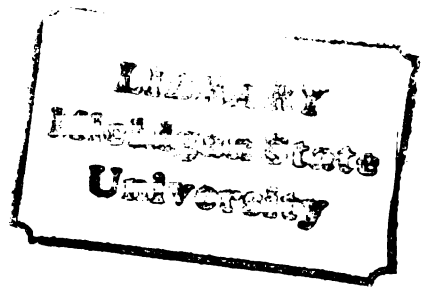


This is to certify that the

thesis entitled

Oxidation of Cholesterol in Heated Tallow

presented by

Thomas Clavin Ryan

has been accepted towards fulfillment
of the requirements for

Masters degree in Food Science

J. I. Gray
Major professor

Date 2/23/82

OXIDATION OF CHOLESTEROL IN HEATED TALLOW

By

Thomas Clavin Ryan

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Food Science and Human Nutrition

1982

ABSTRACT

Oxidation of Cholesterol in Heated Tallow

By

Thomas Clavin Ryan

The oxidative stability of tallow cholesterol and triglycerides was investigated under various processing conditions including heat cycling, frying and add-back procedures. Intermittent heating was found to be more damaging to tallow constituents than was continuous heating. French fried potatoes heated in tallow exhibited a preferential absorption for cholesterol and cholesterol oxidation products and slowed down the rate of tallow degradation. Cholesterol oxidation products isolated from heated tallow systems were identified by chromatographic means as 5-cholesten-3β, 7α-diol, 5-cholesten-3β,7β-diol, 5-cholesten 3β -ol and 3,5-cholestadiene-7-one, with trace amounts of 5-cholestan-3β ,5α , 6 β-triol observed.

Analysis of french fried potatoes obtained from a fast-food franchise utilizing tallow as a frying medium revealed the presence of 5-cholesten-3β,7α -diol, 5-cholesten-3β , 7β -diol, 5-cholesten-3β -ol, and 3,5-cholestadiene-7-one.

Results of this study indicate that cholesterol readily oxidizes under frying conditions and that oxidation products are preferentially absorbed by foods fried therein. Several cholesterol oxidation products were found in commercially prepared fried foods.

To my family, Mr. and Mrs. Thomas J. Ryan, Debra L. Ryan-Smith, Cynthia M. Ryan and Timothy K. Ryan

ACKNOWLEDGMENTS

The author wishes to express his sincere gratitude and appreciation to Dr. J.I. Gray for his unlimited input as research chairman and major professor during the course of this study. The patience, encouragement, and insight contributed by Dr. Gray was invaluable in the completion of this thesis.

Appreciation and gratitude are also extended to the members of the guidance committee, Dr. L.R. Dugan, Dr. A.M. Pearson, Dr. M.E. Zabik and Dr. B.R. Harte for their critical review of this dissertation.

The author extends his heartfelt gratitude and admiration to Dr. I.D. Morton, Chairman, Department of Food Science, Queen Elizabeth College, London, for the critical role he played in the initiation of this study and the professional influence which he left on his return to England.

The author is also indebted to several individuals for their assistance in this research, including Arun Mandagere and Susan Cuppett for their help in the various areas of instrumentation employed, and Julie Luby for her aid in various procedures and the literature search.

The author also wishes to acknowledge Mrs. Loraine Stephens for her assistance in typing this thesis.

Last, the author is especially appreciative to Teresa Kalil, for her support and understanding during the completion of this thesis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figures Page

INTRODUCTION

Recent studies have indicated that when cholesterol is heated in air it readily undergoes oxidation to form products which may be angiotoxic (Imai et al. 1976; Peng et al., 1978) and/or carcinogenic (Smith and Kulig, 1975; Bischoff, 1969). Cholesterol is present in food systems of animal origin, many of which are subjected to conditions which may be conducive to the oxidation of cholesterol. Beef tallow, containing 0.15 to 0.20% cholesterol by weight (Punwar and Derse, 1978) is commonly used as a frying medium in commercial frying operations. The elevated temperature over the extended periods of time to which tallow is subjected may be compatible with the formation of oxidation products from cholesterol.

In addition to the oxidation of cholesterol under frying conditions, tallow triglycerides undergo autoxidation, thermal oxidative, thermal polymerization, and hydrolytic changes (Porkorny, 1980; Perkins, 1967). The products of these reactions have been suspected of introducing potentially hazardous compounds into the diet via fried foods, as evidenced by an overwhelming number of studies reviewed by Artman (1969).

The major objective of this study was to evaluate the oxidative changes in tallow triglycerides and cholesterol during exposure to frying conditions. Processing parameters such as temperature cycling, frying, and add-back procedures were utilized to monitor their effects on the oxidation of the tallow system. Various physical and chemical analysis and chromatographic techniques were employed to assess the oxidative changes in tallow constituents exposed to the various processing parameters.

A second objective of this study was to determine the presence of cholesterol oxidation products in commercially processed foods, such as french fried potatoes.

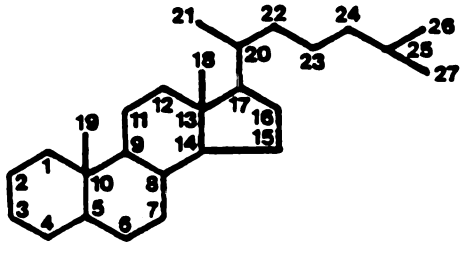
REVIEW OF LITERATURE

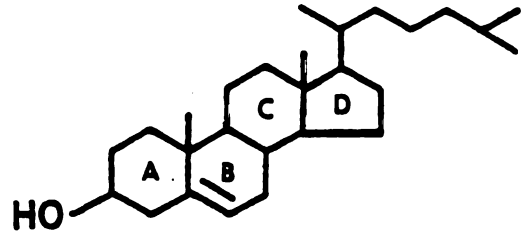
Heated fats and oils, especially those used in deep-fat frying processes, have generally been regarded as having the poorest nutritional value of any edible lipids (Melnick, 1957). Frying processes subject these fats and oils to high temperatures and adequate oxygen concentrations thus promoting physical and chemical changes at sites of unsaturation in the lipid moiety. The compounds formed under frying conditions not only decrease the effectiveness of the fat or oil as a frying medium, but may also pose as hazards to human health. Animal fats used as frying media may present an additional hazard due to the inherent presence of cholesterol. Recent studies indicate that cholesterol in the presence of oxygen, spontaneously forms products, some of which have been implicated as angiotoxins, carcinogens, and mutagens (Smith, 1980). Thus the review of pertinent literature will approach both the chemistry and toxicology of cholesterol and its oxidation products as well as the chemical and physical changes in frying fats and oils and the toxicological aspects of products formed during these changes.

Cholesterol Oxidation

Description of Cholesterol

Cholesterol (5-cholesten-3β-ol) is a C_{27} simple neutral lipid composed of a cyclopentanophenanthrene ring system, an eight carbon side chain, attached to the D ring at the C_{17} position, having two tertiary carbons, and methylated at the C_{10} and C_{13} positions. The alcoholic function is at the C_3 position and an unsaturated bond exists at the C_5-C_6 position.





Cholesterol is distributed in all cells of animal tissues, with higher concentrations found in nervous tissue. Cholesterol is an integral component in cell membranes and may function as a precursor to bile acids and various steroid hormones.

Autoxidation of Cholesterol

Cholesterol is an unsaturated lipid and is therefore susceptible to oxidation by free radical processes via reaction mechanisms analagous to those involved in the autoxidation of unsaturated fatty acids (Smith, 1980). The sensitivity of cholesterol to oxygen has been noted

in various reviews. Smith (1980) reported that Schulze and Winterstein investigated the sensitivity of crystalline cholesterol to air as early as 1902. Bladon (1958) mentioned early studies by Lifschultz in 1907 describing the isolation of "oxycholesterol," believed to be a pure chemical species formed in cholesterol which had been exposed to air. Bergstrom and Samuelsson (1961) cited the work of Lamb (1914) implicating oxygen as a necessary reactant for oxidative changes in cholesterol colloidally dispersed in an aqueous system. Bischoff (1930) confirmed these findings by showing that no "oxycholesterol" formed from cholesterol in the absence of air.

A series of experiments were conducted by Bergstrom and Winterstein from 1941-45 which were later reviewed by Bergstrom and Samuelsson (1961). Molecular oxygen was utilized as the oxidizing species in a number of experiments on aqueous colloidal dispersions of cholesterol. "Oxycholesterol" was found in this oxidizing system. Crystallization and chromatographic techniques revealed that aeration of cholesterol for five hours at 85^{o}C caused an eighty percent conversion of cholesterol to "oxycholesterol." Furthermore, "oxycholesterol" was found to contain 5-cholesten-3β, 7α-diol, 5-cholesten-3β, 7β -diol, 5-cholesten-3β -ol-7-one, and 3,5-cholestadiene-7-one. These studies also showed that prooxidant ions such as cupric and ferric species catalyzed the oxidative reaction. Bergstrom and Winterstein postulated an oxidative mechanism based on free radical processes whereby molecular oxygen attack at the C_7 position yields an unstable hydroperoxide. They attributed the presence of 5-cholesten-3β-ol-7-one to the dehydration of the 7-hydroperoxide, and the epimeric 3,7-diols to form directly from the simple decomposition of the 7-hydroperoxide.

The epimeric nature of these compounds was believed to be from either two stereoisomeric hydroperoxides, or due to rearrangement from a common precursor during decomposition. Trace amounts of cholestan-3β, 5α,6β-triol and 3,5-cholestadiene-7-one reinforced the validity of this proposed scheme.

Bladon (1958) also recognized the free radical induced nature of the commonly formed oxidation products. A similar mechanism was thus proposed with a 7-hydroperoxide as the primary oxidation intermediate. However, Bladon (1958) theorized that the epimeric 3,7-diols commonly formed were the product of the reaction of a 7-hydroperoxide with a propagating species such as an unoxidized cholesterol molecule. Dehydration and oxidation of these compounds accounted for the various secondary oxidation products found in autoxidized cholesterol.

The elucidation of the reaction mechanism involved in sterol autoxidation was greatly assisted by application of thin-layer chromatographic techniques (Smith, 1980). Analysis of a 12-year old sample of originally pure cholesterol by two-dimensional thin-layer chromatography revealed the presence of more than 30 distinct compounds including 3,5-cholestadiene-7-one, 5-cholesten-3β -ol-7-one, cholestan 3β ,5α ,6β-triol, and the epimeric 5 cholesten-3β,7α-diol and 5-cholesten-3β,7β-diol (Smith et al., 1967).

Confirmation of the reaction mechanism was hindered due to the inability to isolate 7-hydroperoxycholesterol, which was the proposed primary oxidation product. Van Lier and Smith (1970b) induced hydroperoxide formation by heating thin layers of cholesterol in air for 7 days at 100°C. A series of fractionations and recrystallizations produced a fraction containing a 5-hydroperoxide

which underwent complete isomerization to 7-hydroperoxycholesterol during Sephadex LH-20 separation. The identity of this compound was confirmed by gas chromatography.

A specific and sensitive thin-layer chromatographic technique was developed by Smith and Hill (1972) to identify sterol hydroperoxides. N,N-Dimethyl-p-phenylenediamine and N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochlorides were utilized as visualizing agents, yielding characteristic colors upon application. Autoxidized cholesterol samples were found to contain 7 -hydroperoxides as the primary autoxidation products.

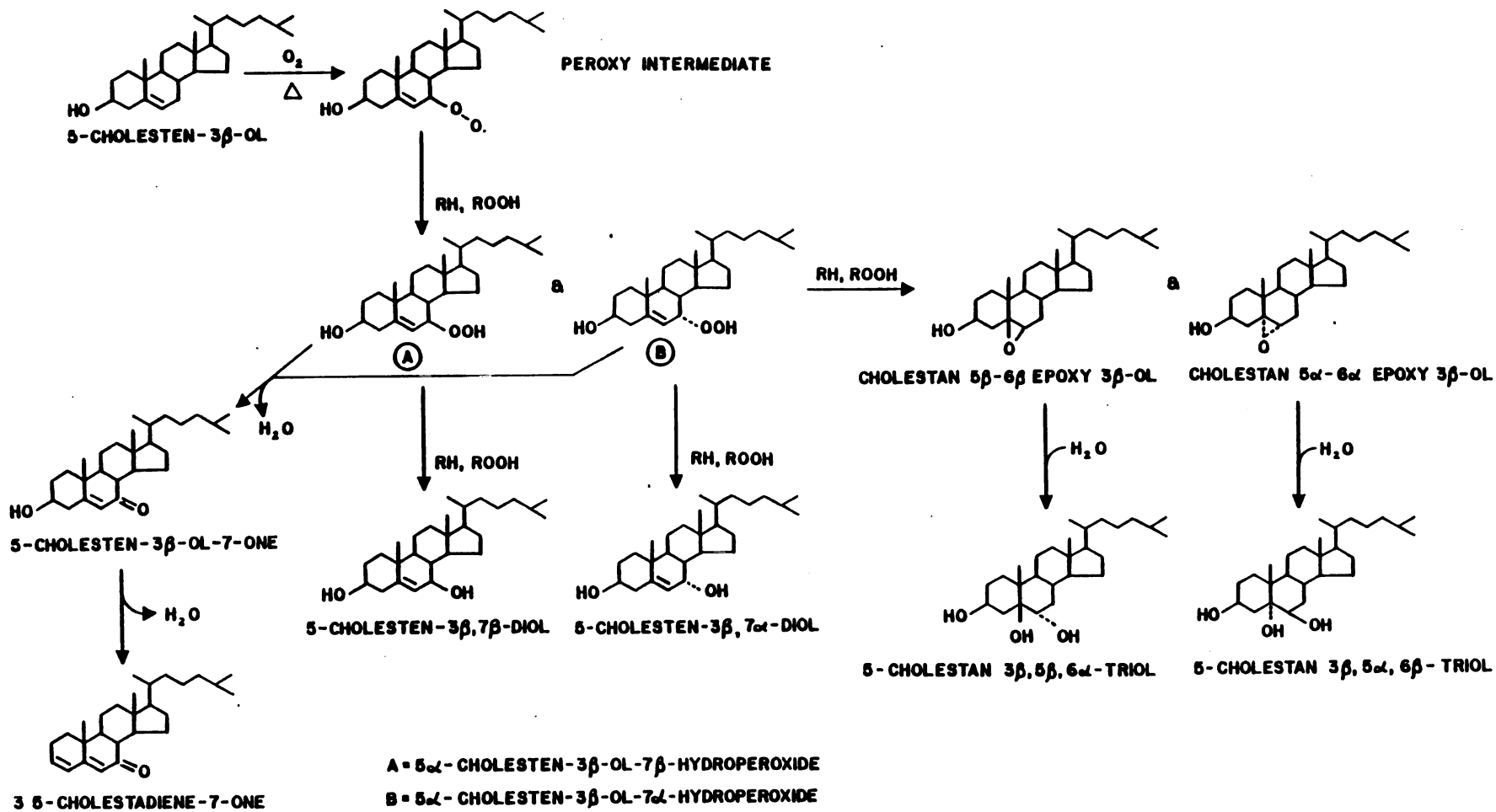
The overall autoxidation scheme for cholesterol was reviewed by Smith (1980). It was generally recognized that two distinct oxygen dependent processes occur in autoxidation, with hydroperoxides representing the primary products.

$$O_2 + RH \longrightarrow ROOH \qquad (1)$$

$$RCH(OH)+O_2 \longrightarrow RC=O + H_2O_2 \qquad (2)$$

The major reactions and products formed are shown in Figure 1. The initial reaction involves the abstraction of the allylic C_7 hydrogen and reaction with ground state dioxygen to form a C_7 peroxy radical. This peroxy radical is stabilized by hydrogen abstraction to form the epimeric 7 α and 7β-hydroperoxide. Smith et al. (1973b) identified these as the initial products of cholesterol autoxidation. It was also shown that both cholesterol hydroperoxides were formed initially, with the 7α-hydroperoxide predominating due to greater thermodynamic stability. Also, the 7α-hydroperoxide was found to isomerize to the 7β -epimer in solution form, solid state, and vapor state (Teng et al., 1973a and b; Smith et al., 1973a). Formation of secondary oxidation

Figure 1. Oxidative mechanisms of cholesterol


5-CHOLESTEN-3β-OL
PEROXY INTERMEDIATE
RH, ROOH
A
&
B
RH, ROOH
CHOLESTAN 5β-6β EPOXY 3β-OL
&
CHOLESTAN 5α-6α EPOXY 3β-OL
H2O
5-CHOLESTEN-3β-OL-7-ONE
RH, ROOH
RH, ROOH
H2O
H2O
5-CHOLESTEN-3β,7β-DIOL
5-CHOLESTEN-3β,7α-DIOL
5-CHOLESTAN 3β,5β,6α-TRIOL
5-CHOLESTAN 3β,5α,6β-TRIOL
3,5-CHOLESTADIENE-7-ONE
A = 5α-CHOLESTEN-3β-OL-7β-HYDROPEROXIDE
B = 5α-CHOLESTEN-3β-OL-7α-HYDROPEROXIDE

products was reported to proceed by formal reductions and dehydrations (Smith, 1980). 7α-and 7β-hydroperoxides are reduced to the respective diols during thermal degradation. Direct dehydration of either hydroperoxide epimer yields 5-cholesten-3β-ol-7-one (Van Lier and Smith, 1970a; Smith et al., 1973b). Epimerization of the 5-cholesten-3β,7β-diol to the 5-cholesten-3β, 7α-diol species may occur. Dehydration of 5-cholesten-3β-ol-7-one formed 3,5-cholestadiene-7-one (Van Lier and Smith, 1980).

The formation of epimeric cholesterol 5,6-epoxides during the autoxidation of cholesterol is facilitated by direct attack of an unoxidized cholesterol molecule by either 7α or 7β-hydroperoxy cholesterol to form cholestan-5,6α-epoxy-3β-ol and cholestan-5,6β epoxy-3β-ol respectively. Hydration of these epoxy compounds yield the triol epimers, cholestan-3β, 5α, 6β-triol and cholestan-3β, 5β,6α -triol (Smith and Kulig, 1975).

Common autoxidation products such as the epimeric 7α-and 7β-hydroperoxides, epimeric 3β-7-diols, and 7-ketone derivatives of cholesterol are formed via the action of other biological or chemical species by reactions not associated with autoxidation. Cholesterol in the presence of enzymes, such as soybean lipoxygenase (Teng and Smith, 1973), rat liver microsomal lipid peroxidases (Smith and Teng, 1974), and horseradish peroxidase (Teng and Smith, 1976), is converted to compounds normally associated with the autoxidative reactions. Singlet oxygen ($^1O_2$) forms a 5α-hydroperoxide which subsequently isomerizes to 7α-hydroperoxycholesterol. Again, C_7 autoxidation products form (Kulig and Smith, 1973). Dioxygen cation (O_2+), although unlikely to occur in biological systems, oxidizes cholesterol to the epimeric

3,7-diols, the 7-ketone, and the epimeric 5,6-epoxide derivative of cholesterol (Sanche and Van Lier, 1976). 5,6-Epoxide formation was also observed by the action of a two electron reduced species anion ($O_2^=$) (Smith and Kulig, 1976; Smith et al., 1978). A hydroxyl radical (.OH) oxidized cholesterol to the epimeric 3β -7-diols, the 7-ketone, the epimeric 5,6-epoxides, and triol derivatives of cholesterol with no detection of a 7-hydroperoxide intermediate (Ansari and Smith, 1979).

Smith (1980) stated that due to the multiplicity of origin for many of the products found in the autoxidation of cholesterol, only the presence of the cholesterol 7-hydroperoxide clearly demonstrates the action of autoxidation processes in a lipid system.

Biological Effects of Cholesterol Oxidation Products

Angiotoxic and Atherosclerotic Effects

The atherosclerotic effects of diets containing cholesterol have been well documented since 1913. In many of these studies, USP-grade cholesterol added to the diet was found to have a profound effect on the amount and rate of deposition of atherosclerotic materials. Smith et al. (1967), however, reported the presence of 3? autoxidation products in USP-grade cholesterol that had been stored at room temperature for 12 years. The possibility that these oxidative derivatives of cholesterol may have influenced the atherosclerotic characteristics of USP-grade cholesterol challenged the validity of previous studies (Taylor et al., 1979).

Two studies cited by Peng et al. (1978) indicated an inherent difference between hypercholesteremia induced endogenously and exogenously. Endogenous hypercholesteremia was found to cause mild atherosclerotic effects while diet-induced hypercholesteremia produced

severe atherosclerosis. The possibility existed that compounds present in USP-grade cholesterol or in foodstuffs containing large amounts of cholesterol in the diet may have caused this difference.

Studies of atheromatas from human aortic sections showed conclusively that in addition to cholesterol, trace amounts of autoxidation products were also present. Among the compounds identified were 5-cholesten-3 β,7α-diol, 5-cholesten-3β , 7β-diol (Henderson, 1954; Brooks et al., 1971), 5-cholesten-3β-ol-7-one (Brooks et al., 1966; Van Lier and Smith, 1967) 3,5-cholestadiene-7-one , and 5-cholestan-3 β, 5 α,6β-triol (Henderson and MacDougall, 1954; Henderson, 1956).

MacDougall et al. (1965) investigated the cytotoxicity of 103 steroids closely associated with cholesterol. Utilizing rabbit aortic cell cultures, 36 compounds were found to be toxic, and thus possible lesion inducers. Severe cell toxicity, resulting in cell death, which is regarded as the primary event in atherosclerotic lesioning, was exhibited by cholestan-3 β,5 α,6β-triol. Cholesterol and 5-cholesten-3β ,7β -diol were less toxic, while 5-cholesten-3β,7α-diol exhibited no toxic effect.

Taylor et al. (1979) implied the exogenous origin of autoxidation product of cholesterol in atheromatas, referring to studies in which antioxidant systems in vivo effectively prevented endogenous production of cholesterol oxidation products.

As a result of these findings, studies of the contaminants in USP-grade cholesterol regarding their angiotoxic and atherosclerotic effects were undertaken. Imai and co-workers (1976) fed concentrated contaminants from USP-grade cholesterol to rabbits. Results indicated

an increase in frequency of dead or dying aortic smooth muscle cells (angiotoxicity) and induced focal edema 24 hours after administration of the contaminant at a gavage of 250 mg/kg body weight. Administration of old and new samples of USP-grade cholesterol produced similar effects with the older sample exhibiting the greater atherosclerotic response. This was attributed to the greater concentration of autoxidation contaminants in the older sample. Purified cholesterol administered at equivalent levels caused no increase in the frequency of dead or dying cells, exhibiting only slight increases in aggregate debris. Administration of cholesterol-contaminant concentrates over a seven-week period induced intimal diffuse fibrous lesions, while purified cholesterol showed no such effects. These findings reinforced the theory that initial atherosclerotic lesioning may be caused by exogenously produced cholesterol oxidation products rather than their cholesterol precursors.

Peng et al. (1978) identified the contaminants in USP-grade cholesterol as autoxidation products of cystalline cholesterol by thin-layer and gas-liquid chromatographic techniques. Compounds identified included 5 -cholestan-3β, 5α, 6β-triol, 5- cholesten- 3β,7α -diol, 5-cholesten-3β , 7β -diol, 5-cholesten-3β-ol-7-one, 5-cholesten 3β , 25-diol, 5 and 7-hydroperoxycholesterol, and 3,5-cholestadiene-7-one. A mixture of these compounds showed extensive cytotoxicity to cultured rabbit aortic smooth muscle cells, indicating potent angiotoxicity. Purified cholesterol exhibited no effect on the cell cultures. Separation and purification of the compounds in the concentrate by preparative thin-layer chromatography and subsequent

administration of the pure compounds to cell cultures indicated that 5-cholesten-3β ,25-diol and 5-cholestan-3β , 5α , 6β-triol were responsible for the observed angiotoxic effect. The other compounds produced effects ranging from mild toxicity to no measurable toxicity in the cell cultures.

Further studies were conducted by Imai and co-workers (1980) in which cholesterol autoxidation products were injected intravenously into rabbits. The use of injection over feeding was to overcome uncertainties associated with biotransformations, retention, excretion, and absorption variances occurring in oral administration. Aortic sections examined after 24 hours showed that cholesterol hydroperoxides exhibited no angiotoxicity. A 10 week study indicated that freshly purified cholesterol or the vehicle used for intravenous administration produced no changes in major arteries or minor branches. Concentrated impurities from USP-grade cholesterol, synthetic 5-cholestan-3β,5,α 6β -triol, and 5-cholesten-3β , 25-diol however produced cell death after 24 hours, followed by necrosis, inflammatory reactions, and at 10 weeks, cellular proliferation leading to fibromuscular thickening. 5-Cholesten-3β-ol-7-one was found to affect minor arterial branches, but showed no demonstrable effect in major branches of the pulmonary artery.

Kummerow (1979a) reviewed the dietary implications of coronary heart disease, specifically atherosclerosis. Cholecalciferol (Vitamin D) had been recognized as an angiotoxin after extensive research with rabbits and swine (Kummerow, 1979b). Thus, atherosclerosis may be a life-long process initiated in fetal stages by high levels of cholecalciferol. This process may continue at accelerated rates due to consumption of oxidized sterols in the diet. Therefore, Kummerow

(1979a) suggested a greater concern over angiotoxins in the diet with less emphasis on cholesterol intake.

The mechanism by which cholesterol autoxidation products may cause intimal aortic cell death has only recently been investigated. Chen et al. (1974) noted that certain oxygenated sterols influenced cholesterol biosynthesis by inhibiting the enzyme, 3-hydroxy-3-methylglutaryl-Co-enzyme A reductase (HMg-CoA reductase). Subsequent investigations indicated a definite structural requirement for compounds having this capacity. It was shown that the prerequisite structure for inhibitors of the enzyme included a side chain of at least 6 carbons, a ketone or hydroxyl function at the C_3 position, and a second ketone or hydroxyl function at positions 6,7,15,20,22,24,25, or 26 (Chen et al., 1974; Kandutsch et al., 1978; Kandutsch and Chen, 1978). Many products of cholesterol autoxidation have structures which were proven to inhibit this enzyme. The presence of these compounds in exogenous cholesterol has been associated with suppression of hepatic cholesterol synthesis as well as inhibition of cholesterol biosynthesis in cell cultures. (Kandutsch et al., 1978). Chen et al. (1974) observed 5-cholesten-3 β,25-diol to be a potent inhibitor of HMg-CoA reductase, with a lesser inhibitory effect exhibited by 5-cholesten-3 β,20 α-diol, 5-cholesten-3 β-ol-7-one, 5-cholesten-3β,7α -diol, and 5-cholesten-3β,7β-diol as well as other recognized cholesterol autoxidation products.

Results from these studies have led to theories which implicated the autoxidation products of cholesterol as initiators of atherosclerosis via HMg-CoA reductase inhibition. Chen et al. (1974) concluded that the inhibition of cholesterol synthesis leads to membrane fragility, which results in improper membrane growth and

subsequent cell death. Alteration of sterol concentrations in membranes also changes cell fluidity thus affecting transport, as well as membrane-localized enzymes, which could lead to cellular disorders. Kandutsch et al. (1978) stated that low cholesterol concentrations may also affect the cells ability to reproduce. Peng et al. (1979) supported these theories and postulated an alternative mechanism. Replacement of cholesterol in membranes by autoxidation products could easily occur due to the presence of both polar and apolar functional groups on the same molecule, leading to cellular disfunction. This theory is supported in that cholestan-3β, 5α, 6β-triol has a greater toxic effect than 5 cholesten-3β, 25-diol, despite its lower ability to inhibit HMg-CoA reductase.

Carcinogenic Effects

Certain steroidal compounds have been suspected of possessing carcinogenic properties. As cited in the review by Lane et al. (1950), Roffo (1938) suspected that "oxycholesterol" may exhibit carcinogenic effects. Subsequent studies by Veldstra (1939) and Larsen and Barret (1944) showed that 3,5-cholestadiene induced papillomas in mice and rats. Conflicting data were presented by Kirby (1943, 1944) when both 3,5-cholestadiene and cholesterol heated to 270-300^{o}C showed no papilloma induction when fed to rats over a two year period.

Bischoff (1963, 1969) reviewed the results of a large number of studies which investigated the possible carcinogenicity of many cholesterol derivatives. Many factors were shown to influence the carcinogenic potential of cholesterol derivatives. It was determined that administration of cholesterol derivatives via oily vehicles enhanced the carcinogenic effect when compared to similar dosing via

aqueous dispersions. The common oily vehicle used was sesame oil which contained compounds such as sesamol and samin, which may have had a co-carcinogenic effect. Also noted was the increased carcinogenic effect of many compounds injected as super-saturated solutions. This phenomenon was attributed to non-specific smooth surface effects characteristic of most active as well as inert compounds in crystalline form, commonly referred to as solid-state carcinogenesis. Non-saturated solutions of cholesterol derivatives exhibited no carcinogenicity.

The results of screening studies for many cholesterol oxidation products showed that an oxygen linkage at the C_6 position was common to all potent carcinogenic derivatives. Cholestan-5,6α -epoxy-3β -ol was found to be a potent carcinogen via oily and aqueous administration. Bischoff (1969) could not however verify the causative structural feature as the epoxy group. The carcinogenicity of this compound was proven subcutaneously in rats and mice. It was noted that inherent contamination by the β-epimer was established which may have accentuated the carcinogenic effect. Other recognized autoxidation products of cholesterol were found to be generally non-carcinogenic.

Black and Douglas (1972, 1973) observed the formation of 5-cholestan-5,6α -epoxy-3β -ol from cholesterol in the skin of human and hairless mice after exposure to ultra-violet radiation. The formation of this compound has been correlated to tumorogenesis.

Chan and Black (1974, 1976) investigated the action of cholesterol-5,6-epoxide hydrase on 5-cholestan-5,6 α-epoxy-3β -ol, based on the premise that the carcinogenicity of this compound arises through the formation of an arene oxide intermediate. The hydrase enzyme converted

the epoxide to a more polar species allowing rapid excretion. Hydrase activity was stimulated by factors which led to the formation of 5-cholestan-5,6α-epoxy-3β-ol or its β-epimer, and was found to function by converting these compounds to their respective triols.

Cholesterol Oxidation Products in Foods

Increased understanding of both the autoxidation reactions of cholesterol and the adverse biological effects of the oxidation products has generated concern over the presence of these compounds in foodstuffs containing cholesterol. Cholesterol is generally associated with foods of animal origins, although recent analysis of palm and palm kernel oils indicates that cholesterol is present in certain plants in trace amounts (Seher and Homberg, 1968; Punwar and Derse; 1978). Smith (1980) emphasized a need to evaluate cholesterol-containing foods, especially those that are processed, for the presence of cholesterol oxidation products. Processing of foods, such as spray-drying, retorting, curing and deep-fat frying, subject cholesterol in the food to conditions conducive to oxidation. To date, there is limited data on the presence of cholesterol oxidation products in foods.

Acker and Greve (1963) isolated cholesterol hydroperoxides and "7-hydroxy cholesterol" from egg-containing dough mixes which were subjected to light under extreme conditions. Finely ground samples exposed to sunlight showed high levels of conversion to these products at the expense of cholesterol, while only limited formation was noted in similarly tested unground samples. Light barrier packaging of the dough mix effectively protected cholesterol from oxidative changes over a two year period. Chicoye et al. (1968) showed that induced photooxdiation of spray-dried eggs caused the formation of

5-cholesten-3β-ol-7-one, 5 cholesten-3β ,7 β-diol, 5-cholesten-3β , 7α-diol, cholestan-5,6 α-epoxy-3β-ol and cholestan-3β , 5α , 6β-triol. Fresh egg yolk and unirradiated spray-dried egg powder contained insignificant levels of these compounds, even when stored for one year at 25°C. Tsai et al. (1979) isolated cholestan-5, 6α-epoxy-3 β-ol, as well as the β-epimer, from dry egg products by high performance liquid chromatography.

Dairy products and meat also have been investigated to a limited degree for the presence of cholesterol oxidation products. Flanagan et al. (1975) isolated 4-cholesten-3-one and 3,5-cholestadiene-7-one from stored samples of nonfat dry milk and anhydrous milk fat. Tu et al. (1967) analyzed freshly cooked meat for 5-cholesten-3β,7α -diol, 5-cholesten-3β, 7β -diol, and 5-cholesten-3β-ol-7-one, but found no measureable amounts in lipid extracts. A comprehensive list of oxidized sterols isolated from foods is given in Table 1.

Several researchers have speculated that certain food processing procedures can promote the formation of cholesterol oxidation products. Taylor et al. (1979) reported that dried powdered foods stored in air at room temperatures may be major sources of angiotoxic sterol oxidation products in the diet. Powdered eggs and milk, as well as products containing dried whey or eggs, should be evaluated in regard to their cholesterol oxidation product content. Other possible sources of these angiotoxins included smoked meats, fish, and air-aged cheeses. The authors recommend low temperature storage coupled with oxygen barrier packaging or nitrogen flushing. The addition of antioxidants such as glutathione is also recommended as a deterrent to the formation of these deleterious compounds.

Table 1. Oxidized sterols in foodstuffs[a]

Foodstuff	Sterol
Egg Products	
Egg yolk	5α-Cholestane-3β,5α,6β-triol
	Cholesta-3,5-dien-7-one
Egg dough	Cholest-5-ene-3β,7-diols
	Cholesterol hydroperoxides
Spray-dried eggs	Cholest-5-ene-3β,7-diols
	3β-Hydroxycholest-5-en-7-one
	5,6α-Epoxy-5α-cholestan-3β-ol
	5α-Cholestane-3β,5,6α-triol
Heat-dried eggs	5,6α-Epoxy-5α-cholestan-3β-ol
	5,6α-Epxoy-5α-cholestan-3β-ol
Dry egg mix	5,6-Epoxy-5α-cholestan-3β-ol
	3β-Hydroxycholest-5-en-7-one
	5α-Cholestane-3β,5α,6β-triol
Milk Products	
Anhydrous milk fat	Cholest-4-en-3-one
	Cholesta-3 ,5-dien-7-one
Nonfat dry milk	Cholest-4-en-3-one
	Cholesta-3,5-dien-7-one
Butterfat	5α-Cholest-7-en-3-one
Other Products	
Pork fat	3 -Hydroxycholest-5-en-7-one
Brewer's yeast	Ergosterol 5 ,8 -Peroxide
	Cerevisterol
	5 ,8 -Ergosta-6,22-diene-3 ,5,8-triol
Baker's yeast	Cholesta-3,5-dien-7-one
Beef	Cholesta-3,5-dien-7-one

[a]. Adapted from Smith (1980)

Another potentially large source of cholesterol oxidation products in the diet is deep-fat fried foods. Punwar and Derse (1978) evaluated various fats and oils for cholesterol content (Table 2). Frying fats derived from animal origins were found to contain appreciable amounts of cholesterol. Frying operations involve high temperatures (180^{o}C) in the presence of air. Kummerow (1979a) stated that potatoes fried in such animal fats probably contain cholesterol oxidation products, since french fried potatoes absorb cholesterol from the oil medium. Punwar and Derse (1978) found 13.9 mg cholesterol/100g french fried potatoes obtained from a commercial franchise. Slover et al. (1980) surveyed fast-food products from various franchises and found cholesterol levels in french fried potatoes ranging from 7.2 to 16.4 mg cholesterol/100 g french fried potatoes. Kummerow (1979a) has stated that 600 million pounds of fat are used for frying each year. Also, oils used to fry chicken and fish retain cholesterol from the product which may later be absorbed by other products fried in the same medium. Kummerow concluded that concern over cholesterol in the diet should be superceded by the possible presence of angiotoxic cholesterol oxidation products formed during processing.

Table 2. Cholesterol content of various fats and oils [a]

Fat and/or Oil	Cholesterol Content (mg/100g)
Corn oil	ND[b]
Coconut oil	ND
Cottonseed oil	ND
Clarified shortening	77.8
Edible rendered bacon fat	108
Edible tallow	141
Lard	75.0
Leaf lard	101
Palm oil	1.97
Palm kernel oil	1.34
Peanut oil	ND
Pure vegetable shortening	ND
Soybean oil	ND
White Clover lard	97.7
Salmon oil (human food grade)	485
Menhaden oil (human food grade)	521
Herring oil	766

a From Punwar and Derse (1978)
b ND, not detected

Heated Fats and Oils

The deterioration of heated fats and oils utilized in deep-fat frying operations is generally considered to be via simultaneous oxidative, polymerization, and hydrolytic reactions (Pokorny, 1980). Many studies published over the last three decades have investigated the nature of these chemical reactions, while an equal number have attempted to assess the biological significance of these altered fats when consumed as part of a fried food. During the course of a number of these investigations, various fats and oils were exposed to abusive extremes of temperature, aeration, or both concurrently. Thus, the value of these studies lies only in understanding the various degradative changes occurring in heated fats and oils, while contributing only a limited insight into processes occurring in actual frying procedures.

Chemical Reactions

A. Oxidative Reactions

1. Autoxidation

Perkins (1967) classified autoxidation processes as those occurring in fats and oils exposed to oxygen at temperatures below 100°C. Autoxidation occurs at sites of unsaturation in fatty acyl groups which compose the triglyceride moiety. The attack by oxygen and subsequent decomposition at these sites involves a three-step free radical chain mechanism as proposed by Farmer (1942):

Initiation

$$RH + O_2 \longrightarrow R\cdot + \cdot OOH$$

Propagation

$$R\cdot + O_2 \longrightarrow ROO\cdot$$

$$RH + ROO\cdot \longrightarrow ROOH + R\cdot$$

$$ROOH \longrightarrow RO\cdot + \cdot OH$$

Termination

$$R\cdot + R\cdot \longrightarrow R\ R$$

$$R\cdot + ROO\cdot \longrightarrow ROOR$$

$$ROO\cdot + ROO\cdot \longrightarrow ROOR + O_2$$

RH refers to any unsaturated fatty acid in which the H is labile by reason of being on a carbon atom adjacent to a double bond. R· refers to a free radical formed by removal of a labile hydrogen. The onset of this reaction mechanism is characterized by absorption of oxygen by the fat. During this induction period, antioxidant species in the fat are consumed while free radicals or their precursors accumulate. Rapid oxygen uptake proceeds at elevated radical concentrations, evidenced by increased peroxide values.

Various researchers have noted decreased induction periods and increased rates of oxidation under certain conditions including increased temperature (Gunstone and Hilditch, 1945), increased surface-volume ratios (Pokorny, 1966), increased unsaturation and addition of preformed hydroperoxides to unoxidized oils (Gunstone and Hilditch, 1945).

Artman (1969) has comprehensively reviewed the autoxidation process. Radical abstraction of an allylic hydrogen from an unsaturated fatty acid forms a fatty acyl radical. Direct addition of

atmospheric oxygen, or addition after double bond rearrangement, yields a peroxy radical which abstracts a new allylic hydrogen from an unoxidized unsaturated fatty acid. The resulting hydroperoxide may then undergo homolytic cleavage, forming two new radicals which induce an autocatalytic propagation of the autoxidation mechanism. Termination reactions occur when the concentration of radicals are sufficiently great to allow kinetically preferential radical-radical interaction.

Products of autoxidation, although dependent on the type of oxidized fat or oil, generally include short chain aldehydes, ketones, alcohols and acids which render the oil aesthetically unacceptable. Trace amounts of esters, hydrocarbons, aromatics, cyclohexanes and lactones have also been isolated from autoxidized samples (Artman, 1969).

Dimeric and polymeric compounds also form in autoxidized lipids at the expense of unsaturated bonds. The formation of these compounds is concommitant with decreases in iodine value and increases in viscosity of the oil. Conflicting reports of the chemical nature of these polymers have been cited in the literature. Artman (1969) stated that both carbon-carbon linked polymers and carbon-oxygen linked polymers have been isolated from autoxidized lipid systems. O'Neill (1954) isolated ether linked dimers from oleate, but found only carbon linked species in linoleate. Chang and Kummerow (1953) cleaved methyl oleate polymers with strong acids, thus indicating ether or peroxy linkages. Kartha (1960) observed oxygen linkages in polymerized methyl oleate held at 25°C while carbon linkages predominated under similar conditions at 60°C. Triolein and methyl oleate exhibited completely different polymeric products when oxidized under similar conditions.

Autoxidation reactions are involved in the degradation of frying media held at ambient temperatures. Artman (1969) described the susceptibility of intermittently heated fats and oils to autoxidative reactions. Formation of hydroperoxides occurs more readily in thermally damaged oil. Buildup of hydroperoxides during cooling cycles of frying fats, followed by reheating, accelerates the liberation of radicals and subsequent degradation of the fat at higher temperatures.

2. Thermal oxidation

Oxidation of a fat or oil in the presence of oxygen at temperatures in excess of 100°C is referred to as thermal oxidation (Perkins, 1967). Temperature, time, rate of aeration, and time-temperature cycling influence the rate of thermal oxidative reactions.

It is generally accepted that oxidative reactions at elevated temperatures proceed via reactions similar to autoxidation; however, the transient nature of hydroperoxides at frying temperatures suggests the development of other primary oxidation products. Artman (1969) noted differences in both reaction rate and type of products between thermal oxidation and autoxidation. Furthermore, Artman postulated direct radical formation at elevated temperatures without the intermediacy of hydroperoxides.

Fats and oils, when heated to high temperatures, absorb oxygen thus increasing the peroxide value of fat or oil systems. Further heating causes the decomposition of peroxides, accompanied by loss of weight due to volatilization of short chain scission products (Artman, 1969). Double bonds conjugate and oxygenated products accumulate as evidenced by increased ultraviolet absorption. The oxygenated products include carbonyls, hydroxy, and epoxy derivatives (Perkins, 1967). With continued heating, conjugation of unsaturated fatty acids such as

linoleic acid occurs. The levels of conjugated acids increase, reach a maximum, then decrease as various polymerization and decomposition reactions take place. This phenomenon is represented by decreasing iodine values with increased time of heating. Browning occurs in the fat and can be attributed to α,β-and α, α'-unsaturated carbonyl compounds as well as highly polar dimeric compounds (Perkins and Kummerow, 1959).

Oxidative polymerization reactions increase the viscosity of the fat or oil, seriously deteriorating the foaming stability. Investigations of the higher molecular weight products formed in heated fats indicate a variety of compounds. Frankel et al. (1960) isolated carbon-linked dimers which were acyclic in nature, some of which contained polar functional groups. Micheal, (1966a and b) reported both polar and non-polar dimers. Non-polar dimers were characterized as Diels-Alder adducts with six-membered unsaturated rings, much like those formed in anaerobically heated fats. Polar dimers included both carbon and oxygen linked dimers with polar functional groups. These were found to be responsible for the increased foaming tendencies of thermally oxidized oils. Melnick (1957a) stated that oxidative polymers cause serious flavor problems in frying fats and oils.

Many products formed in thermal oxidative reactions have also been identified in lipid systems undergoing autoxidation (Perkins, 1967). Low molecular weight alkanes, saturated and unsaturated aldehydes, ketones and alcohols have been isolated from the volatile fractions of various thermally oxidized fats and oils. In addition, pyrolysis products such as methylcyclopentanes, cyclohexanes, benzene derivatives, acrolein, water, carbon monoxide and carbon dioxide have also been reported (Artman, 1969). A wide variety of compounds formed

via conjugation and trans isomerization have also been isolated and identified, indicating the complex reaction processes occurring in heated fats. Several researchers has suspected the presence of cyclic monomers in thermally oxidized fats and oils. Firestone et al. (1961) heated cottonseed oil and found a fraction which did not adduct with urea. Micheal et al. (1966) oxidized methyl linoleate at 200°C hours and identified aromatic as well as alicyclic monomers in the system. Artman and Alexander (1968) found alicyclic, cyclic, and aromatic compounds in thermally oxidized partially hydrogenated soybean oil.

Micro-constituents of fats and oils are also susceptible to oxidative changes at elevated temperatures. Non-saponifiable compounds such as sterols and carotenoids show marked changes as a consequence of heating (Artman, 1969). It has been suggested that sterols may oxidize to volatile or polymeric species when thermally abused. Fioriti and Sims (1967) oxidized cholesterol for nine weeks at 82°C and documented an 80% conversion to oxidation products including epoxy, ketone, and polyhydroxy derivatives.

B. Thermal Polymerization

Anaerobic thermal polymerization reactions are those occurring in fats and oils heated in excess of 100°C in the absence of air (Perkins, 1967). The prevalence of purely thermal reactions in frying fats is unknown, yet conditions such as low oxygen concentrations in frying fats and occulsion of air by steam generated from the fried product may facilitate these reactions. Consequently, various heating studies on fats and oils have been conducted in vacuo or under inert atmospheres. Roth and Rock (1964a) studied anaerobically heated fats, finding little change after prolonged exposure to frying temperatures. Results of various studies indicate that the development of toxic

compounds in thermally abused fats and oils require much higher temperatures than those used in frying (Artman, 1969).

Thermal polymers form via Diels-Alder reactions between unsaturated fatty acyl groups after conjugation has taken place (Perkins, 1967). These reactions proceed at the expense of non-conjugated double bonds in the triglyceride moiety, as indicated by decreased iodine values and increased viscosity levels as heating time progresses (Sims, 1957; Smouse, 1975). These polymerization reactions may occur between two triglycerides by intermolecular dimerization or by reaction between unsaturated fatty acyl groups on the same triglyceride molecule, referred to as intramolecular dimerization. Transesterification of the latter yields higher molecular weight polymers similar to those formed via the intermolecular process (Artman, 1969).

Many studies have been carried out on the thermal polymerization of fats. Perkins (1967) described thermally induced polymerization as it involves linoleic acid content in the triglycerides. Conjugation of the double bonds occurs in the presence of heat. The conjugated species then may react with another unsaturated fatty acyl group or undergo an intramolecular cyclization. This results in a six-membered cyclic compound of either monomeric or dimeric nature, respectively. These polymeric intermediates contain one endocyclic double bond and one exocyclic double bond, the latter possessing the ability to act as a dienophile. Trimerization reactions may then occur, leading to high molecular weight polymeric compounds. Other researchers have isolated similar compounds in thermally oxidized fats. Evans et al. (1965) isolated Diels-Alder products from thermally abused fats, as well as non-cyclic dehydropolymers and hybrid dimers in which one monomeric unit had cyclized prior to dimerization. Sahasrabudhe (1965) found

1,2-disubstituted cyclohexanes in anaerobically heated polyunsaturated fatty acids, indicating Diels-Alder mechanisms at work.

In addition to polymeric substances, volatile and monomeric species have also been isolated and characterized from thermally abused fats and oils. Volatile constituents include typical pyrolysis products of lipids such as short chain aldehydes, ketones, olefins, carbon monoxide, carbon dioxide, and water (Artman, 1969). Many of these compounds are highly reactive and may become incorporated in concommitantly formed polymers. Non-volatile monomers isolated from thermally abused fats include free fatty acids liberated in transesterification reactions. Long chain ketones may result via decarboxylative dimerization of these fatty acids. Acrolein is commonly identified as a pyrolysis product of heated fats, formed from the glycerol moiety (Artman, 1969).

Concern over the presence of thermal polymers in frying fats began when Crampton et al. (1953) reported that they suppress growth and exhibit toxic effects in rats. Melnick (1957a) regarded thermally induced polymers as the inherent danger in fried foods. This was due to the fact that thermal polymers do not exhibit the strong objectionable odors and flavors associated with oxidative polymers. To the contrary, Melnick (1957a) stated that thermal polymers enhance the flavor stability of frying oils. Thus the safeguard of unpalatability is negated when thermal polymerization reactions predominate in heated fats and oils.

C. Hydrolytic Reactions

Hydrolysis of heated fats liberate free fatty acids via cleavage of esterified fatty acyl groups from triglyceride molecules in the presence of water. These long chain fatty acids as well as the

resultant mono-and diglycerides limit the functional life of a frying fat by reducing the smoke point (Artman, 1969). Hydrolysis of triglycerides has also been associated with increased foaming in fats used in frying operations (Smouse, 1975).

The presence of water in fats, whether introduced through the frying of foods or as a product of oxidative reactions, facilitates hydrolytic reactions in heated fats (Artman, 1969; Pokorny, 1980). The accelerated deterioration of fats by the addition of water is well documented in the literature. Perkins and Van Akkeren (1965) injected water into heated fats and found more deterioration than encountered in similar fats which were utilized in frying operations. Yukui (1965) whose research was reviewed by Artman (1969), noted the strong hydrolytic action of water in heated fats. It was found that water caused both hydrolytic and oxidative deteriorations, the extent depending on temperature and degree of exposure to air. Hydrolytic reactions predominate when heated fats are exposed to water under low oxygen atmosphere. At elevated temperatures the effect of water is minimized due to its rapid volatilization.

Used frying fats exhibit elevated free fatty acid levels with average values being around 1% (Bates, 1952). It was important to note that acid values not only reflect the hydrolytic activity in an oil, but also oxidative changes since various scissionings of dismutated hydroperoxides liberate fatty acyl compounds having carboxyl functions (Smouse, 1975).

Effects of Processing Variables on Frying Media Stability

A. Fat Turnover

The loss of frying medium via absorption and the resultant replenishment with fresh oil is referred to as fat turnover. This

process maintains the quality of fats utilized in large volume continuous frying operations by both increasing the proportion of fresh fats in the frying medium and by the absorption of deleterious compounds into the fried product. Constant rates of fat removal and replenishment result in a steady-state condition of the fat, which does not deteriorate extensively with continued use.

Factors such as size of frying loads, frequency of frying, product, and product dimension influence the fat turnover rate, and consequently the life of the frying fat. High frying loads increase the frequency of add-back to the fryer. Use of high frying loads in a continuous process allows steady-state conditions to develop rapidly (Melnick, 1957b; Perkins and Van Akkeren, 1965). Prolonged use of commercial frying media have been reported under these conditions (Artman, 1969).

Product constitutent differences exhibit a profound effect on turnover rate of a frying fat (Pokorny, 1980). Foods which absorb high levels of fat during frying facilitate more frequent or substantial levels of oil add-back. Similar products having different surface areas demonstrate differences in absorption rates. In potato frying operations, french fried potatoes contain approximately 13-14% fat after frying (Watt and Merrill, 1963). Fried sliced potatoes of 12 mm thickness contain 7% fat (Strock et al., 1966) while 1 mm slice contain 30-40% fat as cited by Artman, 1969. Consequently, oils used in potato chip frying operations exhibit extended frying life. Melnick et al. (1958) surveyed oil samples from various chippers and found only slight changes between used oils and the corresponding fresh oils. This stability of used oils was attributed to the high rate of fat turnover.

Non-continuous frying operations, mainly encountered in restaurants or fast food establishments, expose fats to extended periods of time at

high temperature, with small and infrequent frying loads. The decreased stability and functional life of such oils clearly demonstrates the protective effect of high turnover rates (Artman, 1969).

B. Temperature Cycling

Melnick (1957b) noted the greater stability of frying oils used in continuous operations, compared to those used intermittently. This effect was studied by Perkins and Van Akkeren (1965) by heating cottonseed oil both continuously and intermittently. The intermittently heated samples were found to decompose more rapidly than the continuously heated controls. The decreased stability of intermittently heated samples was attributed to buildup of fatty acyl peroxides on the surface of the oil at ambient temperatures, followed by reheating. At elevated temperatures, the peroxides decompose, damaging the oil via liberation of hydroxyl and carbonyl compounds. This damage, estimated by hexane insolubles concentration or amount of polar material present, was equivalent after 62 hours of intermittent heating and 166 hours of continuous heating.

C. Heating Systems

Intense localized heating systems, such as high temperature heating elements, may influence the deterioration of a fat or oil used in frying. Such elements not only expose part of the fat to extremely high temperatures, but also increase agitation of the fat via convection currents (Artman, 1969). Circulating systems utilizing heat exchangers eliminate localized heating, but generally increase agitation and thus the aeration of a fat (Roth and Rock, 1964b).

D. Substrate Effects

Compositions of fried substrates may influence the stability of frying oils in various ways (Pokorny, 1980)

1. Via release of antioxidants or prooxidants from the substrate into the oil or by absorption of such compounds in oils by the fried substrate.

2. Via catalytic effects of various functional groups of the substrate on free radicals and secondary reactions in the frying oil.

3. Via adsorption or chemisorption of oxidation products on the fried substrate.

Constituents in fried substrates which enhance oxidation in frying oils include polyunsaturated lipids and phospholipids introduced through migration and water which promotes hydrolysis reactions. Constituents possessing antioxidant activities include essential oils from herbs and spices, sterols, carotenoid pigments, and tocopherols.

Biological Aspects of Used Frying Oils

Many studies conducted to assess the biological hazards of heated fats and oils generally employed samples which were intentionally abused by extremes of temperature and aeration. These studies indicated that toxic compounds exhibiting adverse physiological effects on test animals were formed. However, a number of these researchers expressed the irrelevence of their results in the application to fats and oils used under normal frying conditions. Therefore, this review will address only those studies conducted under actual or simulated frying conditions.

The accumulated results of studies over the last three decades indicate that fats and oils used under practical frying conditions are not harmful to test animals. Deuel et al. (1951) fed to male and

female rats margarine which had been used for 10 weeks for deep fat frying, finding no adverse effects. Kaunitz et al. (1956) isolated the distillate of fat used in frying for 80 hours at 190°C. Administration of the distillate via dietary supplementation resulted in only slight decreases in both net dietary energy and growth rate Keane et al. (1959) collected fat samples used up to 24 days in commercial frying operations. The response of rats fed fat for 7 weeks at 18 percent of the diet indicated that the used fats had higher caloric efficiencies, yielding better growth rates with no evidence of toxicity. Poling et al. (1960,1962), obtained thirty-four fat samples from various frying operations. These samples caused no liver enlargement or loss of available biological value in rats on supplemented feed for 7 days. This was in great contrast to results obtained with laboratory heated fats without frying. In cottonseed oil used to fry potato chips, Rice et al. (1960) noted decreased feed intake, increased liver size, and decreased growth in rats fed a 20% fat diet, but only after long-term heating in which the fat foamed violently during frying. Artman (1969) cited the work of Kajimoto and Mukai (1964) who reported a correlation between foaming levels during frying and fat degradation. Fats exhibiting severe foaming substantially decreased growth rats of weanling rats when fed at 10 percent of their diets. Also cited by Artman (1969) was the extensive work of Mameesh et al. (1965, 1967) in which cottonseed oil, heated 8 hours daily at 195°C for 25 days, was used to fry broad bean cakes. The used oil was fed to rats at 10% supplementation in a diet of bread, milk, and salt. Unfried bean cake was fed to the control group. Lower feed intake and lower fat absorbability caused a lower growth rate in rats fed the heated fats. Also noted was lower levels of fat in the

livers, but liver size did not change. Autopsy results of the rats receiving fat heated for 204 hours indicated hyperplastic lymphoid tissue on the small intestine and evident changes in liver nuclei after 8 weeks of feeding. Similar results were observed in fat heated for 68 hours, but only after 17 weeks of feeding. The vast differences in these results compared to those reported by other authors who fed abused oils was attributed to the suppressive effect of frying on the formation of toxic compounds

Perhaps the most comprehensive study was conducted by Nolan et al. (1967) to assess long-term effects of feeding various frying oils. Five fats were heated intermittently at 182^{o}C and were used to fry potatoes, onions, and fish. The frying schedule and intermittent heating corresponded to the most abusive commercial practices. Four of the fats were heated until foaming persisted, rendering the fats impractical and unsafe by commercial standards. This time ranged from 49-116 hours. The fifth fat contained methyl silicone which prevented foaming, so frying was discontinued after 216 hours. Each fat and unheated control was administered in the diet of 100 rats at 15 percent from time of weaning to 2 years of age. Rats fed heated fats exhibited slightly poorer growth rates and feed efficiencies than controls, which was attributed to the concentration of non-absorbable polymers in the heated fats. No increase in mortality, increase in tumor incidence, or irregularities monitored by extensive biochemical or histological examination was evident. Analysis of the used fats indicated low levels of distillable, non-urea-adductable materials. Separation, concentration, and administration of these compounds to weanling rats at extremely high levels gave adverse toxic effects. The author

concluded that the absence of adverse effects in the two year study clearly indicate that such substances offer no hazard in a chronic feeding situation analogous to human consumption of used frying fats.

MATERIAL AND METHODS

Beef Tallow

A 100 gallon drum of refined edible beef tallow was obtained from a commercial supplier and stored at $4^{o}C$. The same lot of tallow was used for the duration of the study to avoid variations among tallow samples.

Standard Cholesterol Oxidation Products

Standard 5-cholesten-3β -ol, 5-cholesten-3β, 7α -diol, 5-cholesten-3β, 7β-diol, 5-cholesten-3β-ol-7-one, 3,5-cholestadiene-7-one, 5-cholesten-3β, 25-diol, 5-cholestan-5α,6-epoxy-3β-ol, cholestan-3β, 5α, 6β-triol, 5α- cholestan-3β-ol, 5 α-cholestane-3-one, 5α -cholestan-3,6-dione, 5α-cholestan-3β-ol-7-one, cholestan-3β, 5α-diol -6-one, 5-cholesten-3β, 20 -diol, 5α- cholestane,5,24-cholestadiene-3β -ol, 4-cholesten-3-one, 5-cholesten-3-one, 5-cholesten-3β, 4α -diol, and 5-cholestan-5β , 6-epoxy-3β-ol were purchased from Steraloids Inc., WIlton, New Hampshire, USA.

Chromatography Material and Chemicals

Silica gel H was purchased from Merck, Darmstadt, Germany, while Redi/Plate Precoated Silica Gel GF (20 cm x 20 cm) were purchased from Fisher Inc., Pittsburgh, Pa. All other reagents and chemicals used in

the analysis were analytical grade.

French Fry Potatoes

Hearty-House Grade A, 3/8" Crinkle Cut Potatoes were acquired from Vita-Bite Foods, Portland, Oregon. The french fry potatoes contained; potatoes, partially hydrogenated vegetable shortening (soybean, palm and/or cottonseed oil) and/or beef fat, disodium dihydrogen pyrophosphate (to promote color retention), and dextrose. The french fry potatoes were stored at -20°C until use.

Commercial French Fried Potatoes

French fried potatoes samples were acquired from two commercial fast-food franchises in the East Lansing-Okemos area. These samples were stored in polyethylene bags at -20°C until required for analysis.

A three phase approach was utilized to investigate the effect of frying temperatures on the oxidative stability of cholesterol in tallow. In Phase I of the study, the effect of continuous versus intermittent heating on the cholesterol stability in tallow was investigated. Phase II involved the analysis of both tallow and french fries produced in the laboratory under practical operational conditions, in regard to the oxidative state of cholesterol. Phase III of the study dealt with investigations of commercially produced french fried potatoes by application of techniques and methodology attained in the two previous phases, to determine the prevalence of cholesterol oxidation products in commercially prepared foods.

Phase I Analysis of Continuously and Intermittently-heated Tallow

Two heating trials were carried out in this phase of the study. In Trial I, tallow was heated continuously for 300 hours at 180±2°C, samples being removed for analysis every 50 hours. In Trial II, tallow was heated intermittently for 8 hours per day, for a total heating time of 275 hours. Samples were removed for analysis every 25 hours. No tallow was added back to the fryer in this study. In both trials, 7 kg of tallow were heated in a General Electric Hotpoint Deep-Fat Fryer (Model No. HK3). Collected samples were stored at -20°C until analysis.

Viscosity

Viscosity of all samples was determined using a Nametre 7006 Direct Readout Viscometer in conjunction with an Exacal 100 controlled temperature circulating water bath at 50±.01°C. Approximately 40g of heated tallow samples were placed in a beaker and allowed to equilibrate to 50°C for 15 minutes prior to immersion of the viscometer head. Once immersed, the sample was allowed to equilibrate for 15 minutes prior to recording the viscosity.

Color

A model D-25 Hunter Color Difference Meter with an inverted head was used to measure color differences in the heated tallow samples. A standardized yellow tile (L=78.4, a_L=-1.9, b_L=25.0) was utilized as a color reference. Approximately 70g of tallow samples were placed in an optical glass cylinder cup (7.4 cm x 1.9 cm). Color measurements were taken, aided by covering the samples with an inverted white-lined can for standard optical background.

Iodine Value

Iodine values for all tallow samples were determined using the official AOAC Method (#28.021).

Peroxide Value

Peroxide values for all tallow samples were determined using the official AOAC Method (#28.023)

Free Fatty Acid Value

Free fatty acid values for all tallow samples were determined using the official AOAC Method (#28.030)

Fatty Acid Analysis

All tallow samples were methylated by the boron trifluoride-methanol method of Morrison and Smith (1964) utilizing the preparative conditions for triglycerides. Analysis of fatty acid methyl esters was carried out with a 5830A Hewlett Packard gas chromatograph using a glass column (2m x 4mm i.d.) packed with 15% diethyleneglycol succinate on Chromosorb W 80/100 mesh (Supelco Inc., Bellefonte, Pa.) The analysis was carried out isothermally at 190^{o}C, with an injection port temperature of 210^{o}C, and flame ionization detector temperature of 300^{0}C. Nitrogen carrier gas flow rate at the detector was 40 ml/minute.

Extraction of Cholesterol and Cholesterol Oxidation Products from Tallow

The non-saponifiable fractions which contained cholesterol and any oxidative derivatives were obtained from 100g of each tallow sample by the saponification and extraction procedure described by Itoh et al. (1973). One thousand ml of 1.0N alcoholic KOH were refluxed with the tallow sample for 1 hour followed by multiple extractions with isopropyl ether. After extraction, the non-saponifiables in solution were evaporated to dryness in a Buchi Rotovapor R rotary evaporator

(Buchi Inc. Switzerland), redissolved in a known volume of ethyl acetate, flushed with nitrogen and stored at -20°C until analysis.

Thin-Layer Chromatography of Cholesterol Extracts

Analytical thin-layer chromatography plates (20cm x 20cm) were prepared using silica gel H spread at a thickness of 0.4mm. The plates were allowed to dry overnight, followed by activation at 120°C for 2 hours. One-dimensional thin-layer chromatograms were obtained for all heated tallow non-saponifiable fractions. Similarly, selected standard cholesterol oxidation products were also run on thin-layer plates. Twenty µl aliquots of samples and standards were spotted onto the plates which were developed three times in an ethyl acetate-heptane (1:1, v/v) solvent system, with air drying between irrigations. The thin-layer plates were then sprayed with 50% H_2SO_4 and heated for 15 minutes at 120°C for color development. Rf values and spot colors were recorded.

Two dimensional thin-layer chromatography was performed on the standard cholesterol oxidation products and a representative non-saponifiable fraction from a tallow sample which had been intermittently heated for 75 hours. Twenty µl aliquots were spotted on previously described thin-layer plates. Three developments in the first dimension using ethyl acetate-heptane (1:1, v/v) was followed by two developments in the second dimension using ethyl acetate-benzene (2:1, v/v). Visualization by 50% H_2SO_4 was performed as previously described.

Gas Chromatography of Cholesterol Oxidative Products

Packed and capillary columns were utilized in the gas-liquid chromatographic analysis of standard cholesterol oxidation products and the non-saponifiable fractions of all heated tallow samples. Packed

column gas chromatography analyses were performed using a Hewlett Packard 5830A gas chromatograph equipped with a flame ionization detector and a 2m x 4mm i.d. glass column packed with 3% SP-2100 on 100-120 mesh Supelcoport (Supelco, Inc., Bellefonte, Pa.). The chromatograph was operated isothermally at 260°C with a nitrogen flow rate of 40 ml/minutes at the detector. Temperatures of the detector and injection port were 350°C and 280°C, respectively.

Capillary gas chromatographic analysis was performed using a Hewlett Packard 5840A gas chromatograph equipped with a flame ionization detector. A glass capillary column (30m) coated with SP-2100 (Supelco, Inc., Bellefonte, Pa.) was used and the chromatograph was operated isothermally at 235°C with a back pressure of 0.5 $kg/_m2$. Temperature of the detector and injection port was 400°C and 120°C, respectively.

Phase II-Analysis of Tallow and French Fried Potatoes Used in Frying

Seven kg of tallow were placed in a deep-fat fryer and heated to 180±2°C. The tallow was heated intermittently for 12.5 hours per day for a total of 200 hours. Two 700g samples of french fried potatoes were fried for seven minutes per each 12.5 hour heating period. The fried samples were wrapped in aluminum foil and held at -20°C until analysis. Tallow samples were collected at the end of each 25 hour heating period, and stored at -20°C until analysis. Fresh tallow was added back to the original mark in the fryer when necessary, and the entire tallow volume was filtered every other day.

Tallow Analysis

The analysis of the tallow used to french fry potatoes was carried out as previously described with the following exceptions:

1. Fatty acid analysis was omitted due to poor resolution encountered in previous studies.

2. Sample weight for cholesterol oxidation product analysis was reduced from 100g to 15g.

3. One-dimensional thin-layer chromatography was performed using precoated Silica gel G plates (0.25mm thickness).

4. Only packed column gas chromatography was performed.

Analysis of French Fried Potatoes

Fat Analysis

The fat content of the french fried potatoes was determined by solvent extraction. A 100g aliquot of four frying samples (representing a 25 hour period of heating) were homogenized for 3 minutes in a Virtis homogenizer (Model #45) with 150 ml of hexanes-diethyl ether (9:1 v/v). The solvent extracts were filtered and combined. The homogenate was reextracted for 2 minutes with a similar volume of solvent. Combined extracts were filtered and dried for three hours over anhydrous sodium sulfate. The solvent was removed in a Buchi rotary evaporator. The fat was weighed, flushed with nitrogen, and stored at -20^{o}C until analysis. Unfried french fry potatoes were similarly extracted.

Fatty Acid Analysis

Fatty acid analysis, as described previously, was performed on lipid extracted from unfried french fry potatoes.

Extraction of Cholesterol and Cholesterol Oxidation Products

Non-saponifiable fractions of fat absorbed by french fried potatoes were obtained by methods described earlier with the exception that the sample weight was 15.0g.

Thin-layer Chromatography of Cholesterol Extracts

Thin-layer chromatography was performed as previously described with the exception that precoated Silica gel G plates (0.25mm thickness) were used.

Gas Chromatography of Cholesterol Extracts from Tallow-Fried French Fried Potatoes

Packed column gas chromatography was performed on non-saponifiable fractions of french fry lipids as described previously. Dilutions (1:1) with ethyl acetate were necessary for on-scale chromatograms.

Phase III-Analysis of Commercial French Fried Potatoes

French fried potato samples were obtained from two local fast-food franchises, collected every two days and stored at -20^{o}C until analysis. Six samples covering a 12 working day period were obtained from each franchise. Fat contents, thin-layer and gas-liquid chromatographic analyses were performed as described previously in Phase II.

RESULTS AND DISCUSSION

Phase I - The Effects of Continuous and Intermittent Heating on the Oxidative Stability of Tallow.

A. Effects on Tallow Triglycerides

The analysis of intermittent and continuously heated tallow clearly demonstrates the damaging effect of temperature cycling on the oxidative stability of frying fats. In addition, intermittently heated tallow was found to deteriorate at a much faster rate than tallow heated continuously. Perkins and Van Akkeren (1965) also noted that intermittent heating caused a more rapid decrease in the quality of cottonseed oil compared to continuous heating. This disparity in rate of deterioration can be attributed in part to the type of degradation reactions which predominate at various temperatures in the heating cycle. Frying temperatures around 180^{o}C are conducive to thermal oxidation and polymerization reactions in both intermittent and continuously heated tallow. Exposure to air at ambient temperatures facilitates autoxidation reactions in the intermittently heated tallow as well (Perkins, 1960, 1967).

Tallow as a fat has a solid crystalline structure at ambient temperatures. While in this state, surface-layer tallow triglycerides are directly exposed to autoxidative attack by atmospheric oxygen. Intermittent heating subjects tallow to such conditions. In addition, Artman (1969) stated that fats and oils previously damaged by heating are more susceptible to autoxidative reactions.

At ambient temperatures, hydroperoxides accumulate on the surface of the solid tallow matrix. When reheated, these hydroperoxides decompose, resulting in the liberation of free radicals and secondary oxidation products into the melted tallow moiety. Subsequently, these products of autoxidation may become involved in thermal oxidative or polymerization reactions at elevated temperatures. In the case of prolonged intermittent heating, this process repeats itself many times over, with autoxidation reactions occurring more readily as the total heating time increases. Likewise, the products formed in these processes accumulate in the heated tallow, resulting in increased concentrations of deleterious compounds.

Fryer design may also significantly influence the deterioration rate of intermittently heated tallow (Artman, 1969). Direct exposure heating elements, like those employed in this study, generate extremely high localized temperatures. The solid matrix of tallow at ambient temperatures completely surrounds these elements. When reheating occurs, those tallow triglyercides in contact with or adjacent to the element are exposed to temperatures sufficiently high as to induce thermal polymerization of unsaturated fatty acyl groups. This condition persists until an adequate amount of the fryer contents has melted to allow suitable mobility for convection currents in the liquid tallow to be established. Convection currents also contribute to the incorporation of oxygen in the heated tallow via entrapment at the fryer surface.

Continuously heated tallow deteriorates much more slowly than intermittently heated tallow, although the degradative mechanism is similar. The major deterrent to rapid deterioration is the lack of

autoxidative reactions in continuous heating. Propagation of the oxidative mechanism is limited due to the constant high temperature of the heated tallow. As indicated by Artman (1969), hydroperoxides which are the primary product of autoxidation reactions, are transient at best at frying temperatures. The lability of these compounds precludes their formation of secondary oxidation products. Localized heating effects may influence the stability of continuously heated tallow only at the onset of heating. Convection currents which were present for the duration of the continuous heating trial, slowly induced incorporation of oxygen into the tallow matrix, providing adequate oxygen concentrations for thermal oxidation to occur.

The results obtained in the analysis of heated tallow are presented in Table 3. Peroxide values for tallow samples heated intermittently or continuously did not exceed 3.65, reflecting very low levels of peroxides. Samples obtained for analysis were removed from the fryer while the tallow was still hot, which may explain the low values observed. Perkins (1967) noted that heated fats and oils exhibit low peroxide values, which was attributed to the aforementioned lability of such compounds at elevated temperatures. The fluctuation in peroxide values in both heating trials is attributed to this lability. A slight decreasing trend in peroxide value was noted as heating time increased. A possible explanation for such a trend may be the accumulation of oxidation products in the tallow. Higher concentrations of these products would facilitate their spontaneous reaction with newly formed hydroperoxides. Peroxide values for both heating trials may be indicative of both hydroperoxides formed as the

Table 3. Chemical and physical characteristics of continuously and intermittently heated tallow

Time of Heating (Hours)	Iodine Value		Peroxide Value (meq/kg)		Free Fatty Acid (%)		Viscosity*	
	Int.	Cont.	Int.	Cont.	Int.	Cont.	Int.	Cont.
0	44.3	44.5	0.0	0.0	.70	.69	28.0	27.3
25	42.9	---	3.7	---	.67	---	30.2	---
50	41.6	44.0	2.8	3.5	.63	.644	32.5	29.0
75	41.7	---	3.3	---	.65	---	34.2	---
100	40.5	43.2	3.3	3.3	.66	.71	39.0	33.0
125	39.8	---	3.0	---	.67	---	40.2	---
150	39.7	42.4	2.9	2.4	.71	.70	43.5	37.3
175	37.7	---	3.3	---	.74	---	50.5	---
200	38.8	41.9	3.4	3.0	.75	.712	59.1	41.7
225	36.4	---	2.7	---	.76	---	68.2	---
250	35.9	40.9	2.5	2.9	.86	.95	85.5	49.6
275	34.8	---	1.6	---	.99	---	110	---
300	---	40.0	---	2.3	---	.840	---	65.3

* Units - centipoise x g/cm 3 x 10^{-2}

primary oxidation product of unsaturated fatty acyl groups and peroxyl functions formed by the dismutation of such hydroperoxides.

Iodine values for the two heating trials clearly indicate that the loss of unsaturation is greater for a given time interval in the intermittently heated tallow (Table 3). The comparative loss of unsaturation in continuous and intermittently heated tallow is represented graphically in Figure 2. Intermittently heated tallow exhibited a decrease in iodine value from 44.3 to 34.8 after 275 hours of heating, representing a decrease of 24.1%. Continuously heated tallow showed a decrease of 10.1%, dropping from 44.5 to 40.0 after 300 hours of heating. This difference in the rate of decrease of iodine value can be attributed to the action of autoxidative reactions in intermittently heated tallow at ambient temperatures.

Concommitant with the loss of unsaturation, viscosity of the tallow samples increased (Table 3). This phenomenon was also noted by Perkins (1967). Intermittently heated tallow exhibited a greater increase in viscosity than did continuously heated tallow over a similar period of time. A comparative graphical representation of viscosity changes for both heating trials is shown in Figure 3.

Viscosity levels in heated fats and oils are indicative of the amounts of dimeric and polymeric compounds in the lipid moiety (Smouse, 1975). As seen from the data, intermittent heating appears to be more conducive to the formation of these compounds. From the previous discussion, products of autoxidation at ambient temperature, and recurrent localized heating facilitate polymerization reactions in

Figure 2. Effect of heating time on the iodine value of tallow heated intermittently (B) and continuously (C).

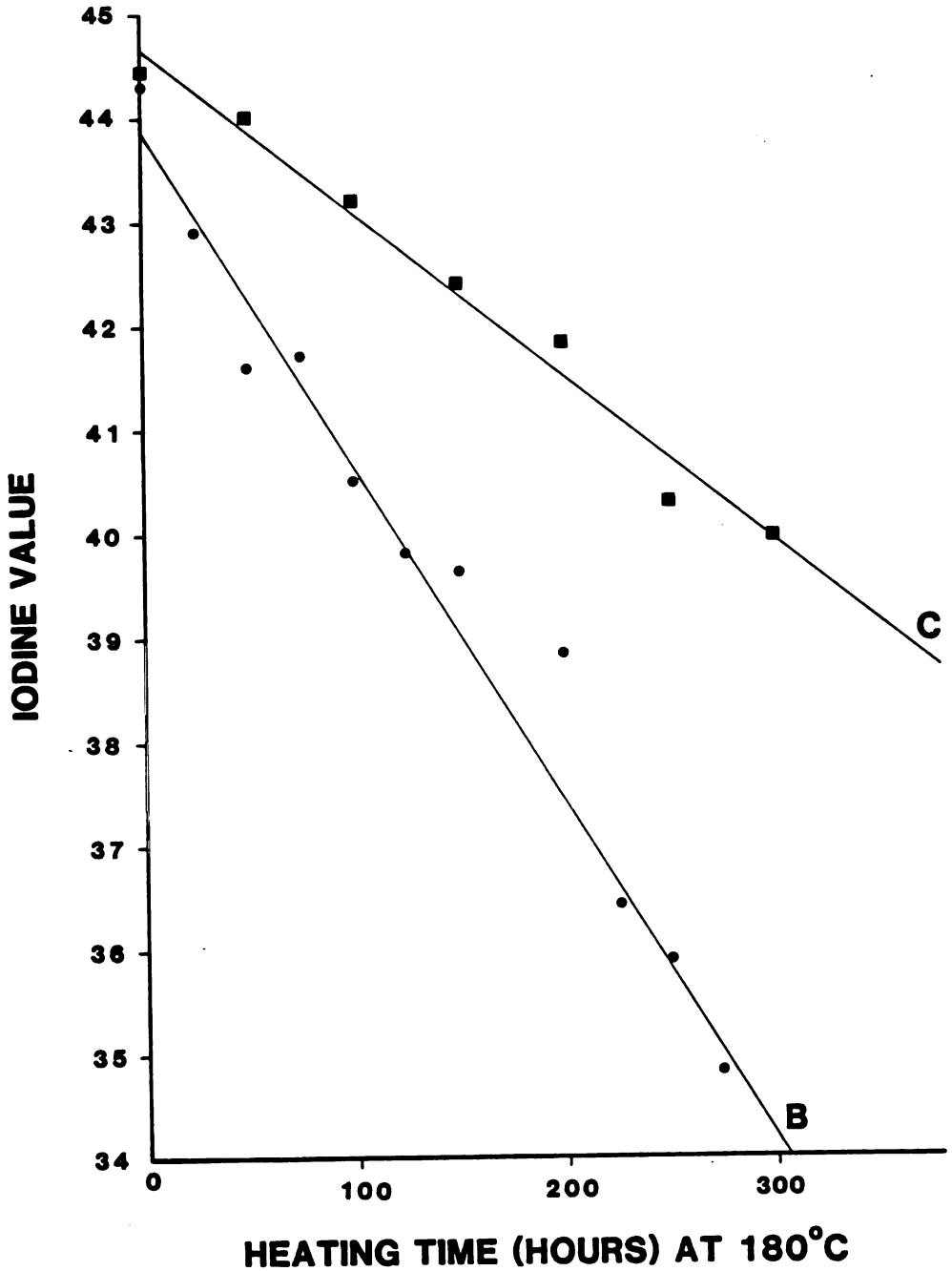
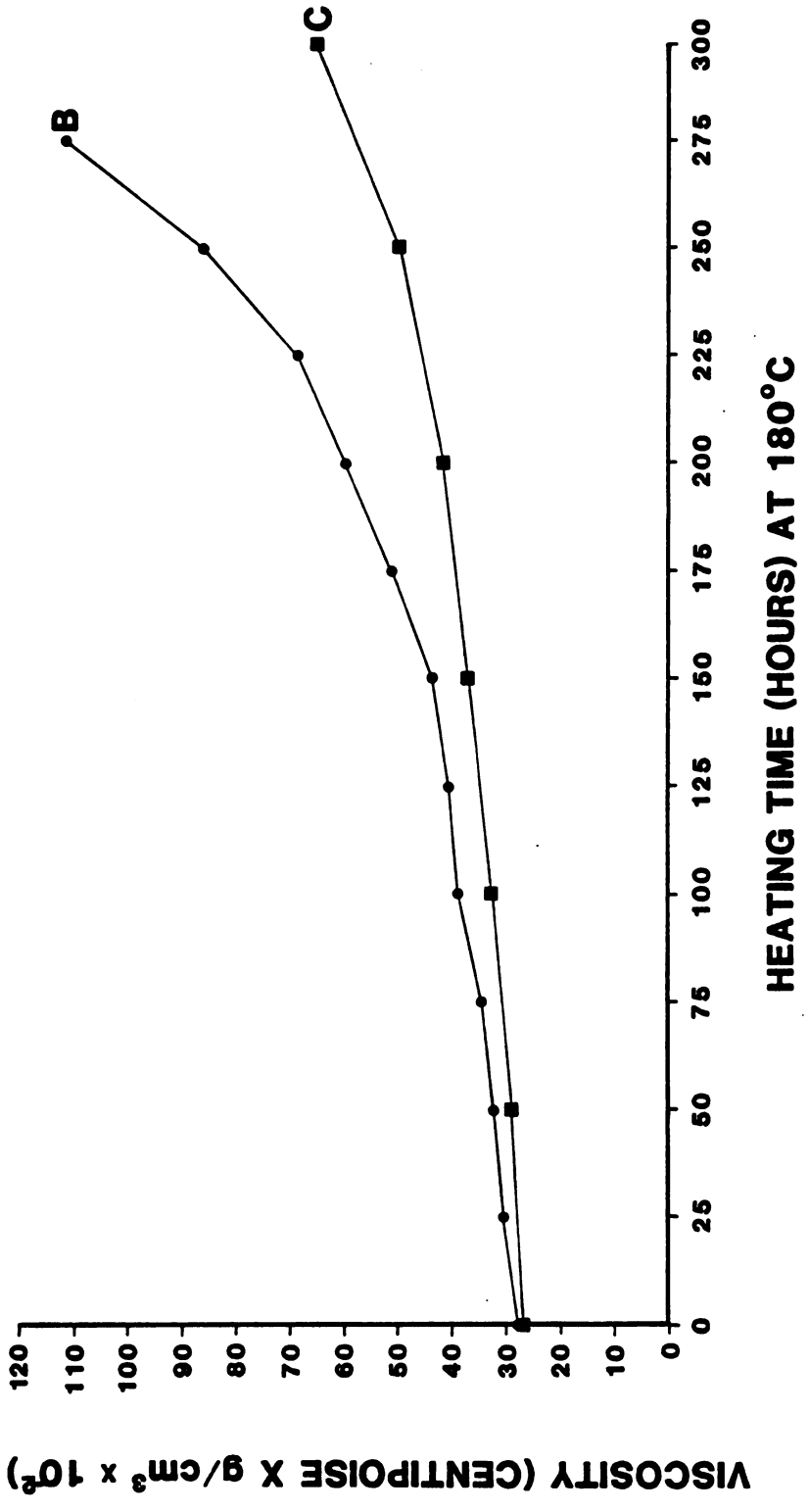

45
44
43
42
41
40
39
38
37
36
35
34
IODINE VALUE
0
100
200
300
HEATING TIME (HOURS) AT 180°C
C
B

Figure 3. Effect of heating time on the viscosity of tallow heated intermittently (B) and continuously (C).


B
C
0
25
50
75
100
125
150
175
200
225
250
275
300
HEATING TIME (HOURS) AT 180°C
0
10
20
30
40
50
60
70
80
90
100
110
120
VISCOSITY (CENTIPOISE X g/cm³ x 10²)

an intermittently heated fat system.

Analysis of iodine values and viscosity data for the two heating trials possibly indicates an inherent difference in the type of reactions occurring in each heating system. Tallow heated intermittently for 125 hours exhibited a 4.5 iodine value unit drop, with a corresponding viscosity increase of 12.2 centipoise x g/cm 3 x 10^{-2}. Tallow heated continuously for 300 hours also exhibited a 4.5 unit drop in iodine value, but had a corresponding viscosity increase of 38.0 centipoise x g/cm 3 x 10^{-2}. These data may indicate that, although the loss of unsaturation per unit time is greater in intermittently heated tallow, the reaction involving those double bonds are different in the two heating systems. A greater proportion of the unsaturated linkages lost via continuous heating appeared to be involved in dimerization or polymerization reactions. An equivalent loss of unsaturated bonds via intermittent heating produced approximately one-third the increase in viscosity noted in continuously heated tallow. This may be attributed to the autoxidative reactions encountered in intermittently heated tallow at ambient temperatures. Therefore, the cause for this disparity may be due to the differences in the types of reactions occurring in the two heating systems. Loss of unsaturation in intermittently heated tallow occurs via thermal oxidation and polymerization reactions at elevated temperatures, and by autoxidation reactions at ambient temperatures (Perkins and Van Akkeren, 1965; Perkins, 1967). Continuous heating of tallow allows only thermal oxidative and polymerization reactions, as autoxidation reactions are incapable of occurring under constant frying temperature conditions.

Free fatty acid values for the intermittent and continuously heated tallow samples increased with heating time (Table 3). In both trials, the free fatty acid control remained below 1%, which correlated well with the findings of Bates (1952). It was noted that with prolonged heating, the tallow samples became increasingly darker which interfered with accurate endpoint determination by phenolphthalein. Although the recorded values are indicative of the amount of hydrolysis occurring in the triglyceride moiety, free fatty acids may become involved in various decomposition reactions (Chalmers, 1951) or polymerization reactions as indicated by the many studies of fatty acid polymers reviewed by Artman (1969). Short chain fatty acids, liberated via hydrolysis or homolytic cleavage of hydroperoxides, may volatilize at frying temperatures (Chang et al., 1978).

Color data for the heated tallow samples are found in Table 4. Color changes were similar for the continuous and intermittently heated tallow samples. It was observed that the tallow which was heated intermittently changed color at a greater rate than the continuously heated samples. Artman (1969) reported that the browning phenemonon accompanies the degradation of a heated fat. Mukai et al. (1965), as cited by Artman (1969), attributed this darkening to the presence of α, β, and α,α unsaturated carbonyl compounds formed via scissioning of hydroperoxides. Also implicated as color contributors in heated fats were highly polar dimeric compounds formed in polymerization reactions of secondary oxidation products (Perkins and Kummerow 1964; Frankel et al., 1960).

Table 4. Hunter colorimeter data of continuously and intermittently heated tallow

Time of Heating (hours)	L		aL		bL	
	Int.	Cont.	Int.	Cont.	Int.	Cont.
0	+42.7	+42.9	-6.1	-6.0	+13.2	+10.9
25	+40.7	--	-3.9	--	+10.8	--
50	+40.0	+38.4	-4.2	-3.7	+13.1	+14.7
75	+38.9	--	-4.2	--	+16.3	--
100	+37.9	+35.0	-4.5	-2.2	+18.7	+19.3
125	+36.6	--	-3.5	--	+20.5	--
150	+34.6	+30.2	-1.3	+3.7	+21.3	+19.2
175	+30.6	--	+4.8	--	+19.7	--
200	+30.8	+26.8	+4.9	+8.3	+20.1	+17.6
225	+27.6	--	+9.5	--	+18.0	--
250	+24.9	+23.0	+12.9	+12.8	+16.3	+14.8
275	+18.9	--	+17.2	--	+12.1	--
300	--	+16.1	--	+17.1	--	+10.0

Thompson et al. (1967) reported poor resolution of fatty acid methyl esters derived from heated fats and oils when analyzed by gas chromatography. Hydroxy, keto, dibasic and polymeric acids which form during heating are retained in the column. Further cis-trans isomerization as well as conjugation of polymeric fatty acids have been implicated as sources of poor resolution.

Similar results were observed in the heated tallow samples. Analysis by gas-liquid chromatography (GLC) became difficult after only 25 hours of heating in the intermittent system and after 50 hours in the continuous system. Despite the poor resolution, a decrease in unsaturated fatty acids in the sample was noted with prolonged heating (Table 5). This substantiates the literature on the subject as well as the iodine value data obtained in this phase of the study.

B. Effects of heating on the stability of cholesterol

Concurrent with the oxidation of heated tallow triglycerides, the oxidative state of cholesterol present in tallow also changes. Cholesterol levels in tallow have been reported to be 141 mg/kg by Punwar and Derse (1978). Cholesterol under conducive conditions, such as high temperatures and exposure to oxygen, may oxidize to form angiotoxic (Imai et al., 1976; Peng et al., 1978) or carcinogenic compounds (Bischoff, 1969; Smith and Kulig, 1975).

Separation of cholesterol and any oxidative derivatives was achieved using the saponification technique described by Itoh et al. (1973). Both continuously and intermittently heated tallow samples and an unheated tallow control were saponified. Analysis of the tallow non-saponifiables was performed using thin-layer chromatography (TLC) and gas-liquid chromatography (GLC).

Table 5. Fatty acid analyses of intermittently and continously heated tallow

Intermittent (Hours)	14:0	14:1	16:0	16:1	18:0	18:1	18:2	18:3	S/U
0	4.0	1.3	26.6	8.8	15.9	41.7	1.7	--	0.87
50	4.5	3.0	26.8	10.5	16.2	36.6	2.4	--	0.90
100	4.4	2.4	27.5	9.2	17.2	39.3	--	--	0.96
150	4.7	1.5	29.4	9.1	17.4	37.9	--	--	1.06
Continuous Hours									
0	3.4	1.3	26.6	8.8	15.9	41.7	1.7	--	0.87
50	4.4	2.6	26.4	11.3	16.3	39.0	2.9		0.89
100	4.4	2.6	27.0	10.0	17.0	39.0	--	--	0.93
150	4.5	2.3	28.4	9.9	17.1	37.9	--	--	1.00

Table 6. Oxidative products of cholesterol extracted from heated tallow fractions - TLC analysis

R_f	Color	Identity
1.00	---	Solvent front
0.82	Beige/brown	3,5-Cholestadien-7-one[a]
0.68	Magenta	Cholesterol[a]
0.60	Yellow	Unidentified[b]
0.42	Yellow	Unidentified
0.28	Blue	5-Cholesten-3β,7β-diol
0.23	Blue	5-Cholesten-3β,7 α-diol

[a] Compounds present in original tallow.
[b] Additional compound present in the 75 hour sample only.

TLC analysis of the tallow non-saponifiables indicated that cholesterol subjected to frying conditions is prone to oxidative changes (Table 6). Unheated tallow was found to contain cholesterol with only a trace amount of 3,5-cholestadiene-7-one, indicating that the processes employed in refining tallow did not seriously alter its oxidative state. Analysis of the intermittent and continuously heated tallow non-saponifiable fractions indicated cholesterol underwent oxidation, and that intermittent heating caused a more rapid change in cholesterol stability. This can be attributed to the higher concentration of free radicals in the intermittently heated system. The rate of cholesterol loss and concommitant appearance of oxidation products was greater in the intermittently heated samples as indicated by TLC analysis. It should be noted that similar compound formation and loss in cholesterol was observed in the continuously heated tallow, but at a

much slower rate. Since the types of products formed from cholesterol in the heated tallow systems were similar, a representative sample was utilized to investigate the identity of the cholesterol derivatives. The 75-hour intermittently heated fraction was chosen.

One dimensional TLC analysis of the intermittently heated tallow non-saponifiable fractions indicated six distinct spots after 75 hours of heating. However, it was observed that one compound present in the 75 hour sample was not apparent after 150 hours of heating, probably due to decomposition by further oxidation. Also noted was the concommittant decrease in the size of the cholesterol spot with increasing intensity of the oxidation products. The characteristics color and Rf values obtained indicated the presence of 5-cholesten-3β , 7α-diol, 5-cholesten-3β,7β -diol, 5-cholesten-3β -ol, and 3,5-cholestadiene-7-one. Noteworthy was the impurity of the standard cholesterol oxides purchased for this study. The impure state of these compounds hindered both thin-layer and gas-liquid chromatographic analysis of the heated tallow extracts.

In order to further establish the identity of the spots, two dimensional TLC was performed (Table 7). The Rf values for the standards used corresponded to six of the seven spots obtained in the two dimensional analysis. Color development was also compatible with the standard cholesterol oxidation products. Compounds identified included 5-cholesten-3β, 7α-diol 5-cholesten-3β,7β -diol, 5-cholesten-3β -ol and 3,5-cholestadiene-7-one. Cholestan-3β,5α,6β -triol was also tentatively identified as the brown spot close to the origin. The low Rf yellow spot may be cholestan-5α,6-epoxy -3β-ol or 5-cholesten-3β-ol -7-one, but impurities in standards prevented positive identification.

Table 7. Two dimensional TLC analyses of oxidation products of cholesterol in tallow heated intermittently for 75 hours and standard compounds.

Compound	Rf value Solvent 1[a]	Rf value Solvent 2[b]	Color
Tallow			
solvent front	1.00	1.00	
3,5-cholestadiene-7-one	0.85	0.72	brown
5-cholesten-3β-ol	0.71	0.52	magenta
unidentified	0.59	0.52	yellow
unidentified	0.47	0.25	yellow
5-cholesten-3β,7β-diol	0.29	0.21	blue
5 cholesten-3β,7α-diol	0.22	0.11	blue
cholesten-3β, 5α, 6β-triol	0.05	0.03	brown
Standards			
3,5-cholestadiene-7-one	0.85	0.72	brown
5-cholesten-3β-ol	0.72	0.53	magenta
cholesterol-α-oxide	0.53	0.25	brown/yellow
5-cholesten-3β,7β-diol	0.30	0.19	blue
5-cholesten-3β,7α-diol	0.24	0.11	blue
cholesten-3β, 5α,6β-triol	0.06	0.04	brown

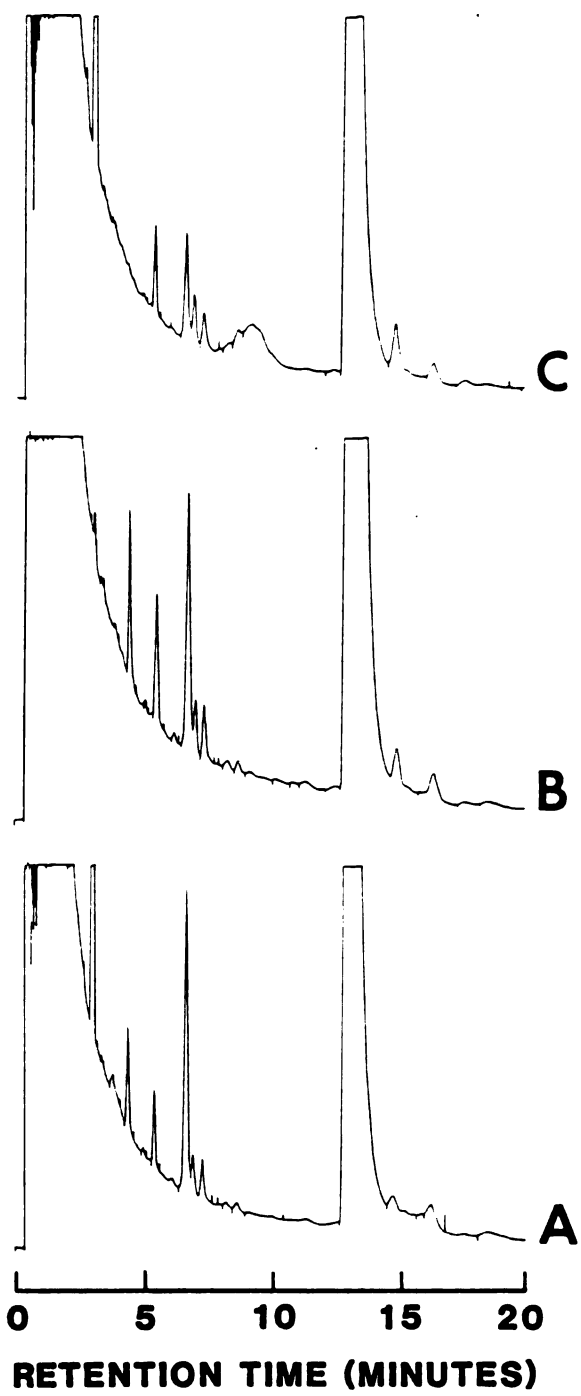
[a] ethyl acetate - heptane (1:1, v/v)
[b] ethyl acetate - benzene (2:1, v/v)

GLC utilizing both capillary and packed columns was employed to analyze the sterol extracts from the heated tallow samples. In both heating trials, an increase in the number of peaks was noted with extended heating. The evolution of peaks occurred at a faster rate in the intermittently heated tallow compared to those samples heated continuously. Chromatograms obtained in capillary GLC analysis for intermittently and continuously heated samples are shown in Figures 4 and 5. Standard compounds were also injected as a means of identification, but results were not conclusive due to the impurity of the standards. Capillary column GLC indicated a greater number of peaks than did packed column GLC.

In order to verify the identity of the cholesterol oxidation products formed in heated tallow, compounds were isolated and concentrated using preparative TLC. The samples obtained were subjected to capillary GLC and then to mass spectrometry. The identities of 3,5-cholestadiene -7-one, 5-cholesten-3β-ol, 5 cholesten-3β, 7α-diol and 5-cholesten-3β, 7β-diol were tentatively established by these procedures. The mass spectra data obtained for the two unidentified yellow spots were inaccurate due to a variety of interferring compounds, thus no definite identification was possible.

Figure 4. Gas chromatograms of tallow non-saponifiable fractions heated intermittently for A) 0 hours, B) 50 hours, C) 100 hours and D) 150 hours.

Figure 5. Gas chromatograms of tallow non-saponifiable fractions heated continuously for A) 50 hours, B) 100 hours and C) 150 hours.


C
B
A
0
5
10
15
20
RETENTION TIME (MINUTES)

As stated in the literature review, cholesterol as an unsaturated lipid is prone to oxidation. Clearly, the results of this phase of the study indicate that frying conditions, consisting of elevated temperatures in the presence of oxygen, facilitate oxidative changes in cholesterol. Cholesterol oxidation products identified from heated tallow are similar to those reported from aged cholesterol (Horvath, 1966; Smith et al., 1967) or intermittently oxidized crystalline cholesterol (Fioriti and Sims, 1967).

Cholesterol in tallow heated intermittently or continously follows an oxidation mechanism similar to that given in Figure 1. Attack of a cholesterol molecule by oxygen yields a peroxy radical at the C_7 position. It is this reaction which causes the difference in rate of cholesterol oxidation in the two heating trials. The intermittently heated system has a greater oxygen concentration due to incorporation of oxygen via autoxidation at ambient temperatures. Furthermore, the subsequent number of free radicals which may effectively abstract the C_7 hydrogen is greater in this system. The abstraction of this hydrogen atom by secondary autoxidation products would greatly facilitate the direct addition of oxygen. Thus, both oxygen and ionizing free radical concentrations are greater in the intermittently heated tallow. Continuously heated tallow incorporates oxygen only by surface entrapment, lowering both the oxygen content and the subsequent radical concentration in the tallow moiety.

With the formation of the cholesterol peroxy radical, abstraction of an allylic hydrogen from an unsaturated fatty acyl group may readily occur. The resulting hydroperoxides spontaneously decompose via formal

reduction to the epimeric 3β , 7-diols evident in the heated tallow fractions. Similarly decomposition of the 3β- 7-hydroperoxides of cholesterol may readily undergo dehydration reactions due to the high temperature conditions in the fryer.

5-cholesten-3 β-ol-7-one was positively identified by TLC results. The final dehydration product, 3,5-cholestadiene-7-one, was positively identified by two dimensional TLC and GC mass spectrometry. Thus the existence of 5-cholesten-3 β-ol-7-one in heated tallow is suspected to be transient at such elevated temperatures.

Smith (1980) stated that reaction of cholesterol-7-hydroperoxide with an allylic hydrogen or hyderoperoxide may cause the formation of the epimeric cholestan-5, 6α -epoxy-3β -ol and cholestan-5,6β -epoxy -3β-ol. Although these compounds were not positively identified in the heated tallow fractions, their presence is suggested by the identification of their hydration product, cholestan-3β , 5α ,6β-triol, in the intermittently heated tallow. Water, formed as a product of various oxidation reactions, may be present in heated tallow systems. Thus, the formation of α or β-oxides of cholesterol in heated tallow systems may be followed by spontaneous hydration, yielding the triol species.

With regards to the analytical procedures employed in this study, it appears that two dimensional TLC analysis is sufficient to positively identify cholesterol oxidation products. Smith (personal communication) has indicated that Rf values and color development after acid treatment adequately confirm the identities of sterols when compared to standard references. Various anomalies have been reported by researchers concerning alternate techniques of analysis of sterols.

Fioriti and Sims (1967) noted the behavior of cholesterol autoxidation compounds during GLC analysis. 5-Cholesten-3β ,7α -diol and 5-cholesten-3β, 7β -diols were found to undergo an on-column decompositon, with the decomposition products stable at operating temperatures. Claude (1966) observed that the 3β , 7-diols decomposed to products having retention times similar to 3,5-cholestadiene-7-one, indicating the formation of a diene-like species. Similarly, Van Lier and Smith (1968) noted the conversion of 5-cholesten-3β -ol-7-one to 3,5-cholestadiene via on-column dehydration. The same reseachers also stated that the presence of the 3β, 7-diols cannot be confirmed due to the previously noted on-column decomposition. In conclusion, it was stated that no single system or derivatization procedure adequately separates important compounds formed in cholesterol oxidation.

Sheppard and Shin (1980) identified the inherent impurities of standard cholesterol oxidation products as a major deterrent to elucidation of the cholesterol oxidative mechanism. Impurities in the standards were encountered in this study which seriously hindered GLC and GC-MS investigations.

Phase II. The Effects of Intermittent Frying on the Oxidative Stability of Tallow.

In the first phase of this study, it was established that intermittent heating of tallow resulted in the rapid degradation of the fat and oxidation of cholesterol. In order to replicate commercial practices more closely, a study involving the production of french fries by frying in intermittently heated tallow was conducted.

A. Effects of Frying in Tallow

The results of the analyses performed on the tallow samples indicate that frying had a suppressive effect on deterioration. Physical and chemical data showed that the action of frying in intermittently heated tallow resulted in a less rapid deterioration than tallow heated intermittently without the introduction of fried substrate. The tallow used for frying did however deteriorate faster than the continuously heated tallow studied in Phase I.

The introduction of fried products to an intermittently heated system results in extension of the fat life due to the constant absorption of the frying medium by the substrate and the subsequent addition of fresh fat or oil to the fryer (Melnick, 1957b; Artman, 1969). Absorption of fat by the subtrate also results in the absorption of degradation products (Hussain and Morton, 1976). Add-back of fat dilutes the degradation products, as well as providing a fresh matrix for frying. Greater frying loads results in more add-back of fat to the fryer which results in a longer frying life (Melnick, 1957b). In addition, steam generated from water in the food substrate may carry volatile degradation products from the fat (Chang et al., 1978), as well as occlude oxygen from the fryer surface, deterring oxidative reactions (Melnick et al., 1958).

Peroxide values for the tallow, presented in Table 8, reached a maximum of 3.8 in the 200-hour study. This low value is the result of factors previously discussed in Phase I of this study. The introduction of french fried potatoes into the tallow or the replacement of fresh tallow into the fryer did not seem to influence the rate of peroxide formation.

Iodine values of the tallow decreased steadily with extended exposure to frying temperature (Table 8). The tallow showed a drop in iodine value from 44.2 initially to 40.9 after 150 hours of heating representing a 7.5% decrease. Tallow samples heated intermittently and continuously exhibited 10.5% and 4.6% decrease, respectively (Table 3). Graphical representation of these data is found in Figure 6.

Similarly, viscosity levels of the utilized tallow increased with prolonged heating (Table 8). This increase was noted as the iodine values for the samples decreased. A comparison of the viscosity data from the used intermittently heated tallow and the viscosity data derived from the two heating trials in Phase I is presented in Figure 7. The tallow in which french fry potatoes were processed increased in viscosity from 27.0 to 51.3 centipoise x g/cm^3 x 10^{-2} after 200 hours of heating, representing a 24.3 unit increase. Intermittent and continuously heated tallow exhibited a 31.1 and 14.4 unit increase after 200 hours of heating, respectively (Table 3).

Free fatty acid values for the utilized tallow indicated no increase in hydrolytic activity due to the introduction of water into the frying medium via french fry potatoes (Table 8). Values did not differ appreciably from those obtained in the analysis of continuous and intermittently heated tallow to which no product was introduced.

Table 8. Physical and chemical characteristics of tallow used in frying study

Time of Heating (Hours)	Iodine Value	Peroxide Value (meq/kg)	Free Fatty Acid (%)	Viscosity[a]
0	44.2	0.0	0.70	27.0
25	44.0	2.2	0.68	28.4
50	43.2	3.8	0.63	29.9
75	42.4	2.4	0.63	31.9
100*	42.0	2.3	0.69	33.8
125	41.2	2.2	0.67	36.3
150*	40.9	2.1	0.71	39.2
175	40.0	1.9	0.72	45.6
200	38.7	2.1	0.74	51.3

* Tallow added back to fryer
[a] Units = centipose x g/cm^3 10_{-2}

Figure 6. Effect of heating time on the iodine value of tallow heated intermittently B), continuously C), and intermittently with frying D).

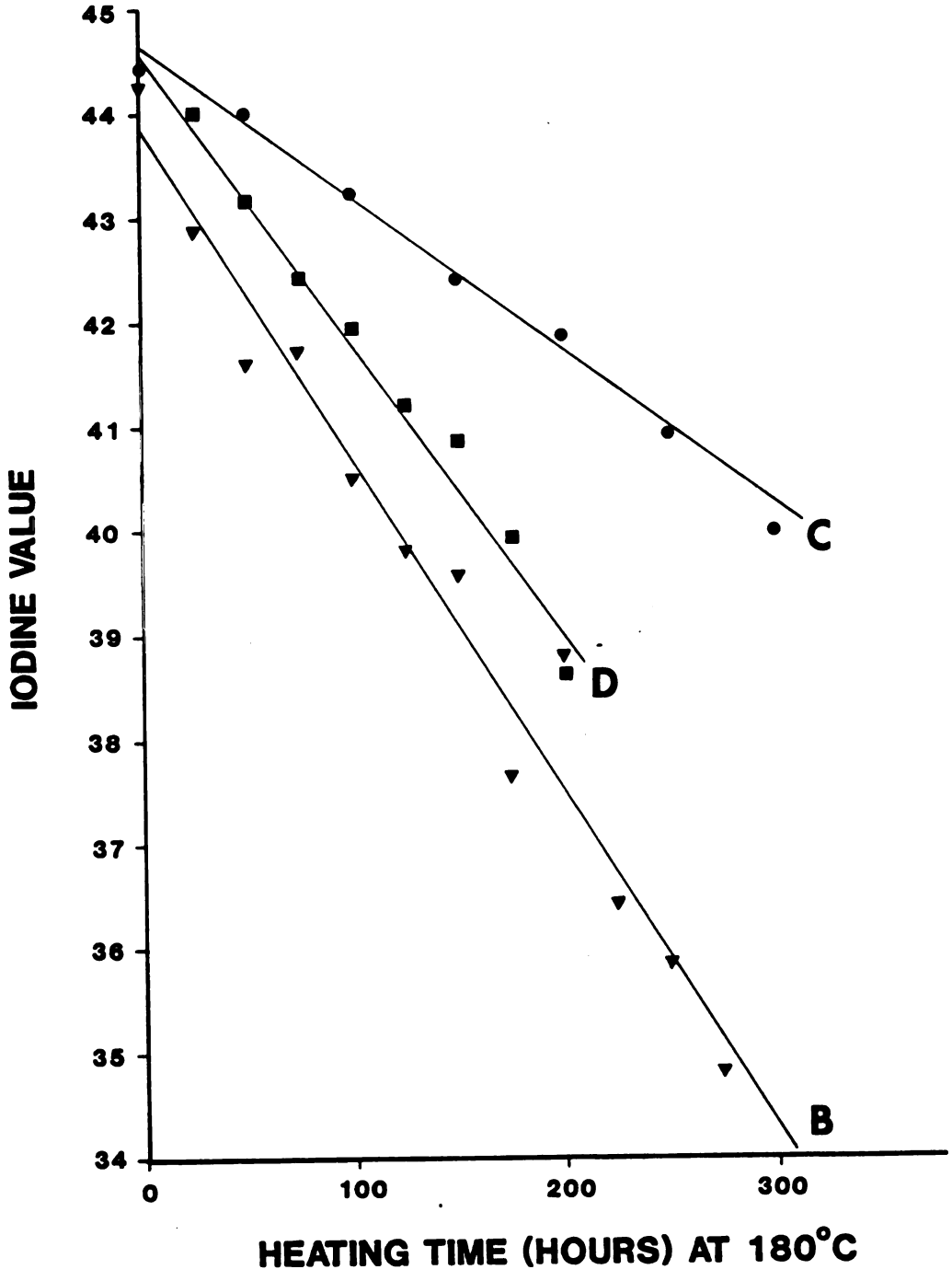
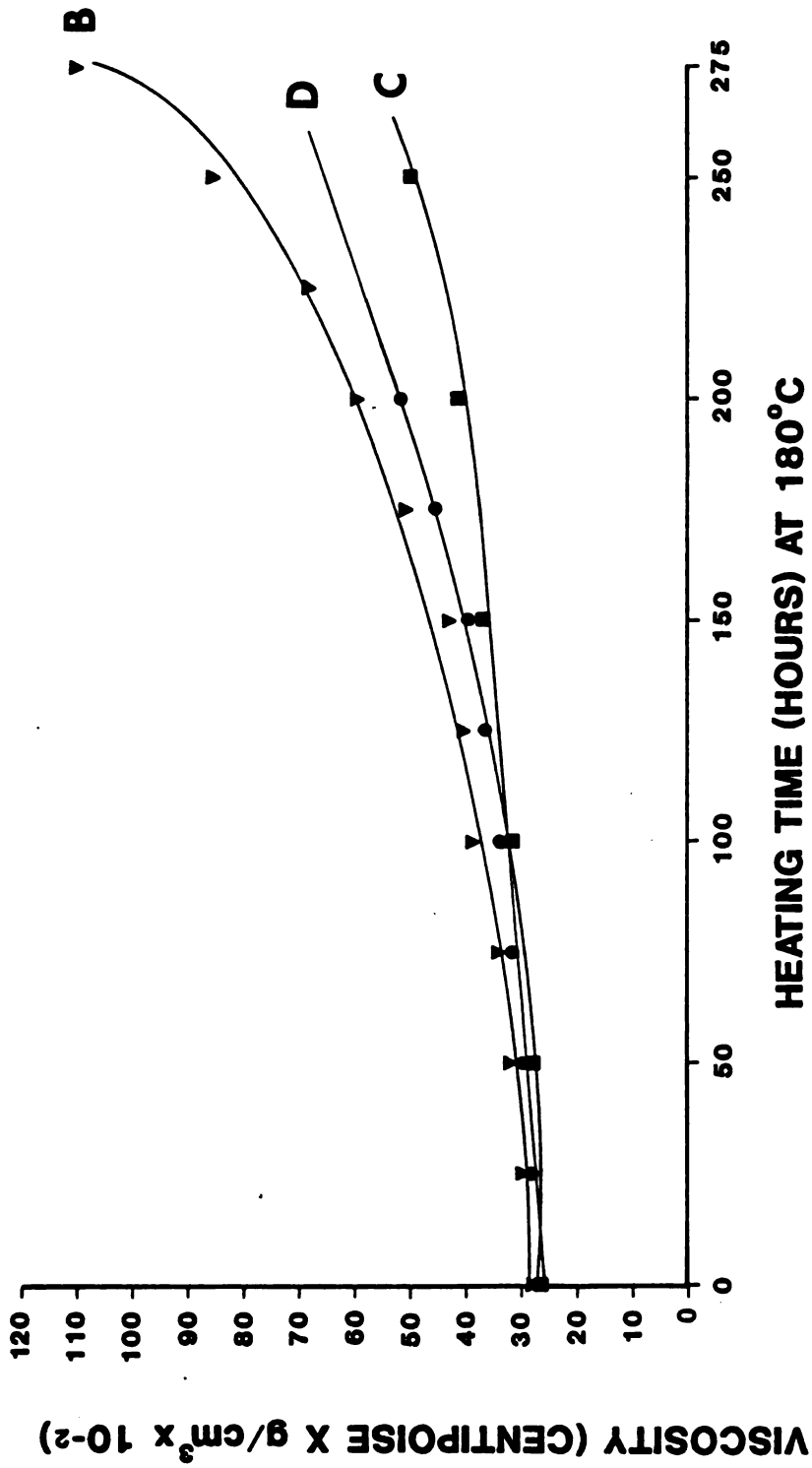

45
44
43
42
41
40
39
38
37
36
35
34
IODINE VALUE
0
100
200
300
HEATING TIME (HOURS) AT 180°C
C
D
B

Figure 7. Effect of heating time on the viscosity of tallow heated intermittently B), continuously C) and intermittently with frying D).


B
D
C
0
50
100
150
200
250
275
HEATING TIME (HOURS) AT 180°C
0
10
20
30
40
50
60
70
80
90
100
110
120
VISCOSITY (CENTIPOISE X g/cm³ x 10-²)

Although these results indicate that hydrolytic activity did not increase, loss of free fatty acids by steam distillation or absorption by the substrate may compensate for the low free fatty acid values (Chang et al., 1978; Pokorny, 1980).

Color data for the utilized tallow are found in Table 9. These data indicate a darkening of the tallow as heating time progressed, as previously noted for heated tallow used in Phase I of this study.

Intense foaming occurred in the tallow medium after 125 hours of intermittent heating. This phenomenon can be attributed to the buildup of polymeric and dimeric compounds in the tallow matrix as evidenced by increases in viscosity levels (Morton, 1977). Smouse (1975) has reported that various vegetable oils foamed after similar heating. This was attributed to polymeric substances as well as free fatty acids in the heated oil system.

Analysis of the non-saponifiable fractions from the tallow samples indicated that oxidation of cholesterol occurred during the frying procedure (Table 10). TLC analyses confirmed the presence of 5-cholesten-3β , 7α -diol, 5-cholesten-3β,7β-diol, 5-cholesten-3β-ol and 3,5-cholestadiene-7-one in the tallow samples after 25 hours of heating. An unidentified yellow spot was also observed with a similar Rf value to the unknown compound noted previously (Table 6). An additional magenta spot was isolated which could not be identified by reference standards. All oxidation products increased with prolonged heating at the expense of cholesterol.

Table 9. Hunter colorimeter data for tallow utilized for frying

Time (Hours)	L	aL	bL
0	+42.1	-4.1	+2.2
25	+37.0	-3.5	+5.6
50	+33.2	-0.8	+5.2
75	+28.7	+3.9	+1.7
100	+24.1	+8.1	-4.7
125	+21.1	+10.6	-9.6
150	+19.4	+11.8	-12.6
175	+17.4	+12.7	-17.3
200	+15.7	+13.3	-21.4

Table 10. TLC analysis of the non-saponifiable fractions of tallow used in frying

Rf Value	Color	Intensity with Time	Identity
1.00	----	--	solvent front
0.98	brown	increase	3,5-cholestadiene-7-one
0.95	magenta	no change	unidentified
0.87	magenta	decrease	5-cholesten-3β-ol
0.68	yellow	increase	unidentified
0.47	blue	increase	5-cholesten-3β,7β-diol
0.39	blue	increase	5-cholesten-3β, 7α-diol

GLC results of the non-saponifiable fractions from the tallow indicate that the overall sterol profile remained somewhat constant for the duration of the study (Figure 8). An increase in the peak eluting immediately after cholesterol was noted. This peak also increased with extended heating in both the intermittent and continously heated samples (Figure 4 and 5). A series of small peaks eluting after the cholesterol peak may be due to plant sterols which migrated from the french fried potatoes to the tallow during frying. The chromatogram of the non-saponifiable fraction of unfried french fry potatoes lipids is found in Figure 9 and will be discussed later in the text.

B. Analysis of French Fried Potatoes

The results of the fat absorption of french fry potatoes fried in tallow are found in Table 11. The fat content of the french fried potatoes ranged from 19.78 to 24.48%, which was appreciably higher than fat contents reported for commercially prepared french fried potatoes by Slaver et al. (1980). The noted higher fat content may be attributed to the larger size and greater surface area of the crinkle cut potatoes used in this study.

TLC analysis of the non-saponifiable fractions of the fat absorbed by french fried potatoes showed that the oxidation products formed in tallow were absorbed by the french fried potatoes as well (Table 12). Similar to the sterol profiles for the tallow samples, all oxidation products present increased with prolonged heating at the expense of cholesterol.

Figure 8. Gas chromatograms of tallow non-saponifiable fractions from tallow samples intermittently heated for frying. (A) 50 hours, (B) 100 hours and (C) 150 hours

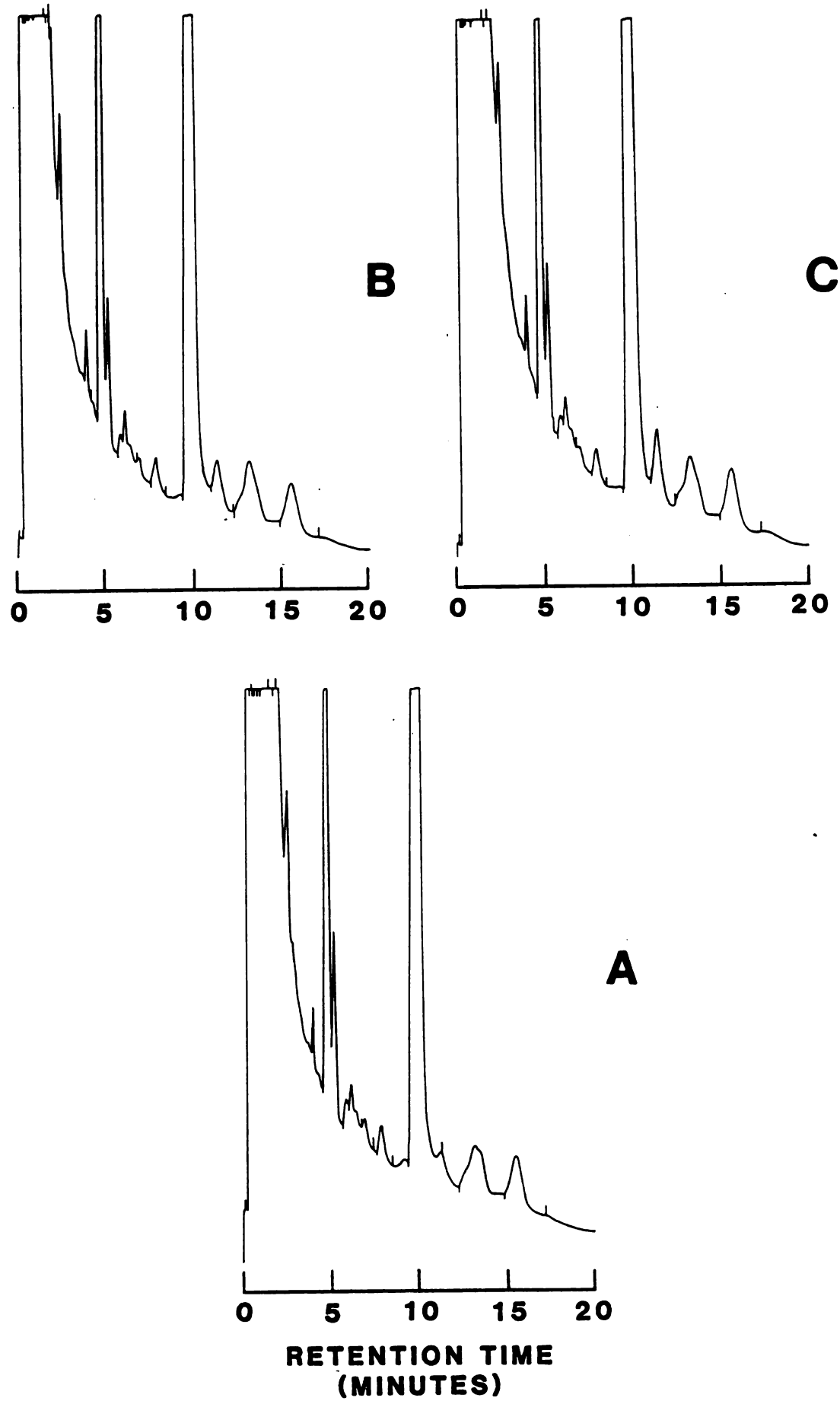

B
C
A
0
5
10
15
20
RETENTION TIME
(MINUTES)

Table 11. Absorption of fat by french fry potatoes fried in tallow

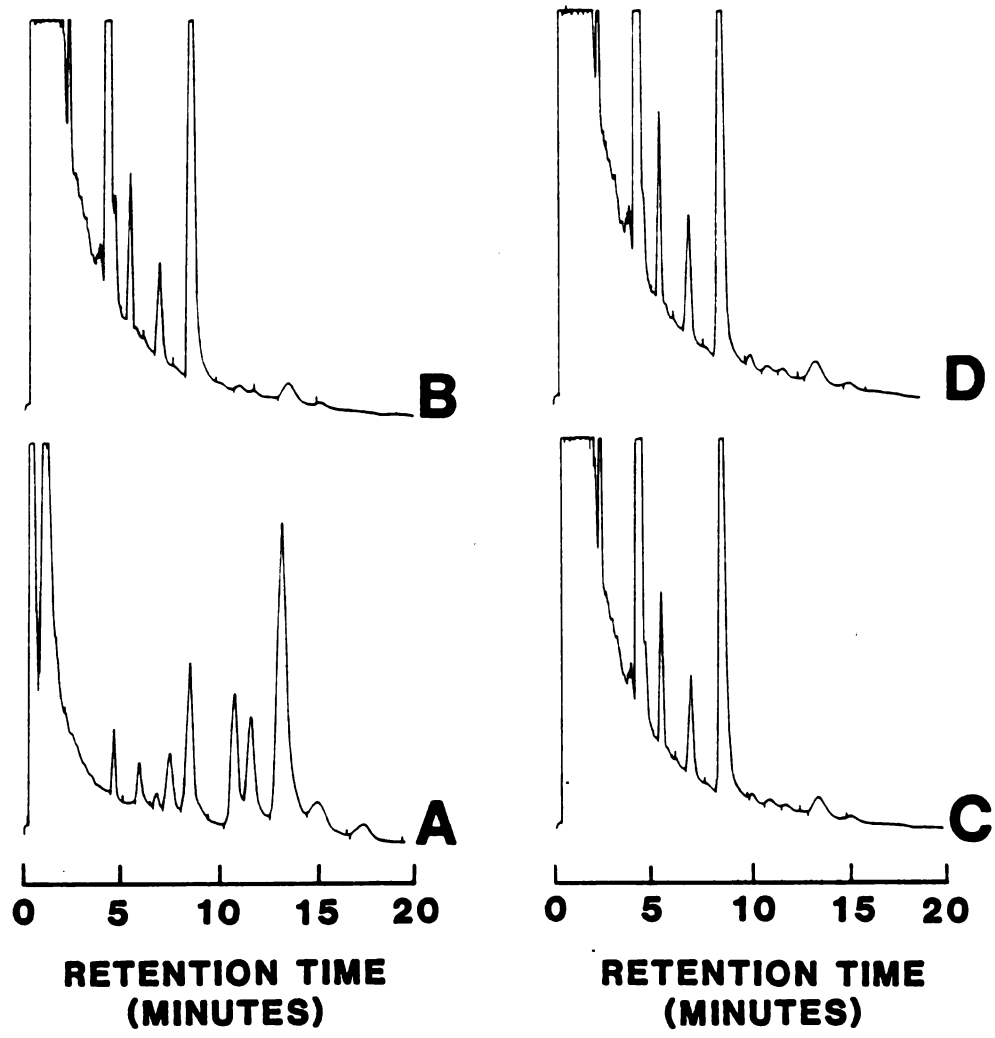
Sample Number	Time Range of Frying (Hours)	Grams of Fat in 400 G Sample	%Fat
Control	---	10.1	2.5
1	0-25	79.1	19.8
2	25-50	87.5	21.9
3	50-75	97.9	24.5
4	75-100	81.1	*20.3
5	100-125	86.7	21.7
6	125-150	81.6	*20.4
7	150-175	88.8	22.2
8	175-200	90.2	22.6

*Fresh tallow added back to fryer

Table 12. TLC analysis of the non-saponifiable fraction of fat extracted from tallow-fried french fried potatoes

Rf Value	Color	Intensity with time	Identity
1.00	--	--	solvent front
.99	beige/brown	increase	3,5-cholestadiene-7-one
.96	magenta	no change	unidentified
.87	magenta	decrease	5-cholesten-3β-ol
.69	yellow	increase	unidentified
.48	blue	increase	5-cholesten-3β, 7β-diol
.41	blue	increase	5-cholesten-3β, 7α-diol
.04	brown	decrease	cholestan-3β, 5α,6β-triol

Figure 9. Gas chromatograms of lipid non-saponifiables extracted from french fried potatoes fried in tallow heated intermittently for (B) 50 hours, (C) 100 hours, (D) 150 hours and (A) unfried control.


B
D
A
C
0
5
10
15
20
RETENTION TIME
(MINUTES)
0
5
10
15
20
RETENTION TIME
(MINUTES)

5-Cholesten-3β, 7α-diol, 5-cholesten-3β, 7β-diol, 5-cholesten-3β -ol and 3,5-cholestadiene-7-one were identified in the french fry potatoes lipid extracts. Cholestan-3β, 5α, 6β -triol was also detected in trace amounts. This compound, as previously described, is the hydration product of cholestan-5,6α -epoxy-3β-ol. The presence of this compound in the french fry potato extract is understandable due to the high concentration of water in the potato matrix.

Chromatograms from the GLC analysis of the french fry potatoes lipid extracts are shown in Figure 9. As seen from these chromatograms, the lipid extract from unfried french fry potatoes contained a number of compounds which could possibly be plant sterols as well as cholesterol. The french fried potatoes utilized in this study were treated with vegetable shortening containing soybean, palm, or cottonseed oil, and beef fat and may adequately account for the sterol profile of the uncooked french fry lipid extract. As a confirmation of vegetable oils in the french fry potato fatty acid analysis was performed on the lipid extracts (Figure 10). The results of the analyses indicate a high concentration of unsaturated fatty acids compared to similar data for tallow (Table 13). The migration of plant sterols from the french fried potatoes into the heated tallow moiety may account for some of the small peaks observed in the GLC sterol profiles for the tallow and lipid extracts from the french fried potatoes. The chromatograms obtained for the lipid extracts for french fried potatoes exhibited sterol profiles similar to those for the tallow samples.

Figure 10. Gas chromatogram of fatty acid methyl esters from a lipid extract of unfried french fry potatoes.

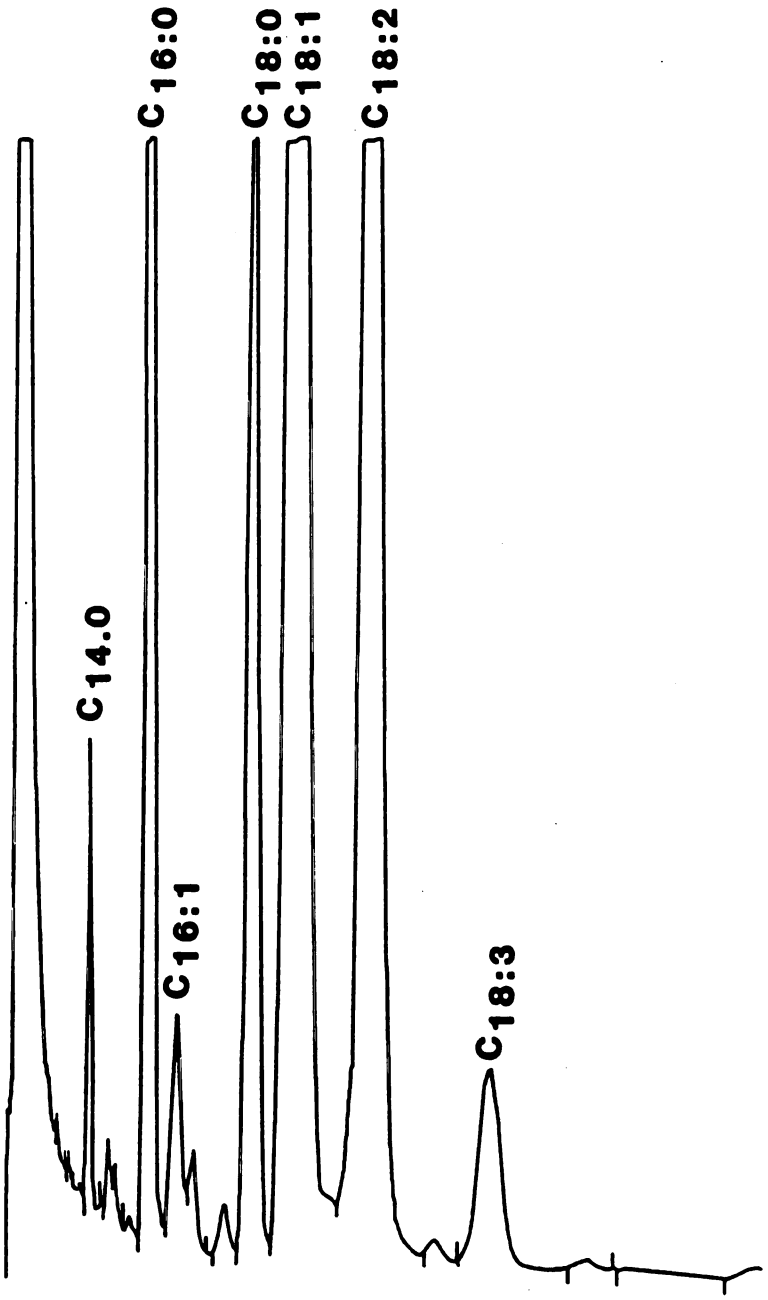

C14.0
C16:0
C16:1
C18:0
C18:1
C18:2
C18:3

Table 13. Fatty acid analysis of tallow and lipid extracts from raw french fry potatoes.

Fatty Acid	C14	C14:1	C16	C16:1	C18	C18:1	C18:2	C18:3	S/U
Tallow	4.0	1.3	26.6	8.8	15.9	41.7	1.7	---	0.87
French fried potato extract	1.1	0.2	15.1	1.5	8.6	43.6	26.1	2.7	0.33

GLC analysis indicated that the concentration of cholesterol oxidation products in the lipid extracts from french fried potatoes was approximately four times greater than the corresponding tallow samples. A dilution factor of four was required for the french fried potato lipid extracts in order to obtain peak sizes similar to the undiluted tallow extracts. This concentration of the cholesterol and its oxidation products in the french fries is in agreement with the findings of Hussain and Morton (1976) who reported that oil absorbed in fried foods is more oxidized than the frying medium itself. Similarly, Pokorny (1980) indicated that preferential absorption of frying fat decomposition products may occur due to polar interaction with the substrate. The increased polarity of cholesterol and its oxidation products relative to the triglyceride moiety may account for this partition effect.

Phase III. The Occurrence of Cholesterol Oxidation Products in Commercially Produced French Fried Potatoes

Results of Phase I and II of this study indicate that intermittent heating facilitates the decomposition of tallow used as a frying medium and the cholesterol contained therein. It was also found that these cholesterol oxidation products are preferentially absorbed by french fried potatoes fried in the heated tallow. To evaluate the implications of this study, commercial french fried potato samples processed by frying in intermittently heated tallow, were obtained and analyzed for the presence of cholesterol oxidation products.

Chromatographic analysis of a non-saponifiable fraction of frying medium samples from a fast-food franchise indicated that beef tallow was utilized as the frying fat (Figures 11 and 12). Thus, french fried potatoes samples from two local fast-food franchises were collected over a two week period and analyzed for cholesterol oxidation products.

Fat absorption data for the two sets of french fried potatoes samples are found in Table 14. Fat content of the french fried potatoes ranged from 16.4 to 20.9%. These values are higher than those obtained by Slover et al., (1980) who reported levels of 11.94 to 13.76% fat in commercial french fried potatoes. This fat content was also substantially lower than those recorded for french fried potatoes analyzed in Phase II of this study (Table 11).

The difference may be attributed to the larger size and surface area of the crinkle cut potatoes used in the laboratory study. The influence of size/surface area on the absorbed fat content was noted in the works of Strock et al., (1966) who reported 7% fat in 12 mm fried sliced potatoes, and Artman (1969) who found 1 mm slices to contain 30-40% absorbed fat.

Figure 11. Gas chromatograms of the non-saponifiable fraction from new fast-food franchise frying medium.

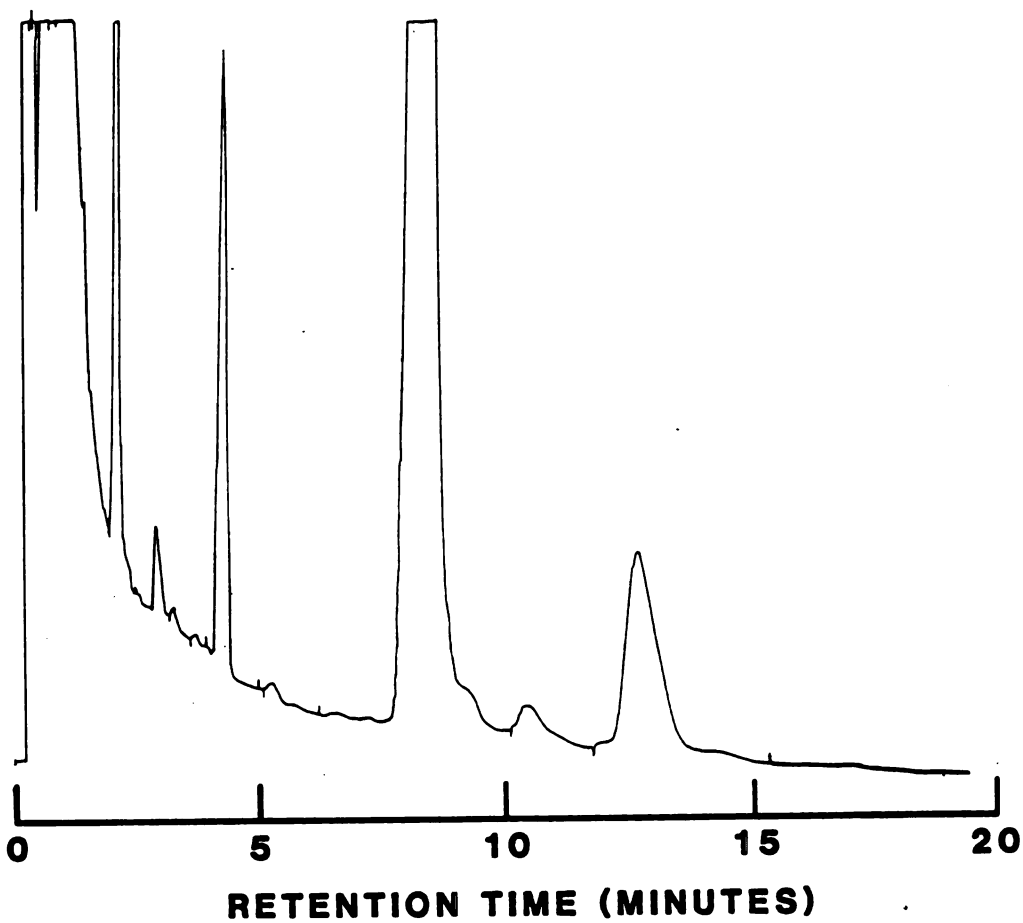

0
5
10
15
20
RETENTION TIME (MINUTES)

Figure 12. Gas chromatogram of the non-saponifiable fraction from discarded fast-food franchise frying medium.

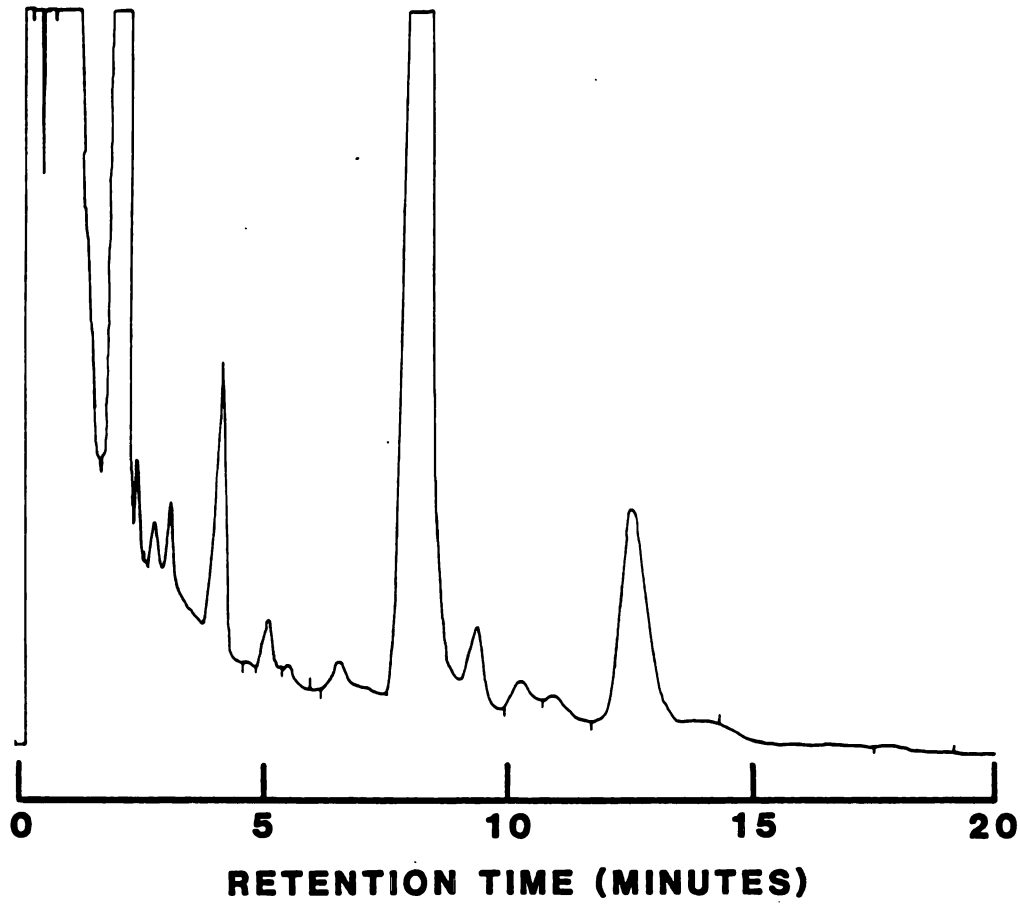

0
5
10
15
20
RETENTION TIME (MINUTES)

Table 14. Fat absorption data of commercially fried french fry potatoes

Sample Sequence A	B	Grams of Fat in 150 g Sample A	B	% Fat A	B
1	1	24.6	25.7	16.4	17.1
2	2	27.5	26.3	18.3	17.5
3	3	31.4	25.2	20.9	16.8
4	4	25.1	29.1	16.7	19.4
5	5	27.9	30.6	18.6	20.4
6	6	25.0	25.6	16.7	17.1

The extracted fat from a fast-food franchise french fried potatoes exhibited color changes over the two week sampling period (Figure 13). It is a evident by the color differences that the lighter sample represents a new batch of the frying medium. It is assumed that the lightest sample indicates the use of a new medium and is therefore indicative of heating time. Variations in color degradation may be attributed to increased use of the medium resulting in the need for more frequent replenishment with fresh fat.

TLC analysis of the non-saponifiable fraction of the lipids extracted from the fast-food franchise french fried potato samples indicated the presence of 5-cholesten-3β, 7β -diol, 5-cholesten-3β , 7β -diol, 5-cholesten-3β -ol, and 3,5-cholestadiene-7-one in the french fried potatoes (Table 15). No cholestan-3β, 5α , 6β-triol was detected. The cholesterol spot was noted to decrease in color intensity as the lipid extracts became darker. The unidentified yellow spot (R_f =0.63) encountered in all previous heated tallow extracts was also present in both sets of french fry samples. An additional unidentified brown spot (R_f =0.88) was also detected in the non-saponifiable fractions of the two series of samples. A yellow spot R_f = 0.92, was observed in only one of the series of samples. The presence of these

Figure 13. Lipid extracts from french fried potatoes obtained from (A) an East Lansing fast-food restaurant and (B) and Okemos area fast-food restaurant.

1 2 3 4 5 6

1 2 3 4 5 6

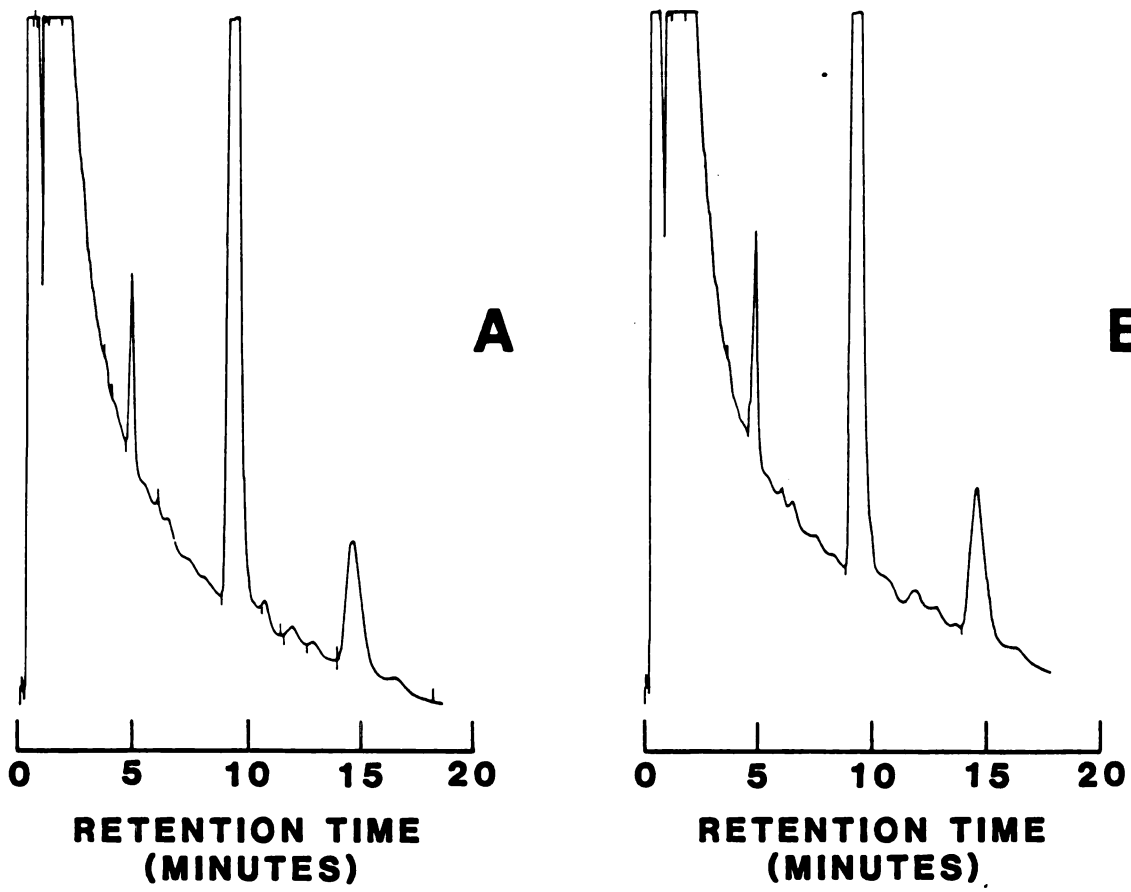
Table 15. TLC analysis of the non-saponifiable fraction of fat extracted from commercial french fry potatoes

Rf Value A	Rf Value B	Color	Intensity with time	Identity
1.00		--	--	solvent front
0.98	0.99	brown	increase	3,5-cholestadiene-7-one
0.92	--	yellow	decrease	unidentified
0.88	0.88	brown	no change	unidentified
0.85	0.84	magenta	decrease	5-cholesten-3β-ol
0.64	0.63	yellow	increase	unidentified
0.47	0.46	blue	increase	5-cholesten-3β, 7β-diol
0.40	0.39	blue	increase	cholesten-3β, 7α-diol

compounds may be attributed to many factors. Hydrogenation of tallow prior to use may form sterol derivatives. The introduction of fish or fruit products into the tallow for frying may result in the migration of sterols from these products into the tallow moiety. These may eventually be absorbed by french fried potatoes fried in the same oil.

The results of GLC analyses of non-saponifiable fractions from lipid extracts of a fast-food franchise french fried potato samples are found in Figure 14. The chromatograms, representing the darkest lipid sample, show a sterol profile similar to those obtained for lipid extracts from the french fried potatoes produced in the laboratory (Figure 9). A dilution factor similar to that used in Phase II of this study was also needed to facilitate GLC analysis.

Figure 14. Gas chromatograms of lipid non-saponifiables extracted from area fast-food franchises french fried potatoes, (A) East Lansing area and (B) Okemos area.


A
0
5
10
15
20
RETENTION TIME
(MINUTES)
B
0
5
10
15
20
RETENTION TIME
(MINUTES)

The result of this study indicates that cholesterol oxidation products are formed in fried foods produced under commercial practices when tallow is used as the frying medium.

SUMMARY AND CONCLUSIONS

The oxidative stability of the triglycerides and cholesterol in edible tallow employed as a deep-fat frying medium was investigated in a three-phase study.

Intermittent heating was found to be more detrimental than continuous heating to tallow in regard to frying stability. Chemical and physical analyses including iodine value, viscosity, peroxide value, free fatty acid, fatty acid analysis, and color indicated that the accumulation of products from degradation reactions occurred more rapidly in intermittently heated tallow. These studies also showed that cholesterol present in tallow readily oxidizes under conditions utilized in frying operations. TLC and GLC analyses indicated that intermittent heating promoted more rapid changes in the oxidative state of cholesterol. Various oxides including 5-cholesten-3β, 7α-diol, 5-cholesten-3β, 7β-diol, 5-cholesten-3β-ol, 3,5-cholestadiene-7-one and cholestan-3β, 5α,6β-triol were identified in the heated tallow samples by two dimensional TLC.

In the second phase of the study, the effect of frying on the oxidative stability of intermittently heated tallow triglycerides and cholesterol was investigated. The act of frying french fry potatoes in intermittently heated tallow was observed to have a suppressive effect on the degradation of the tallow. This was attributed to the absorption of deleterious compounds by the french fried potatoes and the dilution of remaining degradation products by the addition of fresh tallow for that depleted by absorption. TLC analysis of tallow and french fry potato samples indicated that cholesterol oxidizes in the system and

that the formed oxidative derivatives of cholesterol are preferentially absorbed by french fried potatoes. Oxides including 5-cholesten-3β, 7α -diol, 5- cholesten-3β,7β-diol, 5-cholesten-3β -ol, and 3,5-cholestadiene-7-one were identified on both tallow and french fried potato samples. Cholestan-3β, 5α, 6β-triol was also identified in trace amounts in french fried potato.

In the third phase of the study, commercially prepared french fried potatoes from a franchise utilizing tallow as a frying medium were investigated and were found to contain 5-cholesten-3β , 7α-diol, 5-cholesten-3β , 7α -diol, 5-cholesten-3β -ol and 3,5-cholestadiene-7-one.

The conclusions reached as a result of this study are summarized below:

1) Intermittent heating of tallow facilitates a more rapid degradation of tallow triglycerides and oxidation of cholesterol than does continuous heating due to autoxidation at ambient temperatures.

2) A greater proportion of unsaturated linkages lost during heating are consumed in dimerization and polymerization in continuously heated systems than in intermittently heated systems, while the rate of such reactions proceeds much more slowly in the continuously heated system.

3) Oxidation of cholesterol readily occurs at conditions suitable for frying and 5-cholesten-3β,7α-diol, 5-cholesten-3β,7β-diol, and 3,5-cholestadiene-7-one are ubiquitous to heated tallow systems.

4) The introduction of a substrate into heated tallow has a suppressive effect on the degradation of the fat due to absorption by the substrate and dilution by fresh tallow of the degradation products which accumulate with prolonged heating.

5) Cholesterol and cholesterol oxidation products are apparently preferentially absorbed by french fry potatoes fried in the heated tallow moiety. The sterols are approximately four times more concentrated in the french fry potato lipid extract than the heated frying medium.

6) The identified cholesterol oxidation products present in heated tallow systems have not been implicated as having atherosclerotic or carcinogenic properties, although they have been isolated from the lipid fraction of human atheromata.

7) Trace amount of cholestan-3β, 5α, 6β-triol were noted in french fried potatoes fried in tallow. This compound has been well documented as being a potent atherosclerotic and angiotoxic agent. Its presence also indicates the transient existence of its precursor, cholestan-5,6α-epoxy-3 β-ol, a known carcinogen, in heated tallow systems.

8) Commercially processed french fried potatoes which are fried in tallow such as those distributed through fast-food franchises, contain oxidation products of cholesterol including 5-cholesten-3β, 7α-diol, 5-cholesten-3β,7β -diol and 3,5-cholestadiene-7-one, as well as unoxidized cholesterol.

PROPOSALS FOR FURTHER RESEARCH

The study of tallow constituent oxidation during frying processes has raised some questions worthy of further investigation. These include:

1. The possible participation of cholesterol or cholesterol oxidation products in polymerization reactions occurring in heated tallow.

2. The identification and isolation of the different dimeric and polymeric species formed in intermittent and continously heated tallow.

3. The effect of unsaturated bond concentration on the oxidative stability of cholesterol in a heated tallow system.

4. In this study, several compounds were isolated which could not be identified by standard references. Application of GC-MS techniques with free sterols or silylated derivatives may allow resolution of these structures.

5. It has been established that cholesterol in tallow migrates to products fried in tallow. Research is needed to determine if cholesterol-containing foods such as chicken or fish release cholesterol to the frying medium which may eventually be reabsorbed by other food products.

6. Extensive work in the area of cholesterol oxidation is needed in foodstuffs stored at ambient temperatures such as dried sausage and cheese products.

7. An investigation of plant sterols in fried food systems, either naturally occurring in plant oils or via migration from fried vegetable products merits more study.

8. The development of preparative procedures which would allow separation of sterols from lipid matrices and prevent the need for saponification would prove invaluable in future studies.

9. More extensive research in the area of the biological effects of the cholesterol oxidation products formed in all processed foods is needed to assess the true hazard these compounds may pose in the diet. It is important to establish whether cholesterol or its oxidation products are responsible for atherosclerotic, angiotoxic, and carcinogenic effects.

BIBLIOGRAPHY

Acker, L. and Greve, H. 1963. Uber die photooxydation des cholesterins in eihaltigin Lebensmitteln. Fette Seifen Anstrech. 65:1009.

Ansari, G.A.S. and Smith, L.L. 1979. The oxidation of cholesterol by hydroxyl radical. Photochem. Photobiol. 30:147.

Artman, N.R. 1969. The chemical and biological properties of heated and oxidized fats. Adv. Lipid Res. 7:245.

Bates, R.W. 1952. Quality deep fat-fried foods. Food Eng. 24(12):82.

Bergstrom, S. and Samuelsson, B. 1961. The autoxidation of cholesterol- In "Autoxidation and Antioxidants," W.O. Lundberg, Ed., Interscience Publishers, John Wiley and Sons, New York City, N.Y., Vol. 1 pp. 233-248.

Bischoff, F. 1963. Carcinogenesis through cholesterol and derivatives. Prog. Exp. Tumor Res. 3:412.

Bischoff, F. 1969. Carcinogenic effects of steroids. Advan. Lipid. Res. 7:165.

Black, H.S. and Douglas, D.R. 1972. A model system for the evaluation of cholesterol α-oxide in ultraviolet carcinogenesis. Cancer Res. 32:2630.

Black, H.S. and Douglas, D.R. 1973. Formation of a carcinogen of natural origin in the etiology of ultraviolet light-induced carcinogenisis. Cancer Res. 33:2094.

Bladon, P. 1958. The superficial oxidation of cholesterol. In "Cholesterol," R.P. Cook, Ed. Academic Press Inc. New York. p.76.

Brooks, C.J.W., Harland, W.A. and Steel, G. 1966. Squalene, 26-hydroxy-cholesterol and 7-ketocholesterol in human atheromatous placques. Biochim. Biophys. Acta. 125:620.

Brooks, C.J.W., Steel, G., Gilbert, J.D. and Harland, W.A. 1971. Lipids of human atheromata. Part 4: Characterization of a new group of polar sterol esters from human atherosclerotic placques. Atherosclerosis. 13:223.

Chalmers, J.G. 1951. Chemical changes in cottonseed oil on heating to various temperatures. Acta Unio Intern. Contra Cancrum. 7:612.

Chan, J.T. and Black, H.S. 1974. Skin carcinogenesis: cholesterol-5α, 6α -epoxide hydrase activity in mice skin irradiated with ultraviolet light. Science. 186:1716.

Chan, J.T. and Black, H.S. 1976. Distribution of cholesterol 5α, 6α-epoxide formation and its metabolism in mouse skin. J. Invest. Derm. 66:112.

Chang, S.S. and Kummerow, F.A. 1953. The isolation and characterization of the polymers formed during the autoxidation of ethyl linoleate. J. Amer. Oil Chem. Soc. 30:403.

Chen, H.W., Kandutsch, A.A. and Waymouth, C. 1974. Inhibition of cell growth by oxygenated derivatives of cholesterol. Nature. 251:419.

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

Claude, J.R. 1966. Separation et identification de sterols substitves sur les cycles Aet B par chromatographie en couches Minees et chromatographie en phase gazeuse. J. Chromatog. 23:267.

Crampton, E.W., Common, R.H., Farmer, F.A., Wells, A.E. and Crawford, D. 1953. Studies to determine the nature of the damage to the nutritive value of some vegetable oils from heat treatment. J. Nutr. 49:333.

Deuel, H.J. Jr., Greenberg, S.N., Colbert, C.E., Baker, R. and Fisher, N.R. 1951. Toxicology studies on isopropyl and stearyl citrate. Food Res. 16:258.

Evans, C.D., McConnell, D.G., Frankel, E.N. and Cowen, J.C. 1965. Chromatrograpic studies on oxidative and thermal fatty acid dimers. J. Amer. Oil Chem. Soc. 42:764.

Farmer, E.H., Bloomfield, G.F., Sundralingam, A. and Sutton, D.A. 1942. The course and mechanism of autoxidation reactions in olefinic and polyolefinic substances including rubber. Trans. Faraday Soc. 38, 348:356.

Fioriti, J.A. and Sims, R.J. 1967. Autoxidation products of cholesterol. J. Amer. Oil Chem. Soc. 44:221.

Firestone, D., Horwitz, W., Freidman, L. and Shue, G.M. 1961. Heated fats. I. Studies of the effects of heating on the chemical nature of cottonseed oil. J. Amer. Oil Chem. Soc. 38:253.

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

Frankel, E.N., Evans, C.D. and Cowan, J.C. 1960. Thermal dimerization of fatty ester hydroperoxides. J. Amer. Oil Chem. Soc. 37:418.

Gunstone, F.D. and Hilditch, T.P. 1945. The union of gaseous oxygen with methyl oleate, linoleate and linolenate. J. Chem. Soc. 836.

Henderson, A.E. 1956. A histochemical and chromatographic study of normal and atheromatous human arteries. J. Histochem. Cytochem. 4:153.

Henderson, A.E. and MacDougall, J.B.D. 1954. A histochemical and chromatographic study of the lipid distribution in human arteries. Biochemical J. 57:xxi.

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

Hussain, S.S. and Morton, I.D. 1976. Absorption of food by frying. Proc. 4th. Internat. Cong. Food Sci. and Tech. Madrid, Spain Vol. I.

Imai, H., Werthessen, N.T., Subramanyan, V. LeQuesne, P.W., Solaway, A.H. and Kanesawa, M. 1980. Angiotoxicity of oxygenated sterols and possible precursors. Science 207:651.

Imai, H., Werthessen, N.T., Taylor, C.B. and Lee, K.T. 1976. Angiotoxicity and athersclerosis due to contaminants of USP-grade cholesterol. Arch. Pathol. Lab. Med. 100:565.

Itoh, T., Tamura, T. and Matsumato, T. 1973. Methylsterol composition of 19 vegetable oils. J. Amer. Oil Chem. Soc. 50:300.

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

Kandutsch, A.A. and Chen, H.W. 1978. Inhibition of cholesterol synthesis by oxygenated sterols. Lipids. 13:704.

Kartha, A.R.S. 1960. The iodine value decreasing reaction in fat autoxidation. J. Sci. Research (India) 19B, 199-205.

Kaunitz, H., Slanetz, C.A., Johnson, R.E., Knight, H.B., Saunders, D.H. and Swern, D. 1956. Biological effects of the polymeric residues isolated from autoxidized fats. J. Amer. Oil Chem. Soc. 33:630.

Keane, K.W., Jacobson, G.A. and Krieger, C.H. 1959. Biological and chemical studies on commercial frying oil. J. Nutr. 68:57.

Kirby, A.H.M. 1943. Attempts to induce stomach tumors. I. The effect of cholesterol heated to 300°C. Cancer Res. 3:519.

Kirby, A.H.M. 1944. Attempts to induce stomach tumors. III. The effects of a) a residue of cholesterol heated to 300°C., and b) 3,5-cholestadiene. Cancer Res. 4:94.

Kulig, M.J. and Smith, L.L. 1973. Sterol metabolism. XXV. Cholesterol oxidation by singlet molecular oxygen. J. Org. Chem. 38:3639.

Kummerow, F.A. 1979a. Nutrition inbalance and antiotoxins as dietary risk factors in coronary heart disease. Am. J. Clin. Nutr. 35:58.

Kummerow, F.A. 1979b. Possible role of oxidized lipids in atherogenesis. Paper presented at the workshop, Autoxidation Processes in Food and Related Biological Systems. U.S. Army Research and Dev. Command, Natick, Mass.

Lane, A., Blickenstaff, D. and Ivy, D.C. 1950. The carcinogenecity of fats "browned" by heating. Cancer 4:1044.

Larsen, C.D. and Barrett, M.K. 1944. Administration of 3,5-cholestadiene and dicholesteryl ether to mice and rats. J. Nat. Cancer Inst. 4:587.

MacDougall, J.D.B., Biswas, S. and Cook, R.P. 1965. The effect of certain C_{27} steroids on organ cultures of rabbit aorta. Br. J. Exp. Pathol. 46:549.

Melnick, D. 1957a. Absence of thermal polymers in potato-chip frying oils. J. Amer. Oil Chem. Soc. 34:351.

Melnick, D. 1957b. Nutritional quality of frying fats in commercial use. J. Amer. Oil Chem. Soc. 34:578.

Melnick D., Luckmann, F.H. and Gooding, C.M. 1958. Composition and control of potato chip frying oils in continuing commercial use. J. Amer. Oil Chem. Soc. 35:271.

Micheal, W.R. 1966. Thermal reactions of methyl linoleate. II. The structure of aromatic C_{18} methyl esters. Lipids 1:359.

Micheal, W.R. 1966. Thermal reactions of methyl linoleate III. Characterization of C_{18} cyclic esters. Lipids 1:365.

Morrison, W.R. and Smith, L.M. 1964. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron-flouride-methanol. J. Lipid Res. 5:600.

Morton, I.D. 1977. Changes related to different time-temperature combinations. In Physical, Chemical and Biological Changes in Food Caused by Thermal Processing. Hoyem, T. and Kvale, O. Eds. Applied Science Pub. LTD. Essex Eng. pp. 135-151.

Nolen, G.A., Alexander, J.C. and Artman, N.R. 1967. Long-term rat feeding study with used frying fats. J. Nutr. 93:337.

O'Neill, L.A. 1954. Some recent studies on the autoxidation of drying oils. Chem. Ind. (London). p. 384.

Peng, S.K., Taylor, C.B., Tham, P. and Mikkelson, B. 1978. Effect of autoxidation products from USP-Grade cholesterol on aortic smooth muscle cells. An in vitro study. Arch. Pathol. Lab. Med. 102:57.

Peng, S.K., Tham, P., Taylor, C.B. and Mikkelson, B. 1979. Cytotoxicity of oxidation derivatives of cholesterol on cultured aortic smooth muscle cells and their effect on cholesterol biosynthesis. Am. J. Clin. Nutr. 32:1033.

Perkins, E.G. 1960. Nutritional and chemcial changes occurring in heated fats: A review. Food Technol. 14:508.

Perkins, E.G. 1967. Formation of non-volatile decomposition products in heated fats and oils. Food Technol. 21:611.

Perkins, E.G. and Kummerow, F.A. 1959. The isolation and characterization of the polymers formed during the thermal oxidation of corn oil. J. Amer. Oil Chem. Soc. 36:371.

Perkins, E.G. and Van Akkeren, L.A. 1965. Heated fats. IV. Chemical changes in fat subjected to deep fat frying processes: Cottonseed oil. J. Amer. Oil Chem. Soc. 42:782.

Pokorny, J. 1966. The autoxidation of some vegetable oils at elevated temperatures. XIII. Effect of temperature and diffusion on the weight changes during the oxidation of rapeseed oil. Chem. Abstr. 66:20213.

Pokorny, J. 1980. Effect of substrates on changes of fats and oils during frying. La Rivista Italiana Delle Sastanze Grasse. LVII 222.

Poling, C.E., Warner, W.D., Mone, P.E. and Rice, E.E. 1960. The nutritional value of fats after use in commercial deep-fat frying. J. Nutr. 72:109.

Poling, C.E., Warner, W.D., Mone, P.E. and Rice, E.E. 1962. The influence of temperature, heating time and aeration upon the nutritive value of fats. J. Amer. Oil Chem. Soc. 39:315.

Punwar, J.K. and Derse, P.H. 1978. Application of the official AOAC cholesterol method to a wide variety of food products. J. Amer. Oil Chem. Soc. 61(3):727.

Rice, E.E., Poling, C.E., Mone, P.E. and Wayner, W.D. 1960. A nutritive evaluation of over-heated fats. J. Amer. Oil Chem. Soc. 37:607.

Rock, S.P. and Roth, H. 1964a. Factors affecting the rate of deterioration in the frying qualities of fats. I. Exposure to air. J. Amer. Oil Chem. Soc. 41:228.

Rock, S.P. and Roth, H. 1964b. Factors affecting the rate of deterioration in the frying qualities of fats. II. Type of heater and method of heating. J. Amer. Oil Chem. Soc. 41:531.

Sahasrabudhe, M.R. 1965. Introduction: Studies on heated fats. J. Amer. Oil Chem. Soc. 42:763.

Sanche, L. and Van Lier, J.C. 1976. Tracer studies of cholesterol degradation induced by ionized gases. Chem. Phys. Lipids. 16:225.

Seher, A. and Homberg, E. 1968. Die untersuchung von sterin-gemischen mit helfe der Dunnschichi-Chromatographic. Fette Seifen Anstrech. 7:481.

Sheppard, A.J. and Shen, C.J. 1980. Activities of FDA's division of nutrition regarding cholesterol oxides. In Autoxidation in Food and Biological Systems, M.G. Simic and M. Karel, Eds., Plenun Press, New York City, N.Y. pp. 133-140.

Sims, R.P.A. 1957. Possible mechanisms in thermal polymerization of vegetable oils. II. Polymer formation. J. Amer. Oil Chem. Soc. 34:466.

Slover, H.T., Lanza, E. and Thompson, R.H. Jr. 1980. Lipids in fast foods. J. Food Sci. 45(6):1583.

Smith, L.L. 1980. The autoxidation of cholesterol in autoxidation. In Food and Biological Systems, M.B. Sim and M. Karel, Eds., Plenum Press, New York City, N.Y. pp. 119-132.

Smith, L.L. and Hill, F.L. 1972. Detection of sterol hydroperoxides on thin-layer chromatoplates by means of the Wurster dyes. J. Chromatog. 66:101.

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

Smith, L.L. and Kulig, M.J. 1976. Singlet molecular oxygen from hydrogen peroxide. J. Amer. Chem. Soc. 98:1027.

Smith, L.L., Kulig, M.J., Muler, D. and Ansari, G.A.S. 1978. Oxidation of cholesterol by dioxygen species. J. Amer. Chem. Soc. 100:6206.

Smith, L.L., Kulig, M.J. and Teng, J.I. 1973a. Sterol metabolism XXVI. Pyrolysis of some sterol allylic alcohols and hydroperoxides steroids. 22:627.

Smith, L.L., Matthews, J.C., Price, J.C., Bachman, R.C. and Reynolds, B. 1967. Thin-layer chromatographic examination of cholesterol autoxidation. J. Chromatog. 27:187.

Smith, L.L. and Teng, J.I. 1974. Sterol metabolism. XXIX. On the mechanism lipid peroxidation in rat liver. J. Amer. Chem. Soc. 96: 2640.

Smith, L.L., Teng, J.I., Kulig, M.J. and Hill, F.L. 1973b. Sterol metabolism. XXIII. Cholesterol oxidation by radiation-induced processes. J. Org. Chem. 38:1763.

Smouse, T.H. 1975. The frying properties of a series of vegetable oils and the effectivness of selected tests in predicting their frying stability. Cento panel meeting on regional problems of edible fats and oils. PCSIR Laboritories, Lahore, Pakistan.

Strock, H., Ball, C.O., Chang, S.S. and Steir, E.F. 1966. Effects of agitation and temperature in the deep-fat frying of potatoes. Food Technol. 20:545.

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. Nutr. 32:40.

Teng, J.I., Kulig, M.J., Kan, G. and Van Lier, J.C. 1973. Sterol metabolism. XX. Cholesterol 7α -hydroperoxide. J. Org. Chem. 38:119.

Teng, J.I., Kulig, M.J. and Smith, L.L. 1973. Sterol metabolism XXVI. Gas chromatographic differentation among cholesterol hydroperoxides. J. Chromatog. 75:108.

Teng, J.I. and Smith, L.L. 1973. Sterol metabolism. XXIV. On the unlikely participation of singlet molecular oxygen in several enzyme oxygenations. J. Am. Chem. Soc. 95:4060.

Teng, J.I. and Smith, L.L. 1976. Sterol metabolism. XXXIII. On the oxidation of cholesterol by dioxygenases. Bioorganic Chem. 5:99.

Thompson, J.A., Paulose, M.M., Reddy, B.R., Krishnanamurthy, R.G. and Cheng S.S. 1967. A limited survey of fats and oils commercially used for deep-fat frying.

Tsai, L.S., Hudson, C.A., Ijichi, K. and Meehan, J.J. 1979. Quantitation of cholesterol α-oxide in eggs by gas chromatography and high performance liquid chromatography. J. Amer. Oil Chem. Soc. 56:185A.

Tu, C., Powrie, W.D. and Fennema, O. 1967. Free and esterified cholesterol content of animal muscles and meat products. J. Food Sci. 33:581.

Van Lier, J.E. and Smith, L.L. 1967. Sterol metabolism Part 1.-26-hydroxy cholesterol in the human aorta. Biochemistry. 6:3269.

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

Van Lier, J.E. and Smith, L.L. 1970a. Autoxidation of cholesterol via hydroperoxide intermediates. J. Org. Chem. 35:2627.

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

Veldstra, H. 1939. 3,5-cholestadiene from cholesterol oleate and its possible bearing on the formation of carcinogenic substances in heated fats. Nature. 144:246.

Watt, B.K. and Merrill, A.L. 1963. Composition of Foods. Agr. Handbook No. 8, U.S. Dept. of Agr. Wash. D.C.