. Food Sci. 38:1080.

Maerker, G. and Unruh, J. Jr. 1986a. Cholesterol oxides I. Isolation and determination of some cholesterol oxidation products. J. Am. Oil Chem. Soc. 63:767.

Maerker, G. and Bunick, F.J. 1986b. Cholesterol oxides II. Measurement of the 5,6-epoxides during cholesterol oxidation in aqueous dispersions. J. Am. Oil Chem. Soc. 63(6):771.

Maerker, G. 1987. Cholesterol autoxidation- current status. J. Am. Oil Chem. Soc. 64(3):388.

McCay, P.B. and King, M.M. 1980. Biochemical funtion. In "Vitamin E- A Comprehensive Treatise", L.J. Machlin, Ed. Part 5E. In "Basic Clinical Nutrition", Marcel Dekker, Inc. New York. Vol. 1. pp. 289-317.

McCleur, R.H. and Jungalwala, F.B. 1976. High performance liquid chromatographic analysis of glycossphingolipids and phospholipids. Adv. Exp. Med. Biol. 76:533.

McGill, E.A. 1980. The chemistry of frying. Baker's Digest 6:38.

Missler, S.R., Wasilchuh, B.A. and Merrit, C. Jr. 1985. Separation and identification of cholesterol oxidation products in dried egg preparations. J. Food Sci. 50:595.

Naber, E.C. and Biggert, M.D. 1983. Analysis for and generation of cholesterol oxidation products in egg yolk by heat treatment. Poultry Sci. 64:341.

Nourooz-zadeh, J. and Appelqvist, L.A. 1987. Cholesterol oxides in Swedish foods and food ingredients: Fresh eggs and dehydrated egg products. J. Food Sci. 52(1):57.

Park, S.W. and Addis, P.B. 1985a. Capillary column gas-liquid chromatographic resolution of oxidized cholesterol derivatives. Anal. Biochem. 149(3):275.

Park, S.W. and Addis, P.B. 1985b. HPLC determination of C-7 oxidized derivatives in foods. J. Food Sci. 50:1437.

Park, S.W. and Addis, P.B. 1986a. Further investigation of oxidized cholesterol derivatives in heated fats. J. Food Sci. 51(5):1380.

Park, S.W. and Addis, P.B. 1986b. Identification and quantitative estimation of oxidized cholesterol derivatives in heated tallow. J. Agric. Food Chem. 34(4):653.

Park, S.W. and Addis, P.B. 1987. Cholesterol oxidation products in some muscle foods. J. Food Sci. 52(6):1500.

Parsons, P.G. and Goss, P. 1978. Chromosome damage and DNA repair induced in human fibroblasts by UV and cholesterol oxide. Aust. J. Exp. Biol. Med. Sci. 56(3):287.

Pearson, A.M., Gray, J.I., Wolzak, A.M. and Horenstein, N.A. 1983. Safety implications of oxidized lipids in muscle foods. Food Technol. 37:121.

Peng, S.K. and Taylor, C.B. 1984. Cholesterol autoxidation, health and arteriosclerosis. Wld. Rev. Nutr. Diet. 44:117.

Petrakis, N.L., Gruenke, L.D., Craig, J.C. 1981 Cancer Res. 41:2563.

Pryor, W.A. and Lightsey, J.W. 1981. Mechanisms of nitrogen dioxide reactions: initiation of lipid peroxidation and the production of nitrous acid. Science 214(10):435.

Ryan, T.C. 1982. Oxidation of cholesterol in heated tallow. M.S. Thesis, Michigan State University, East Lansing, MI.

Ryan, T.C., Gray, J.I. and Morton, I.D. 1981. Oxidation of cholesterol in heated tallow. J. Sci. Food Agric. 32:305.

Sabine, J.R. 1977. "Cholesterol". Marcel Dekker Inc., New York.

Sato, K. and Hegarty, G.R. 1971. Warmed-over flavor in cooked meats. J. Food Sci. 36:1098.

Schenk, G.O. and Koch, E. 1960. Elektrochem. 64:170.

Sevanian, A., Mead, J.F. and Stein, R.A. 1979. Epoxides as products of lipid autoxidaiton in rat lungs. Lipids 14:634.

Shen, C.S.J. and Sheppard, A.J. 1983. High performance liquid chromatography of some biologically important cholesterol oxides. Chromatographia 17(9):469.

Smith, L.L., Smart, V.B. and Ansari, G.A.S. 1979. Mutagenic cholesterol preparations. Mut. Res. 68:23.

Smith, L.L. and VanLier, J.E. 1970. Sterol metabolism-part 9. 26-Hydroxycholesterol levels in the human aorta. Atherosclerosis 12:1.

Smith, L.L. 1981. "Cholesterol Autoxidation". Plenum Press, New York.

Smith, L.M., Clifford, A.J., Hamblin, C.L. and Creveling, R.K. 1986. Changes in physical and chemical properties of shortenings used for commercial deep-fat frying. J. Am. Oil Chem. Soc. 63(8):1017.

Smith, L.L., Kulig, M.J. 1975. On the derivation of carcinogenic sterols from cholesterol. Cancer Biochem. Biophys. 1:79.

Sugino, K., Terao, J., Murakami, H. and Matsushita, S. 1986. High performance liquid chromatographic method for the quantification of cholesterol epoxides in spray-dried egg. J. Agric. Food Chem. 34(1):37.

Taylor, C.B., Peng, S.K., Werthessen, N.T., Tham, P. and Lee, K.T. 1979. Spontaneously occuring angiotoxic derivatives of cholesterol. Am. J. Clin. Nutri. 32(1):40.

Teng, J.I., Kulig, M.J. and Smith, L.L. 1973. Sterol metabolism. XXII gas chromatographic differentiation among cholesterol hydroperoxides. J. Chromatog. 75:108.

Tsai, L.S., Ijichi, K., Hudson, C.A. and Meehan, J.J. 1980. A method for the quantitative estimation of cholesterol α-oxide in eggs. Lipids 15:124.

Tsai, L.S. and Hudson, C.A. 1981. HPLC of oxygenated cholesterols and related compounds. J. Am. Oil Chem. Soc. 58(10):931.

Tsai, L.S. and Hudson, C.A. 1984. Cholesterol oxides in commercial dry egg products: Isolation and Identification. J. Food Sci. 49:1245.

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

Van Lier, J.E. and Smith, L.L. 1968. Sterol metabolism II. Gas chromatographic recognition of cholesterol metabolites and artifacts. Anal. Biochem. 24:419.

Van Lier, J.E. and Smith, L.L. 1970. Sterol metabolism XI. Thermal decomposition of some cholesterol hydroperoxides. Steroids 15(4):485.

Weiss, T.J. 1983. "Food Oils and Their Uses". Avi Publishing Company, Inc., Westport, Conn.

Wu, J.W., Lee, M.H., Ho, C.T. and Chang, S. Elucidation of the chemical structures of natural antioxidant isolated from rosemary. J. Am. Oil Chem. Soc. 59(8):339.