THE UNIVERSITY OF MANITOBA

Phytosterol and Tocopherol Changes in Modified Canola Oils During Frying and Storage of Fried Products

by

Wei Li

A Thesis
Submitted to the Faculty of Graduate Studies
In partial fulfilment of the requirements
for the Degree of

MASTER OF SCIENCE

Department of Foods and Nutrition
The University of Manitoba
Winnipeg, Manitoba, Canada

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PHYTOSTEROL AND TOCOPHEROL CHANGES IN MODIFIED CANOLA OILS DURING FRYING AND STORAGE OF FRIED PRODUCTS

BY

WEI LI

A Thesis/Practicum submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements for the degree of

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Abstract

The oxidative stability of phytosterols and tocopherols was investigated in this study by 1) heating phytosterol standards; 2) frying potato chips in canola oils and storing the fried products and 3) heating canola oils at frying temperatures to simulate frying. Pure ß-sitosterol, campesterol and stigmasterol were heated at 75°C, 95°C, 120°C, 155°C and 180°C for 1 hour, 5 hours and 12 hours. The changes of phytosterols were determined by HPLC and the oxidation products formed during the heating were identified by GC-MS. ß-Sitosterol and campesterol were stable at 75°C for 12 hours and began to oxidize at 95°C. Elevated temperatures accelerated the oxidation rate of phytosterols. ß-Sitosterol and campesterol produced large amounts of oxidation products when heated at 155°C and 180°C for 1 hour. Stigmasterol was the most stable phytosterol among all the evaluated phytosterols.

The potato chips were fried in regular, low linolenic acid, high oleic acid and hydrogenated canola oils at 180±5°C. The potato chips were collected on the first and fifth day of frying and stored at 60°C for 16 days without light. Major phytosterol changes in canola oils and in fried potato chips were determined by HPLC. During the potato chip frying, 50% to 60% of total sterols disappeared and several oxidation products were observed. Only a small amount of sterols disappeared during the storage of potato chips.

Four canola oils were heated at 190°C for up to 72 hours to simulate frying. At the end of the heating at simulated frying temperature, the losses of total phytosterols were at 30%, 38%, 39% and 60% for low linolenic, regular, high oleic and hydrogenated canola oils, respectively,

and the accumulation of total phytosterol oxidation products were at 923 ppm, 363 ppm, 224 ppm, 346 ppm in hydrogenated, regular, low linolenic and high oleic canola oils, respectively. The stability of sterols decreased in the following order: stigmasterol, cholesterol, ß-sitosterol, campesterol and brassicaterol. The major oxidation products formed from these sterols during heating were: 7-ketocholesterol, 7α- and 7ß-hydroxycholesterols, α- and ß-epoxycholesterols, 7α- and 7ß-hydroxysitosterol, 22 or 25-hydroxysitosterol, 7ß- and 7α-hydroxysitosterols, α- and ß-epoxysitosterols, 7-ketositosterol, 7α and 7ß-hydroxycampesterols, 7-ketocampesterol, α- and 7ß-hydroxystigmasterols, α- and ß-epoxystigmasterols and 7-ketostigmasterol.

Tocopherol changes in canola oils during frying and potato chip storage were determined by normal phase HPLC. At the end of the potato chip frying, the total tocopherol losses were at 47%, 47%, 58% and 92% in low linolenic, regular, high oleic and hydrogenated canola oils, respectively. γ-Tocopherol disappeared faster than α-tocopherol. During the heating at simulated frying temperature, similar changes of tocopherols were observed as during frying. Unsaturation was found to be the major factor affecting the disappearance of tocopherols during the storage of potato chips. Total tocopherol losses in the potato chips at the end of storage were at 10%, 50%, 85% and 96% for hydrogenated, low linolenic, high oleic and regular canola oils respectively. α-Tocopherol was found to decrease at a faster rate than γ-tocopherol during the storage of potato chips, which was different from frying.

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This thesis is dedicated to my parents. Their great love, encouragement and understanding always give me confidence and strength during my graduate studies in the University of Manitoba.

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Appendix: Mass Spectra of Authentic Phytosterol Oxidation Products

Chapter I

Introduction

Phytosterols are a group of sterols that are widely present in foods of plant origin. They are closely related to cholesterol in chemical structure. Cholesterol is subject to spontaneous oxidation during food processing and storage (Smith, 1981, Maerker, 1987, Maerker and Unruh, 1986, Park and Addis, 1986a, 1986b, Missler *et al.*, 1985). About 70 cholesterol derivatives formed during oxidation have been identified (Smith, 1981). There is accumulating evidence that cholesterol oxidation products have adverse effects on human health (Hubbard *et al.*, 1989, Cox *et al.*, 1988, Kubow, 1993). Recent studies have demonstrated that similar oxidation products from phytosterols are formed in foods fried in vegetable oils (Daly *et al.*, 1983, Ghavami and Morton, 1984, Lee *et al.*, 1985, Nourooz-Zadeh and Appelqvist, 1992, Blekas and Boskou, 1989, Li and Przybylski, 1995, Dutta and Appelqvist, 1995). The health effect of these oxidation products is not known and has yet to be established.

Tocopherols, or vitamin E, are the well known endogenous antioxidants present in vegetable oils and biological systems (Timmermann, 1990). Vegetable oils are the major source of vitamin E in the human diet. The presence of tocopherols in food systems can delay the oxidation process in foods. Tocopherol amounts in foods are expected to decrease during processing and storage (Bauernfeind, 1977; Suarna *et al*, 1991).

New genetically modified canola oils, such as low linolenic and high oleic canola oils have been

developed for frying to replace hydrogenated canola oil. Although researchers have investigated the phytosterol oxidation and tocopherol changes in some vegetable oils (Dutta and Appelqvist, 1995, Ghavami, and Morton, 1984, Blekas and Boskou, 1989), the knowledge of behaviour of phytosterols and tocopherols in canola oils during frying and storage of fried products is lacking. The main purpose of this study was to investigate the changes of phytosterols and tocopherols in canola oils during frying and storage of fried products.

Chapter II

Literature Review

2.1. Sterols

2.1.1. Chemical Structure, Classification of Sterols and Their Occurrences

Phytosterols comprise a major portion of unsaponifiable matter of most vegetable oils. Their chemical structures are very similar to that of cholesterol. The major phytosterols in vegetable oils include ß-sitosterol, stigmasterol, campesterol and brassicasterol. The chemical structures of cholesterol and major phytosterols are illustrated in Fig. 2.1 and Fig.2.2, respectively. Their common names and nomenclator names are shown in Table 2.1.

Table 2.1. Common and Nomenclator Names of Some Sterols

Common Names	Nomenclator Names
Cholesterol	Cholest-5-en-3ß-ol
Campesterol	24α-Methylcholest-5-en-3ß-ol
Brassicasterol	24ß-Methylcholest-5,22-dien-3ß-ol
ß-Sitosterol	24α-Ethylcholest-5-en-3ß-ol
Stigmasterol	24α-Ethylcholest-5,22-dien-3ß-ol
Δ^5-Avenasterol	24-Ethylidenecholest-5-en-3ß-ol
Δ^7-Avenasterol	24-Ethylidenecholest-7-en-3ß-ol

Fig. 2.1 Chemical Structures of Cholesterol

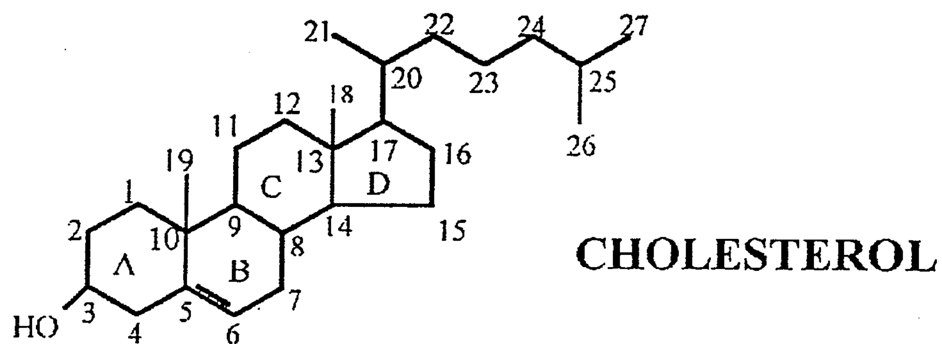
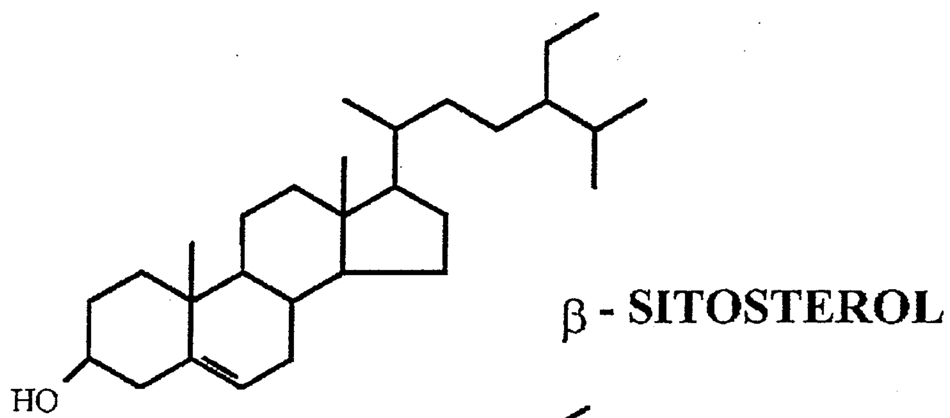
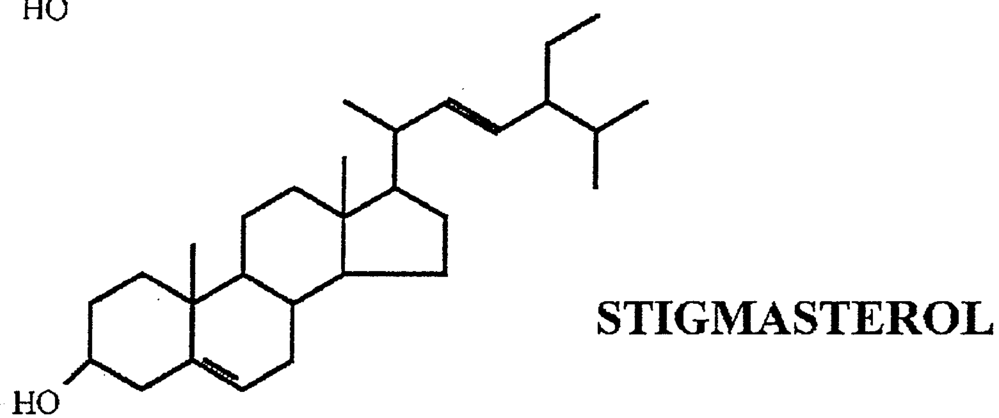
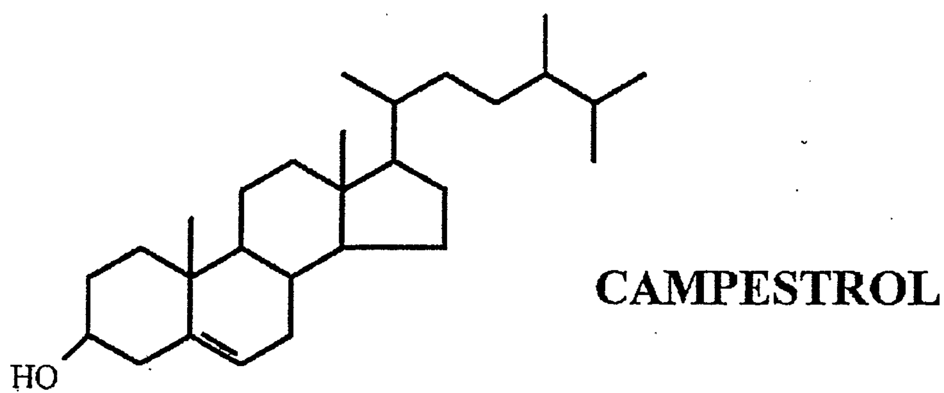
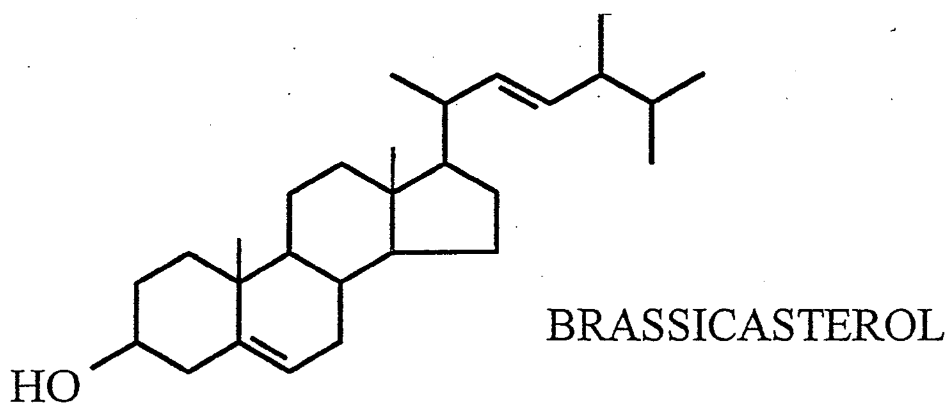


Fig. 2.2 Chemical Structures of Phytosterol









In general, vegetable oils contain more sterols than animal fats (Kochhar, 1983). The sterol contents in some major animal fats and vegetable oils are listed in Table 2.2.

Table 2.2. Sterol Contents in Major Vegetable Oils and Animal Fats

Fat or Oil	Total Sterol Contents (mg/100g)
Animal origin:	
Beef tallow	80 - 140
Lard	110 - 120
Fish oil	300
Vegetable Oils:	
Wheat germ	1200 - 2600
Corn	580 - 1500
Canola	350 - 840
Sunflower	250 - 750
Soybean	150 - 420
Cottonseed	260 - 430
Olive	230 - 310

Adapted from Kochhar (1983).

The contribution of individual phytosterols in vegetable oils is different. ß-Sitosterol is usually the main phytosterol, followed by campesterol and stigmasterol. Brassicasterol is present in relatively large amounts only in plants and seeds of *Crucifer* families (rapeseed). In other vegetable oils, brassicasterol is either absent or present in very small amounts. Table 2.3 listed the distribution of major sterols in selected vegetable oils.

Table 2.3. Distribution of Major Sterols in Selected Vegetable Oils (% of total sterols)[a]

Sterols	HEAR*	Canola	Soybean	Sunflower	Corn
Cholesterol	0.4	0.1	0.3	0.1	0.1
Brassicasterol	13.2	13.8	-	-	-
Campesterol	34.4	35.2	18.1	7.5	17.2
Stigmasterol	0.3	0.5	15.2	7.5	6.3
ß-Sitosterol	47.9	48.2	54.1	58.2	60.3
Δ^5-Avenasterol	2.1	2.4	2.5	4.0	10.5
Δ^7-Avenasterol	-	-	2.0	4.0	1.1
Δ^5-Stigmasterol	-	-	0.5	11.5	0.1
Δ^7-Campesterol	-	-	3.6	2.4	-

* High erucic acid rapeseed oil
[a] Adapted from Przybylski, 1994

Phytosterols are present in free and esterified forms in vegetable oils. In general, 25% to 80% of the phytosterols were in the esterified form of fatty acids (Kochhar, 1983). In soybean oil, the percentage of free campesterol and stigmasterol are considerably higher than their esterified forms, whereas ß-sitosterol, Δ^7-campesterol and Δ^7-avenasterol are present at higher amounts in the form of esters (Kochhar, 1983). In rapeseed oil, brassicasterol exists largely in the nonesterified form (Kochhar, 1983). Δ^5-Avenasterol, Δ^7-avenasterol, Δ^5-stigmasterol and Δ^7-campesterol are present in canola oil in relatively small amounts.

2.1.2. Sterol Contents in Foods

Seed maturity is one of the major factors affecting the levels of phytosterols in plants

(Kochhar, 1983, Lozano *et al.*,1993). Usually, higher amounts of unsaponifiables were found in immature seeds than mature seeds. Lozano *et al.* (1993) investigated the total unsaponifiable and sterol contents in avocado oils. They found that the contents of unsaponifiables in crude oils were 15% to 40% in very young fruits and 4% to 9% in mature fruits. The sterol contents in the oils was 1.1% to 6.2% in immature fruits while 0.8% to 2.0% in mature fruits.

Genetic modification of the seeds also influenced the sterol contents. Kovacs *et al.* (1978) studied the sterol contents in the rapeseed oils that had been transferred from high to low erucic acid varieties over the harvesting period from 1971 to 1977. They found that the total sterol contents increased to 9.75 mg/g in genetically modified varieties as compared to 6.90 mg/g in the unmodified varieties. The contribution of brassicasterol decreased from 11% in regular rapeseed oil to 8% in modified varieties.

During processing of vegetable oils, total and individual sterol contents gradually decreased at the various processing stages. Degumming process of vegetable oils effectively removed sterol glucosides and fully refined oils were free from those compounds (Kochhar, 1983). Crude sunflower and corn oils contained 30-50 mg/100g of glucoside sterol esters, but none of these components were detected in the refined oils (Kochhar, 1983). Physical and alkali refining, bleaching and deodorization, each lowered the sterol contents (Jawad *et al.*, 1984, Johnasson and Hoffman, 1979). The greatest reduction in the sterol content was caused by bleaching where oxidation products might have been formed (Johnasson and Hoffman, 1979, Kochhar, 1983). Bleaching caused the formation of sterol artifacts and the partial modification of

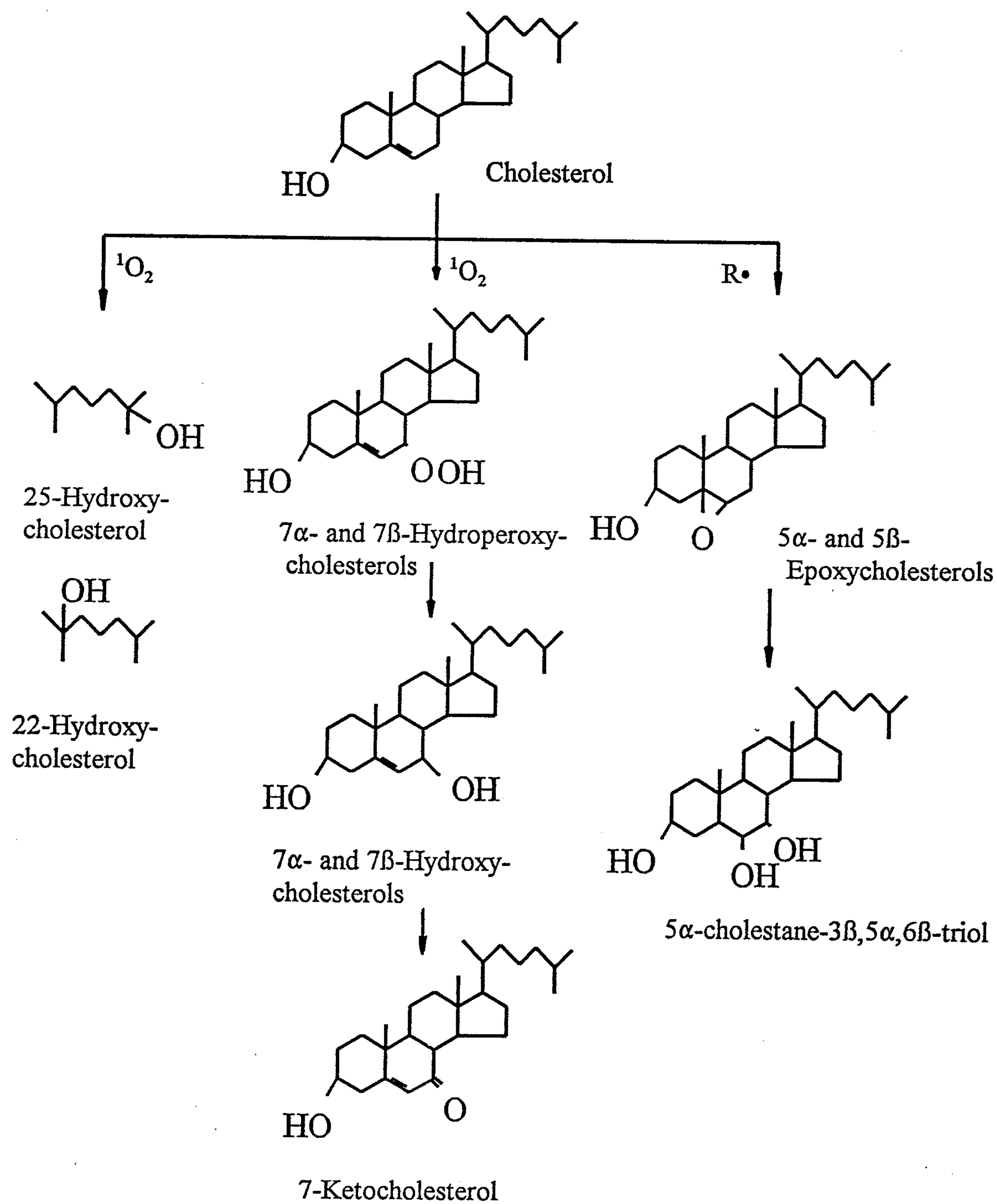
individual sterols and sterol esters by deacylation. Nonpolar steroids and dehydrated phytosterols were formed during bleaching. The oxidized derivatives of sterols, such as sterol ketones may be present in the bleached and deodorized oils (Kochhar, 1983)

2.1.3. Sterol Degradation and Oxidation

2.1.3.1. Oxidation of Sterols

Similar to other lipids, sterols are subject to oxidation especially when they come in contact with oxygen (air) at elevated temperatures. Extensive investigation have been carried out to detect cholesterol oxidation products in foods. Systematic studies and excellent review papers have been published (Smith, 1981,1987, Maerker, 1987, Finocchiaro and Richardson, 1983). The possible pathways of cholesterol oxidation have been reviewed by Paniangvait (1995) and are illustrated in Fig.2.3. Cholesterol oxidation is initiated by hydrogen abstraction in C4 and/or C7 positions due to the presence of unsaturated double bond in the B ring (Maerker, 1987). Because of the influence of hydroxyl group at the C3 position, oxygen attacks at the C4 occur rarely. The attack of oxygen at C7 results in the formation of two epimeric hydroperoxides. These intermediate products are not stable and easily converted to 7α- and 7ß-hydroxycholesterols and 7-ketocholesterol. The hydroperoxides can react with cholesterol free radicals and form α and ß-epoxycholesterols. Epoxycholesterols can be converted into cholestane-3ß,5α,6ß-triol in the presence of acid. When cholesterol oxidizes at the side chain, then 22- and 25-hydroxycholesterols are the most common products, due to the tertiary carbon atoms at these positions. Besides the major cholesterol oxidation products mentioned above,

Fig. 2.3. Pathway of Cholesterol Oxidation



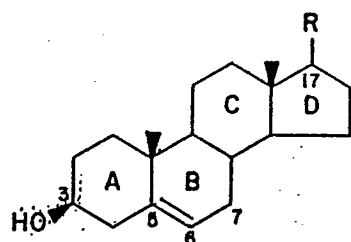
about 70 other derivatives have been identified and characterized (Smith, 1981). The major cholesterol oxidation products are presented in Fig. 2.4. The common and nomenclature names of some cholesterol oxides are listed in Table 2.4.

Table 2.4. Common and Nomenclature Names of Some Cholesterol Oxides

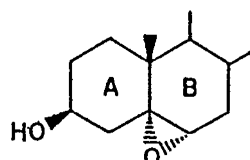
Common Names	Nomenclature Names
7α-hydroxycholesterol	cholest-5-en-3ß,7α-diol
7ß-hydroxycholesterol	cholest-5-en-3ß,7ß-diol
7-ketocholesterol	3ß-hydroxycholest-5-en-7-one
α-epoxycholesterol	5α,6α-epoxy-5-cholestan-3ß-ol
ß-epoxycholesterol	5ß,6ß-epoxy-5-cholestan-3ß-ol
cholestanetriol	5α-cholestane-3ß,5,6ß-triol
22-hydroxycholesterol	cholest-5-en-3ß,22-diol
25-hydroxycholesterol	cholest-5-en-3ß,25-diol

Although the oxidation of cholesterol has been extensively investigated, limited information exits on the oxidation of phytosterols. Since phytosterols differ from cholesterol only in side chain structures, similar oxidation products may be expected. Daly *et al.* (1983) heated ß-sitosterol at 100°C for 48 hours and separated and tentatively identified seven major oxidation products. These oxidation products were 7α and 7ß-hydroxy sitosterol, 7-ketositosterol, 5,6-epoxysitosterol, Δ^4-sitosterol-3,6-dione, Δ^4-sitosterol-3-one and Δ^5-sitosterol-3-one.

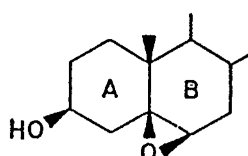
Fig. 2.4 Major Oxidation Products of Sterols



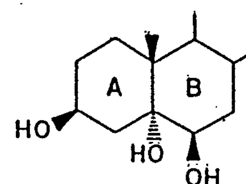
STEROL NUCLEUS



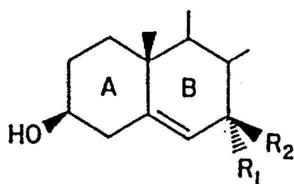
α-EPOXIDE
III



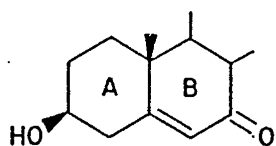
β-EPOXIDE
IV



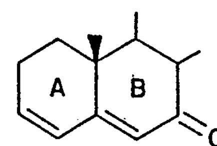
TRIOL
V



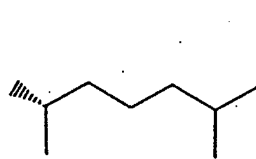
7-HYDROXY
VI R_1 =OH, R_2=H
VII R_1 =H, R_2=OH



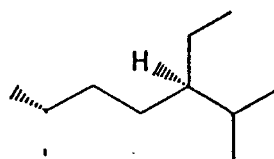
7-KETONE
VIII



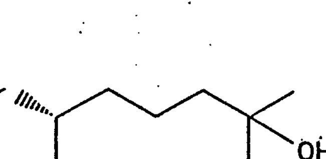
3,5-DIEN-7-ONE
IX



CHOLESTEROL
Ic



β-SITOSTEROL
Is



25-HYDROXY
IIc

SIDE CHAINS

Blekas and Boskou (1989) successfully separated the oxidation products of stigmasterol heated in triacylglycerols by thin layer chromatography (TLC). These authors identified stigmasta-3,5,22-triene, stigmasta-3,5,22-trien-7-one, stigmasta-4,22-dien-3-one, stigmasta-4,6,22-trien-3-one, 5,6-epoxy-stigmasterol, stigmasta-5,22-diene-3ß,7ß-diol, stigmasta-5,22-diene-3ß,7α-diol and 5α-stigmast-22-ene-3ß,5,6ß-triol. Gordon and Magos (1984) studied the oxidation products of Δ^5-avenasterol heated at 180°C in pure triacylglycerols. They successfully isolated epimeric 7-hydroxyavenasterol, 7-ketoavenasterol, cholesta-3,5-diene-7-one, epimeric 5,6-epoxide, cholest-5-en-3-one, cholest-4-en-3-one, cholesta-4,6-diene-3-one and confirmed their identity by ultraviolet-spectroscopy (UV) and gas chromatography - mass spectrometry (GC-MS). More recently, Dutta and Appelqvist (1995) investigated phytosterol oxides in french fries prepared in different vegetable oils. They found that french fries fried in palm/rapeseed oil blend contained 136 ppm and 50 ppm α- and ß- epoxysitosterols and 80 ppm and 24 ppm of α- and ß-epoxycampesterols. The french fries fried in regular and high oleic sunflower oils contained 6 ppm, 7 ppm, and 10 ppm of 7α- and 7ß-hydroxysitosterols and 7-ketositosterol, respectively, and 11 ppm, 12 ppm and 22 ppm of 7α- and 7ß-hydroxycampesterols, and 7-ketocampesterol, respectively.

The kinetics of sterol oxidation have been reported. Yan and White (1990) investigated the cholesterol oxidation rate in heated lard enriched with two and ten times the original amounts of cholesterol. They found that the cholesterol level in the lard were steadily decreasing throughout the heating at 180°C and the formation of cholesterol oxides followed the first order of reaction rate.

2.1.3.2. Oxidation of Sterols During Food Processing and Storage.

Cholesterol oxidation products were first detected in processed egg products and later found in a variety of foods. In general, cholesterol oxidation products are present in low amounts in most processed foods (McCluskey and Devery, 1993). Snack foods, such as potato chips and french fries fried in beef tallow contained high amounts of cholesterol oxidation products (Lee *et al.*,1985, Bascoul *et al.*, 1986). Osada *et al.* (1993) compared the cholesterol and cholesterol oxide contents in fresh and processed marine foods. They found that no detectable amounts of cholesterol oxides were present in fresh marine products. But in dried, canned and pickled fish products, the cholesterol oxides were detected (Table 2.5).

Bascoul *et al.*(1986) observed that 25% cholesterol was lost after 60 hours of frying in beef tallow. They quantified the amounts of cholesterol oxidation products by TLC with FID detector. Major oxidation products of cholesterol decreased in the following order: 90 ppm, 40 ppm, 20 ppm and 15 ppm for 7α and 7ß-hydroxycholesterol, cholestan-3,5-diene-7-one, α- and ß-epoxycholesterols and cholestane-3ß,5α,6ß-triol, respectively. Zhang *et al* (1991) investigated the presence of phytosterol oxides in french fries from two fast food restaurants for 30 consecutive days. They found that total cholesterol oxides in fresh fried fries were ranging from 11 ppm to 39 ppm. The major identified oxides were 7ß-hydroxycholesterol, α- and ß-epoxycholesterols, cholestane-3ß,5α,6ß-triol, 7-ketocholesterol and 25-hydroxycholesterol.

Table 2.5. Cholesterol Oxidation Products (COPs) in Processed Marine Foods[a]

		Sterols (mg/100g)								
		cholesterol	7α-OH[1]	7ß-OH[2]	5ß-epoxy[3]	5α-epoxy[4]	triol[5]	7-keto[6]	unknown	total COPs
Sardine	fresh	193								
	dried	333	2.7	9.8	4.9	1.1		5.3	5.0	28.7
Squid	fresh	799								
	dried	513		5.5	2.2	1.4		1.9	3.6	14.6
	canned boiled	356		2.8	0.7	0.2			7.3	11.0
Alaskan pollack roe	fresh	48.5								
	pickled and spiced	403	3.8	5.8	1.0	0.8	0.3	3.3	4.8	20.9

[1] 7α-OH = 7α-hydroxycholesterol; [2] 7ß-OH = 7ß-hydroxycholesterol; [3] 5ß-epoxy = 5ß-epoxycholesterol;
[4] 5α-epoxy = 5α-epoxycholesterol; [5] triol = cholestane-triol; [6] 7-keto = 7-ketocholesterol.
[a] Adapted from Osada *et al.* (1993b)

Ghavami and Morton (1984) found a considerable amount of phytosterol losses in soybean oil during heating at 180°C for 96 hours. The oxidation products were not analysed in their study. Lee *et al.*(1983) investigated the cholesterol and sitosterol oxidation products in french fries and potato chips collected from five restaurants. In their study, the potato chips were fried in cottonseed oil while the french fries were fried in a mixture of beef tallow and hydrogenated vegetable oils. They found that all the french fries contained various level of sterol oxides (Table 2.6). Four major oxysterols, namely α-epoxycholesterol, ß-epoxycholesterol, 7α-hydroxycholesterol and 7ß-hydroxycholesterol were detected in these french fries. The amounts of oxysterols in the french fries were ranging between 2-81ppm. The potato chips stored at 23°C for 150 days did not contain any detectable amounts of oxysterols. However, the potato chips stored at 40°C for 95 days produced 6 ppm, 13 ppm and 9 ppm of ß-epoxysitosterol, 7α-hydroxysitosterol and 7ß-hydroxysitosterol, respectively. Dutta and Appelqvist (1995) observed that french fries fried in different vegetable oils contained various levels of phytosterol oxidation products. These were as follows: 50-136 ppm α- and ß-epoxysitosterols, 24-80 ppm α- and ß-epoxycampesterols, 6-10 ppm 7α- and 7ß-hydroxysitosterols and 11-12 ppm 7-ketositosterol. Nourooz-Zadeh and Appelqvist (1992) studied the ß-sitosterol oxidation products in soybean and wheat flour and found 53-129 ppm phytosterol oxidation products in the wheat flour stored for 36 months at room temperature. The identification was based on the mass spectra of authentic oxidation products synthesized in their laboratory.

Table 2.6. Levels of Sterol Oxidation Products in French Fries (µg/g of lipids)[c]

Common names:	α-epoxide	ß-epoxide	7ß-hydroxy	7α-hydroxy
Restaurant	cholestan-5, 6α-epoxy-3ß-ol	cholestan-5, 6ß-epoxy-3ß-ol	5-cholestan-3ß,7ß-diol	5-cholestan-3ß,7α-diol
A	19	25	39	tr[b]
	10	27	14	2
B	nd[a]	3	11	7
	9	23	44	21
C	17	18	27	8
	nd	18	30	13
D	tr	tr	3	0
	nd	6	0	0
E	6	9	81	21
	nd	2	62	2

[a] nd= not detected due to co-eluting substances on HPLC, [b] tr = traces on TLC but not quantifiable by HPLC
[c] Adapted from Lee *et al.* (1985)

2.1.4. Health Implication of Sterol Oxidation Products

Cholesterol oxidation products have been found to be cytotoxic, mutagenic and carcinogenic and their effects have been reviewed in a number of papers (Addis and Warner, 1991, Smith, 1989, Colesterolo *et al.* 1992).

There has been accumulating evidence, from *in vivo* and *in vitro* studies, to indicate that some cholesterol oxidation products are powerful atherogenic agents (Smith, 1989). It is known that some cholesterol products are much more powerful in inducing angiotoxicity and atherosclerosis than cholesterol itself. Imai *et al* (1976) was the first to demonstrate that oxidation products of cholesterol play a primary role in arterial wall injury and lesion development. In their study, lesions in arterial walls were observed in rabbits fed with a diet containing partially oxidized cholesterol. However, when carefully purified cholesterol was used to feed the rabbits, it did not result in any lesion formation activities. Peng *et al.* (1978) conducted *in vitro* investigation on the toxicity of cholesterol oxidation products on lesion formation in smooth muscle cells of pigeon arteries. They found that cholestane-3ß,5α,6ß-triol and 25-hydroxycholesterol caused extensive cell damage as compared to purified cholesterol. Jacobson *et al.*, (1985) demonstrated that cholestane-3ß,5α,6ß-triol was so powerful that it could create a high risk of atheroscleroses at very low intake level. Watanabe *et al.*(1988) also reported that cholestane-3ß,5α,6ß-triol was the most toxic of all cholesterol oxides whereas 5α-epoxycholesterol was also recognized as being capable of producing lesions as well as having a mutagenic and a carcinogenic properties.

Some cholesterol oxidation products can cause inhibition of 3-hydroxy-3-methyl glutaryl Co-A

reductase (HMGCoA reductase), a key enzyme involved in cholesterol biosynthesis (Erickson, *et al.*, 1977, Defay *et al.*, 1982). Peng *et al.* (1979) found that 25-hydroxycholesterol was a potent inhibitor of this enzyme followed by cholestane-3ß,5α,5ß-triol. The overall effect of cholesterol oxidation products include: 1) decrease in the amount of cholesterol in the cell membrane, 2) inhibit on normal cholesterol biosynthesis in animal bodies, 3) impair cell membrane function and 4) inhibit cholesterol uptake by cells. These effects may lead to necrosis, abnormal cell proliferation and the formation of atheromas (Huddard *et al.* 1989). Cholesterol oxidation products have also proven to have mutagenic and carcinogenic properties by a number of *in vivo* and *in vitro* studies (Watanabe *et al.*, 1988, Sevanian and Peterson, 1984, Gray *et al.*, 1971, Bischoff, 1969).

The oxidized phytosterols are structurally similar to cholesterol oxides. Substantial amounts of phytosterol oxidation products could be formed during frying and processing of vegetable oils. However, little is known about the health effects of phytosterols products. Further investigation is required in this area.

2.1.5. Determination of Sterols and Their Oxidation Products

2.1.5.1. Preparation of the Samples

The separation and quantification of sterols and their derivatives in foods are difficult because their isolation is frequently impeded by large amounts of triglycerides, phospholipids and other impurities (McCluskey and Devery, 1993). Since sterols and sterol derivatives are present in foods only in small amounts, multiple enrichment steps of samples by saponification, TLC, solid

phase extraction (SPE) and HPLC are usually carried out prior to separation and quantification. Validation of the analysis of sterol oxidation products is crucial because any deviation from the procedure such as temperature increases or exposure to light and oxygen may lead to artifact formation and degradation of oxidation products. Some cholesterol oxidation products such as 7-ketocholesterol are sensitive to alkaline and may be lost during hot saponification (Maerker, 1987).

The analytical procedures used to analyse sterols and their derivatives usually consist of 1) extraction of total lipids; 2) enrichment of sterols and sterol oxides; 3) separation and quantification of cholesterol oxides and 4) confirmation of structural identity.

The lipid extraction is usually the first step toward the analysis of sterols and their derivatives in food and biological samples. The most often used method was the procedure described by Blight and Dyer (1959), in which a mixture of chloroform/methanol (2/1, v/v) was applied as the extracting solvent.

The most commonly used methods to remove the interfering impurities include saponification, column chromatography and preparative thin-layer chromatography. The principle of saponification is to hydrolyse the ester bond of triglycerides and phospholipids in alkaline media and to convert fatty acids into water soluble soaps. In addition, saponification can free the esterified phytosterols in vegetable oils enabling the analysis of the total phytosterol content. After the addition of water to the saponified mixtures, tocopherols, cholesterol, sterols, sterol