THE UNIVERSITY OF MANITOBA

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

by

Wei Li

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

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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.

Finally, to my wife, without her love, understanding, and self sacrifice, I would never complete the project.

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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

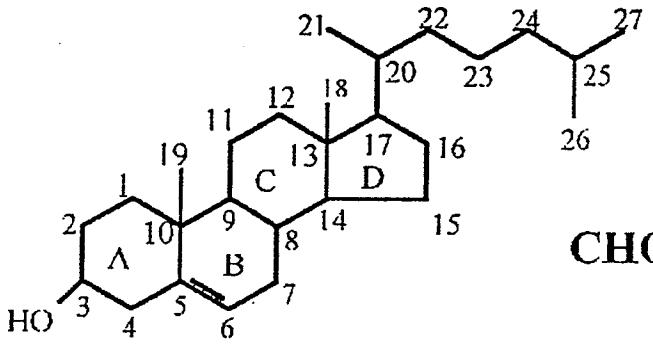
2.1.1. Chemical Structure, Classification of Sterols and Their Occurrences

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

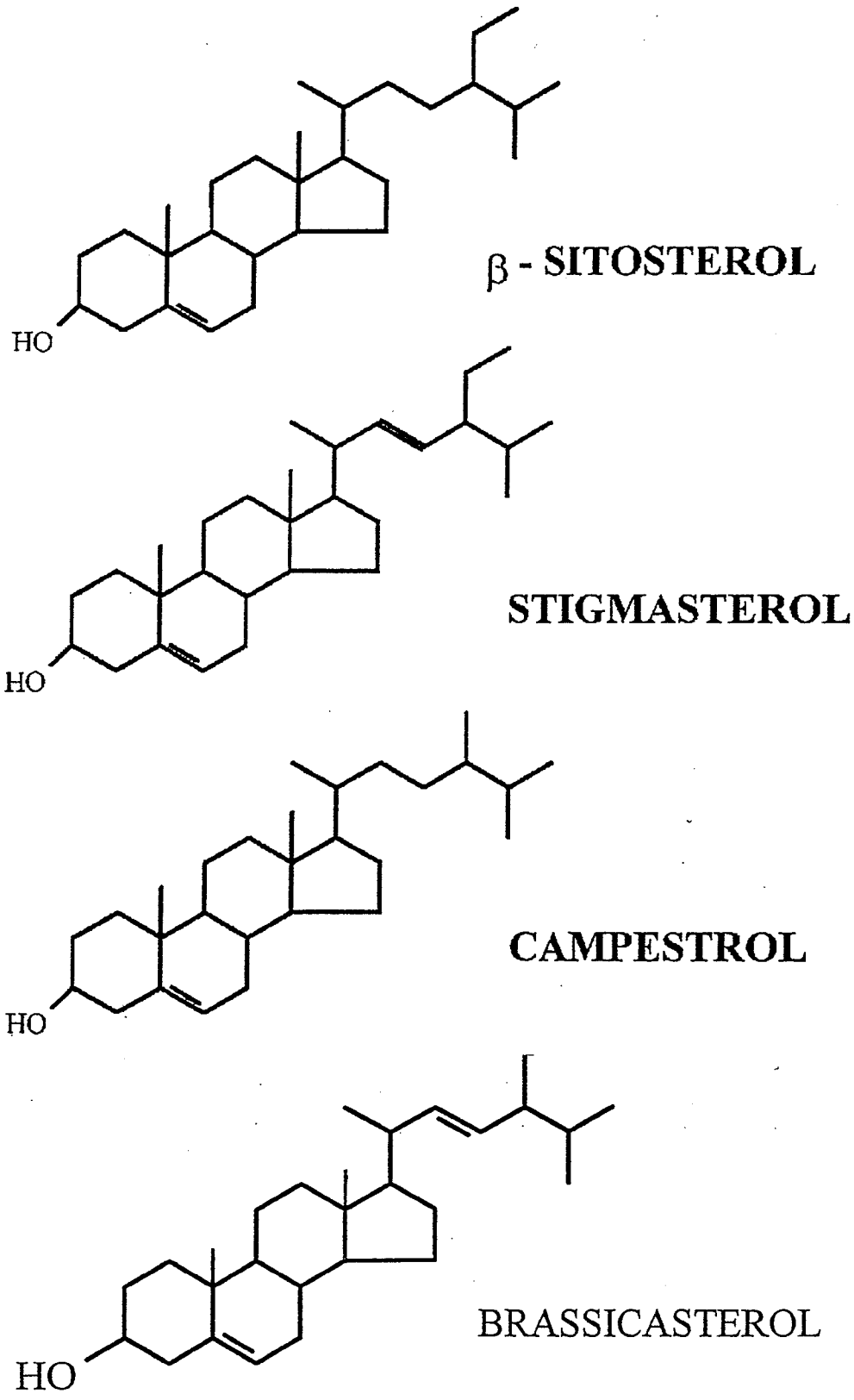
Common Names	Nomenclator Names
Cholesterol	Cholest-5-en-3ß-ol
Campesterol	24α-Methylcholest-5-en-3ß-ol
Brassicasterol	24ß-Methylcholest-5,22-dien-3ß-ol
ß-Sitosterol	24α-Ethylcholest-5-en-3ß-ol
Stigmasterol	24α-Ethylcholest-5,22-dien-3ß-ol
Δ^5-Avenasterol	24-Ethylidenecholest-5-en-3ß-ol
Δ^7-Avenasterol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

Fig. 2.1 Chemical Structures of Cholesterol



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

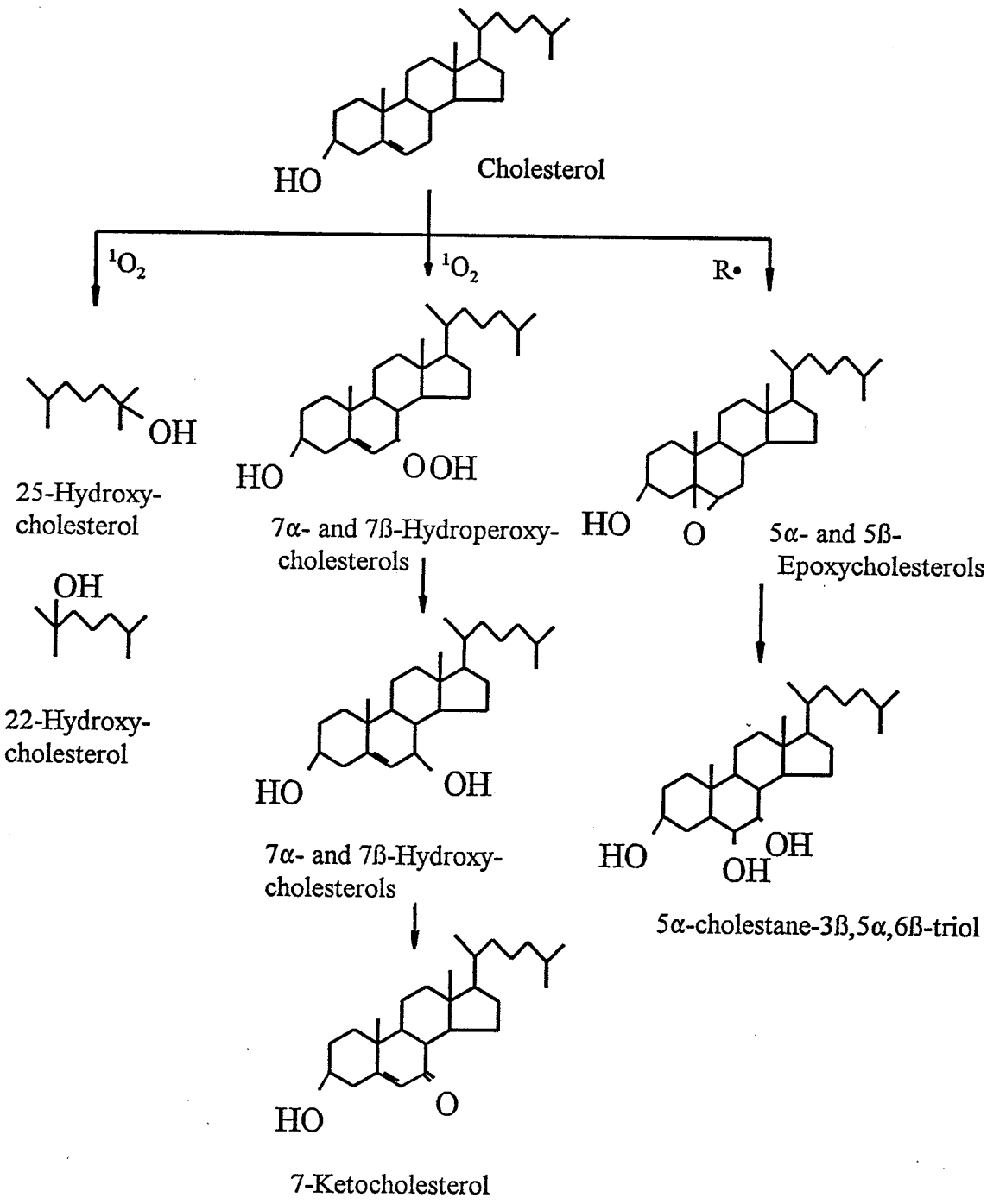
individual sterols and sterol esters by deacylation. Nonpolar steroids and dehydrated phytosterols were formed during bleaching. The oxidized derivatives of sterols, such as sterol ketones may be present in the bleached and deodorized oils (Kochhar, 1983)

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



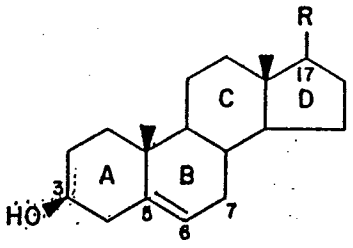
about 70 other derivatives have been identified and characterized (Smith, 1981). The major cholesterol oxidation products are presented in Fig. 2.4. The common and nomenclature names of some cholesterol oxides are listed in Table 2.4.

Table 2.4. Common and Nomenclature Names of Some Cholesterol Oxides

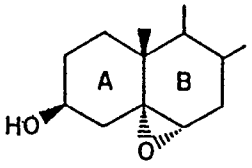
Common Names	Nomenclature Names
7α-hydroxycholesterol	cholest-5-en-3ß,7α-diol
7ß-hydroxycholesterol	cholest-5-en-3ß,7ß-diol
7-ketocholesterol	3ß-hydroxycholest-5-en-7-one
α-epoxycholesterol	5α,6α-epoxy-5-cholestan-3ß-ol
ß-epoxycholesterol	5ß,6ß-epoxy-5-cholestan-3ß-ol
cholestanetriol	5α-cholestane-3ß,5,6ß-triol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

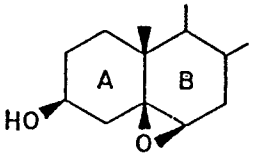
Fig. 2.4 Major Oxidation Products of Sterols



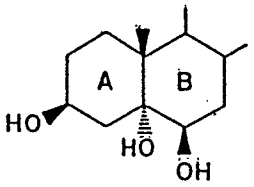
STEROL NUCLEUS



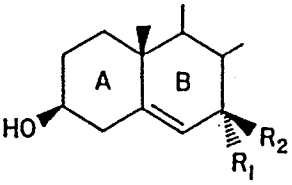
α-EPOXIDE
III



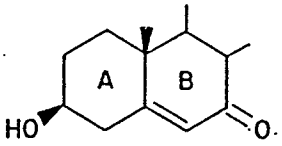
β-EPOXIDE
IV



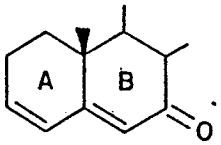
TRIOL
V



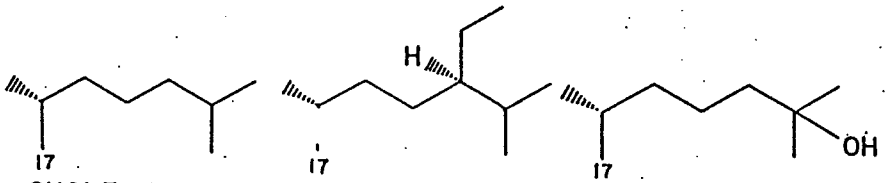
7-HYDROXY
VI R_1=OH, R_2=H
VII R_1=H, R_2=OH



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

oxides and other unsaponifiables are extracted with suitable organic solvents, usually diethyl ether. The hot saponification is still used and often criticized because some oxides are unstable in hot alkaline media. This may be one of the reasons for the formation of cholesterol oxidation artifacts. Maerker and Unruh (1986) studied the effect of hot saponification on isolation and determination of cholesterol oxides and found that epimeric epoxycholesterols, 7-hydroxycholesterols were stable under saponification conditions, but 7-ketocholesterol and 6-ketocholestanol (used as internal standard) were destroyed by the hot alkali. They also observed that saponification contributed to the generation of additional amounts of oxidation products, such as epimeric 7-hydroxycholesterols. Cold saponification was proposed and the recoveries of the most common cholesterol oxides performed (Bergsterom and Wintersteiner, 1941, Park and Addis, 1986a). Park and Addis (1986a) found that the recoveries of cholesterol oxides were no lower than 80% with exception of cholestane-3ß,5α,6ß-triol by cold saponification. They suggested that after using of 5α-cholestane as an internal standard, recoveries of oxides were compensated and did not require any additional correction factors except for cholestane-3ß,5α,6ß-triol. Przybylski and Eskin (1991) observed that by carefully removing oxygen from solvents, containers and solution used and then saponifying at ambient temperature with protection from light, artefacts were not detected at the detection level of 0.1 ppm.

Some researchers used TLC and HPLC to further purify and fractionate unsaponifiables prior to the separation of sterols and their derivatives (Abdel Nabey *et al.*, 1991, Zullo *et al.*, 1989, Koopman *et al.*, 1987). The advantage of TLC and HPLC pre-fractionation is to make mixture

of unsaponifiables less complex. Abdel-Nabey *et al.* (1991) investigated the total unsaponifiables of cottonseed oils. The oils were saponified first and then the unsaponifiables were pre-separated on TLC. The sterol and sterol oxide fractions were removed from TLC, extracted, silylated and then separated and quantified by GC. Koopman *et al.* (1987) carried out the purification of unsaponifiable extracts successfully from serum samples through reversed-phase HPLC, where first 5 mL of solvent following injection was collected for subsequent GC analysis of oxides. Amelio *et al.*(1992) used HPLC to fractionate unsaponifiable into two major fractions: alcohols and sterols. Both fractions were then silylated and separated by GC. Cholesterol, brassicasterol campesterol, stigmastadienol, sitosterol, silostanol, Δ^5-avenasterol, $\Delta^{5,24}$-stigmastadienol, Δ stigmastenol and $^7\Delta$ -avenasterol were separated in the analysed samples.

A number of researchers used column chromatography to enrich the sterol oxides in order to avoid saponification prior to GC analysis (Nourooz-Zadeh and Appelqvist, 1987, 1991, Missler *et al*, 1985, Morgan and Armstrong, 1987, 1989). Morgan and Armstrong (1989) used silica Sep-pak cartridge to enrich the epimeric epoxides from egg yolk lipids. The extracted lipids from egg-yolk were applied into silica cartridge (Sep-pak, Waters Associates), followed by 10 mL of hexane/diethyl ether (95:5, v/v), 25 mL of hexane/diethyl ether (90:10, v/v), 15 mL of hexane/diethyl ether (80:20, v/v) and 10 mL of acetone elution. The collected acetone fraction contained all recoverable cholesterol oxides which were further analysed on GC. Missler *et al.* (1985) purified lipid extracts by silica gel liquid chromatography and then by preparative HPLC prior to GC quantification. The samples were first cleaned up on a glass column (50 cm x 1.75

cm i.d.) packed with silica gel to remove water and polar organic compounds and then applied into HPLC with a column (27.0 x 1.5 cm i.d.) packed with silicic acid. Ethyl acetate was used as mobile phase at a flow rate of 5 mL/min. The fraction with retention time between 10 to 50 minutes was collected and successfully analysed for cholesterol oxides.

2.1.5.2. Determination of Sterols and Their Derivatives

Chromatographic methods are the most often used techniques to separate sterols and their oxidation products (Fenton, 1992).

Gas chromatography (GC) is the most commonly used method to separate and quantify cholesterol, phytosterols and their derivatives (Kovacs, *et al.*, 1978, Fenton and Sim, 1991, Gordon and Griffith, 1991, Przybylski and Eskin, 1991). The majority of researchers performed their separation and quantification of sterols and oxysterols by GC with capillary columns applying nonpolar and polar phases such as DB-1, DB-5, OV-17 and others (Osada *et al.*, 1993, Morgan and Armstrong, 1987, 1989, Park and Addis, 1986a, 1986b, Yan and White, 1990, Nourooz-Zadeh and Appelqvist, 1992, Maerker, 1986). Frega *et al.* (1992) successfully separated the whole unsaponifiable fraction by using a silica capillary column with 50% phenyl-50% methylpolysiloxane as a stationary phase. The samples were saponified and the unsaponifiables were then transferred into trimethylsilyl ethers for GC analysis. Docosanol, tetracosanol, hexacosanol, squalene, α-tocopherol, campesterol, stigmasterol, sitosterol, Δ^5-avenasterol, cycoartenol, 24-methylenecycloartanol and citrostadienol were separated during a single run. The development of capillary GC, especially when combined with direct on-

column injection, has greatly improved the separation and provide better accuracy (Maerker, 1987). Przybylski and Eskin (1991) demonstrated that by using splitless injection on DB-1 capillary column and mass selective detector (MSD) good separation of phytosterol oxidation products can be achieved. The detection limit of sterol oxides reached 0.1 ppm in their study. Identification of sterol oxides is commonly based on the comparison of the retention time of oxysterol standards and the analysed components and often verified by mass spectrometry. The mass spectra of major underivatised cholesterol oxides and their trimethylsilyl derivatives have been published (Park and Addis, 1986a, Tsai and Hudson, 1984). Nourooz-Zadeh and Appelqvist (1992) published some mass spectra of ß-sitosterol oxidation products. Dutta and Appelqvist (1995) presented the mass spectra of authentic phytosterol oxidation products synthezed in their laboratory, including epimeric 7-hydroxysitosterols, epimeric epoxysitosterols, 7-ketositosterol, epimeric 7-hydroxycampesterols, epimeric epoxycampesterols, 7-ketocampesterol, epimeric 7-hydroxystigmasterols, epimeric epoxystigmasterols and 7-ketostigmasterol (Appendix).

HPLC has also been used to separate sterols, both with the reverse and normal phases. Holen (1985) investigated the effect of various mobile phases and temperatures on the separation of eight structurally closely related sterols: desmosterol, ergosterol, brassicasterol, fucosterol, cholesterol, stigmasterol, campesterol and ß-sitosterol on C18 and C8 columns. He found that a temperature of 30°C and a mobile phase of methanol:water (99:1) were the optimal for separation. The advantage of HPLC separation of sterols is that the samples can be directly separated after the saponification without further derivatization. The separation of sterols on

HPLC usually takes a shorter time than that of GC separation. HPLC has also been used to separate sterols oxides (Chen *et al.*, 1994, Csallany *et al.*, 1989). However, the method application is limited because most sterol oxides do not have strong absorbing chromophores and require detection below 210 nm. This restricts solvents that can be used as mobile phases. Some sterol oxides such as α and ß-epoxycholesterols do not absorb UV light at all (Csallany, *et al.*, 1989). Additionally, the separation of all oxidized cholesterol derivatives on one column is hard to achieve because the complicated mixtures require a very high separation efficiency (Sevanian and McLeod, 1987).

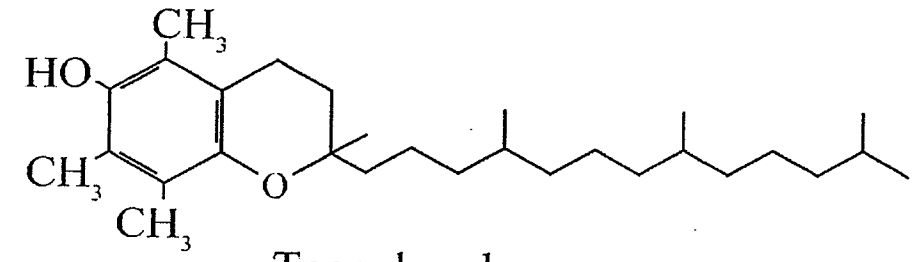
2. 2. Tocopherols:

2.2.1. Chemistry, Occurrences of Tocopherols

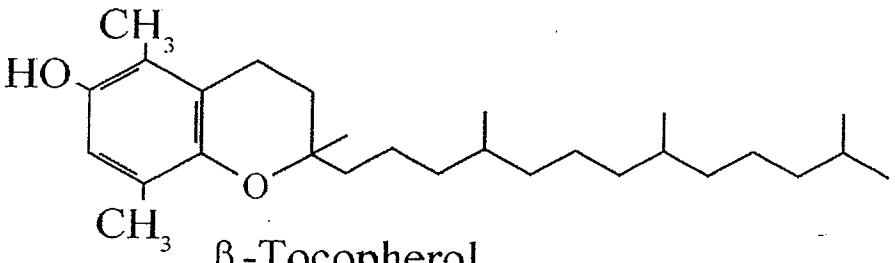
Tocopherols, known as vitamin E, are well known antioxidants in food and biological systems. Eight different compounds having vitamin E activity have been identified and they belong to two families, tocopherols and tocotrienols. Both groups compose of four isomers: α-, β-, γ-, δ- tocopherols and α-, β-, γ-, δ-tocotrienols, depending on the numbers and positions of methyl groups attached to the chromanol. The chemical structures of tocopherols differ from tocotrienols mainly by the three double bonds in the side chains. The chemical structures of tocopherols and tocotrienols are shown in Fig. 2.5 and Fig.2.6

Tocopherols and tocotrienols are present in a variety of plant materials such as nuts, seeds, oils, fruits, vegetables and grasses mainly in free forms (Bauernfield, 1977). Vegetable oils, nuts and seeds are considered superior sources of tocopherols in the human diet (Bauernfield, 1977). Table 2.7 summarizes the tocopherol contents in major vegetable oils.

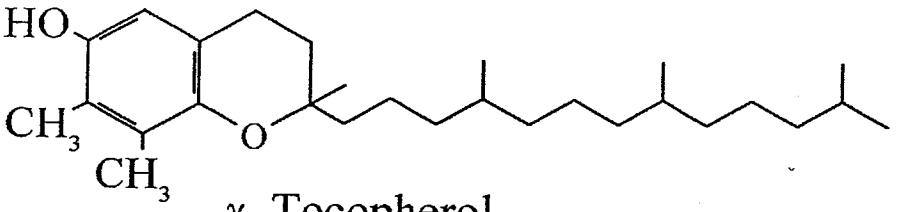
Fig. 2.5. Chemical Structures of Tocopherols



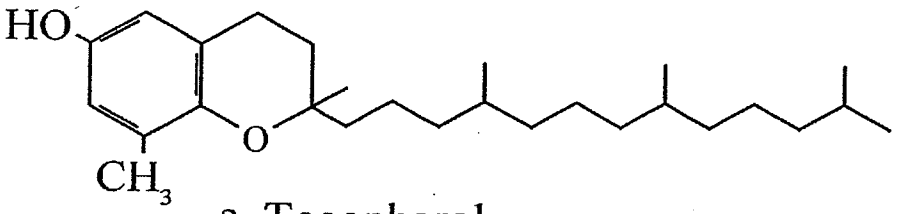
α -Tocopherol



β -Tocopherol

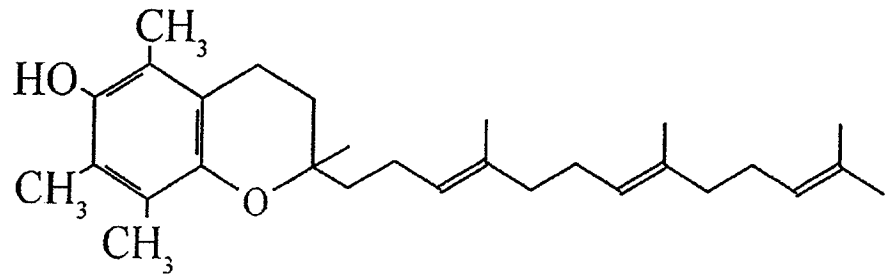


γ -Tocopherol

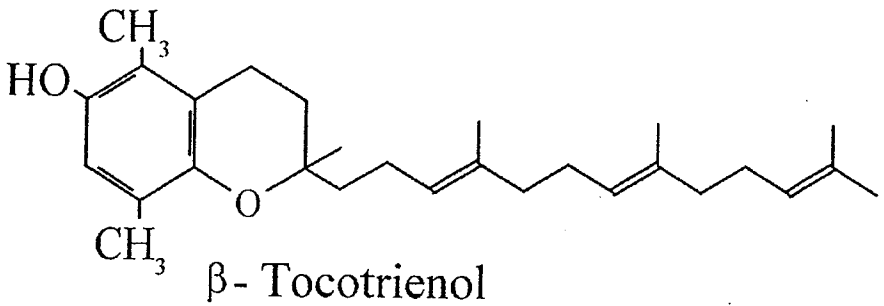


δ -Tocopherol

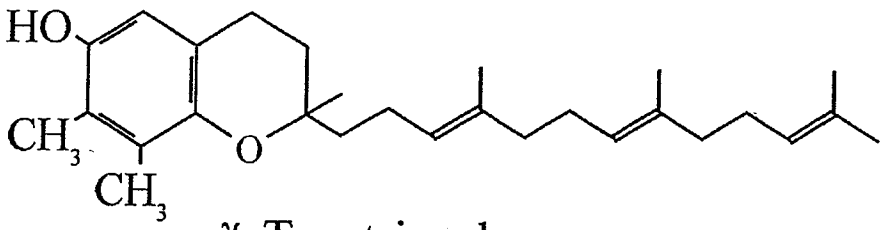
Fig. 2.6. Chemical Structures of Tocotrienols



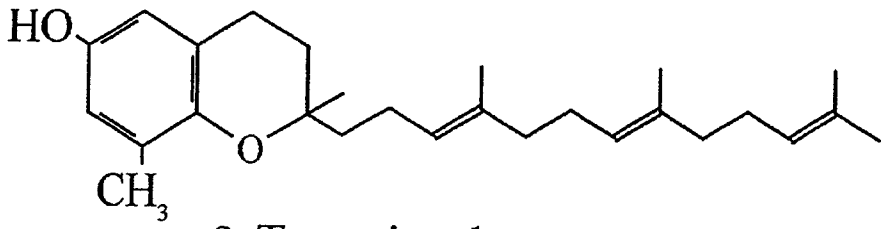
α-Tocotrienol



β- Tocotrienol



γ-Tocotrienol



δ-Tocotrienol

Table 2.7. Tocopherol Contents in Selected Vegetable Oils (ppm)

Oil	α	β	γ	δ
Rapeseed	268.00	-	426.00	-
Canola(Westar)	272.10	0.10	423.20	-
Soybean(Refined)	116.00	34.00	737.00	275.00
Soybean	90.00	-	680.00	230.00
Sunflower	608.00	17.00	11.00	-
Corn	134.00	18.00	412.00	39.00

Adapted from Przybylski, 1994.

The majority of vegetable oils contain mainly tocopherols and only small amounts of tocotrienols (Slover, 1970, Bauernfeind, 1977). Unlike animal fats, few oils have α-tocopherol as the major component. The ß- and γ-tocopherols predominate in many vegetable oils. The tocopherol contents in oil may depend on the maturity of the seeds, growing conditions (Hashim *et al.*, 1993, Kamai-Eldin *et al.*,1991) and processing used (Walker and Slinger, 1975, Jung *et al.*, 1989).

2.2.2. Antioxidant Activities of Tocopherols

Lipids and lipid substances are susceptible to oxidation. The most susceptible components are polyunsaturated fatty acids. This oxidation process causes the deterioration of foods and damage to the cells in a biological system. Tocopherols present in foods and cellular membranes act as antioxidants protecting unsaturated fatty acids and other lipid components

from oxidation (Timmermann, 1990).

In biological systems, α-tocopherol is the most potent antioxidant. Other forms of tocopherols have lower biological effectiveness. The average values of biological activity of tocopherols are given in Table 2.8.

Table 2.8. Biological activity of vitamin E

Compound	%*	Compound	%*
α-Tocopherol	100	α-Tocotrienol	15-30
β-Tocopherol	15-20	β-Tocotrienol	1-5
γ-Tocopherol	1-20	γ-Tocotrienol	1
δ-Tocopherol	1	δ-Tocotrienol	1

Adapted from Schuler 1990
* Relative percentage to α-tocopherol.

Tocopherols contribute to the stability of food systems by protecting them from oxidation. A number of studies have demonstrated that the presence of tocopherols at certain concentrations can significantly delay the oxidation of lipids (Lea and Ward, 1959, Parkhurst *et al.*, 1968, Blekas *et al.*, 1995, Miyagawa *et al.*, 1991). Frankel and Gardner (1989) demonstrated that the presence of tocopherols in oils during storage can reduce the total amounts of oxidation volatile compounds. The mechanism involved in the antioxidant activity of tocopherols has been studied (Burton and Ingold, 1981, Frankel and Gardner,1989, Suarna *et al.*, 1991, 1992). It is generally accepted that tocopherols function as antioxidants by being oxidized to

tocopheroxyl radicals (Suarna, 1991). Tocopherol (TocH) transfers hydrogen from the OH group in the TocH to a peroxyl radical (LOO•) and a tocopheroxyl radical (Toc•) is formed, which combines with another LOO• to form a nonradical products (LOO-Toc) (1,2)

$$LOO\bullet + TocH \rightarrow LOOH + Toc\bullet \quad (1)$$

$$LOO\bullet + Toc\bullet \rightarrow LOO\text{-}Toc \text{ (nonradical products)} \quad (2)$$

(Burton and Ingold, 1981).

Recent studies by Suarna *et al.* (1991) and Suarna and Shouthwell-Keely (1992) reported that some of the oxidation products of tocopherols could be further oxidised by transferring of hydrogen. These authors observed that some tocopherol oxidation products had even better antioxidant activities than tocopherols. The major oxidation products formed from individual tocopherols are different and largely depend on the oxidation stages, condition of oxidation, substrates, and the type of antioxidant present.

2.2.3. Degradation of Tocopherols in Foods During Frying and Storage

A large portion of fats and oils consumed in the North America each year is used for frying (Yan and White, 1990). Frankel *et al.*(1959) considered that the fate of tocopherols in fats was controlled by two principal reactions: 1) between tocopherol and formed reactive hydroperoxides and 2) the spontaneous oxidation of tocopherol by oxygen. Although tocopherols are very effective antioxidants at room temperature, they have little effect at frying conditions (Peled *et al.*, 1975, Marinova, *et al.* 1992). This is because tocopherols are spontaneously oxidized and distilled with steam and/or destroyed at high temperatures.

Ramanujan and Anantakrishnan (1958, cited from Bauernfeind, 1977) heated peanut oil and sesame oil to 175°C for 30 min. and found that 32% and 40% of tocopherols were lost, respectively. More recently, the tocopherol changes in various frying situation were investigated by Miyagawa *et al.*(1991) and Gordon and Kourimaska (1994). These authors concluded that the rate of tocopherol losses were different and affected by the type of frying, products fried and frying conditions.

Tocopherols are also subject to significant losses during storage. Bunnell *et al.*(1965, cited from Bauernfeind, 1977) observed that the losses of tocopherol during the storage of food cooked in vegetable oils were even greater than that during cooking.

2.2.4. Methods to Determine Tocopherols

Tocopherol contents in foods and biological systems are usually determined by electrochemical and chromatographic methods.

In the past, TLC and GC were used extensively for quantification of individual tocopherols in oils and foods. TLC was employed during as it was the only sufficient analytical technique within the reach of many laboratories at that time (Kochhar and Rossel, 1990). The development of GC had created the better separation and sensitivity than TLC, and was then employed regularly for the quantification of tocopherols by many researchers (Slover and Thompson, 1981, Marker, 1988, Lercker and Caboni, 1985). The procedure of GC analysis usually requires saponification followed by the preliminary separation and clean-up of

unsaponifiables by TLC and other methods prior to GC analysis. Most of the above procedures require a long time and extensive sample preparation. Since tocopherols are known to be sensitive to light and prone to oxidation, losses may occur during the lengthy preparation of the samples. These limitations and difficulties prompted the development of HPLC techniques. Nowadays, HPLC is the most often used method to analyse tocopherols and tocotrienols in vegetable oils and foods. A number of researchers have described versatile procedures used to estimate the tocopherol and tocotrienol contents in vegetable oils by HPLC (Thompson and Hatina, 1979, Speek *et al.*, 1985, Pozok *et al.*, 1990, Abdollahi *et al.*, 1993). The most popular and most often cited procedure is that described by Carpenter (1979). The vegetable oils were diluted in *n*-hexane and then injected directly into the HPLC system without further sample preparation. A silica column with a mobile phase of hexane and isopropanol (99:1 or 98.5:1.5) was employed for the separation of tocopherols. The detectors, either UV set at 280 nm or fluorescence detector set at 290 nm excitation and 330 nm emission, were use. All tocopherol and tocotrienol isomers were separated in a single run and the whole procedure took only 10-15 min and was found to be ideal for routine analyses.

Reversed phase HPLC has also been used for tocopherol analysis in foods, especially when other vitamins and components such as sterols are determined simultaneously (Pozo, *et al.*, 1990, Andrikopoulos *et al.*, 1991). However, it should be noted that the use of a reverse phase column and methanol/water as a mobile phase does not separate β- and γ-tocopherol isomers.

Chapter III

Objectives of the Study

3.1. Objectives

The overall objective of this study was to investigate the behaviour of phytosterols and tocopherols in canola oils during frying and storage of fried foods.

3.2. Specific Objectives:

1). To investigate the kinetics of phytosterol oxidation and products formation.

2). To investigate phytosterol changes in frying oils during the frying process.

3). To evaluate the changes in phytosterols during potato chips storage.

4). To identify and quantify phytosterol oxidation products formed during frying and storage of fried products.

5). To investigate tocopherol changes in frying oils during the frying process.

6). To assess the effect of potato chip storage on tocopherols changes.

Chapter IV

Experiment Design and Materials

4.1 Materials.

Name of the Products	*Name of the Company*
Standards:	
1. ß-sitosterol (approx. 60% ß-sitosterol, 20% campesterol),	Sigma, St. Louis, MO, USA;
2. stigmasterol (96%),	Sigma, St. Louis, MO, USA;
3. campesterol (65%),	Sigma, St. Louis, MO, USA;
4. cholesterol (99%),	Sigma, St. Louis, MO, USA;
5. 7-ketocholesterol (95-97%),	Sigma, St. Louis, MO, USA;
6. 25-hydroxycholesterol,	Sigma, St. Louis, MO, USA;
7. 7α-hydroxycholesterol,	Sigma, St. Louis, MO, USA;
8. Cholesterol-α-epoxide,	Sigma, St. Louis, MO, USA;
9. ß-Epoxycholesterol,	Steraloids Inc., Wilton, NH, USA;
10. 7ß-Hydroxycholesterol and	Steraloids Inc., Wilton, NH, USA;
11. Cholestane-3ß,5α,7ß-triol	Steraloids Inc., Wilton, NH, USA;
12. Plant sterol mixture (approx. 20% brassicasterol, 30% campesterol, 45% ß-sitosterol 5% stigmasterol),	Matreya Inc., Pleasant Gap, PA, USA.

Name of the Products	*Name of the Company*
Agents:	
1. Sylon-BTZ (N, O-bis (trimethylsilyl) acentamide : trimethylchlorosilane : trimethylsilylimidazole, 3:2:3)	Supelco (Oakville, ON, Canada);
2. Pyridine (99.9%),	Sigma, St. Louis, MO, USA.
Samples:	
1. Regular canola oil (RCO) Commercially refined, bleached and deodorized (RBD)	CanAmera Foods, Saskatoon, Saskatchewan;
2. Hydrogenated canola oil (HYCO) Commercially RBD	CanAmera Foods, Saskatoon, Saskatchewan;
3. Low linolenic canola oil (LLCO) Commercially RBD	CanAmera Foods, Saskatoon, Saskatchewan;
4. High oleic canola oil (HOCO) Experimentally RBD	Allelix Inc. Clayton-Humko, Tennessee, USA;
5. Potatoes *(norchip* variety)	Southern Potato Co., Winkler, Manitoba, Canada.

4.2. Kinetics of Sterol Oxidation

4.2.1. Heating Phytosterols.

ß-Sitosterol, stigmasterol and cholesterol were dissolved in methanol at a concentration of about 1 mg/mL. One mL of each solution was placed into 20 mL vials (50 mm x 25 mm i.d.) and stored in an oven at 75°C, 95°C, 120°C, 155°C and 180°C for 1 hour, 5 hours and 12 hours. The methanol evaporated during first few minutes of heating and a thin layer of sterol film formed on the vial walls.

4.2.2. HPLC Determination and Quantification of Sterols.

The heated sterol products were dissolved in 5 mL of methanol and 10-20 µL of solutions were injected into HPLC for quantification. The samples were analysed in duplication. A Shimadzu HPLC system comprising of a pump, an autosampler and a UV detector was used. A reverse phase C18 column (SGE, 3 µm, 250 x 4.0 mm i.d., SGE Co., Australia,) was used to separate phytosterols. Isocratic mobile phase of acetonitrile: methanol (50:50, v/v) was used at a flow rate of 0.8 mL/min. Sterols were detected at 210 nm and quantified with external calibration.

4.3. Frying and Storage of Potato Chips

4.3.1. Potato Chip Frying.

The potato chips analyzed were fried in the four canola oils by Petukhov (1996). Briefly, a *Belshaw* Model 611 mini-fryer (Belshaw Bros. Inc., Seattle, Washington, USA) was used. On the first day of frying, the oils were heated to 185±5°C for 30 min. The pre-heated oils were sampled and labelled as day 0. Potatoes *Norchip* variety were sliced to approximately 1.2 to

1.3 mm thickness and fried in batches using a wire holder. The frying temperature was held at 180±5°C. The frying time was 15 minutes per batch, 8 hours frying each day (32 batches) for five consecutive days. The oils were cooled down overnight and reheated to 180±5°C in the morning. About 10-15% of fresh oils were added at the beginning of each day to compensate for losses. Oil samples were taken at the end of each frying day and labelled as day 1, 2, 3, 4 and 5, respectively. The oil samples were flushed with nitrogen and stored at -30°C until analysed. All the batches of potato chips fried from each day were combined, mixed to make a composite batch and sealed into commercial cellophane bags (approx. 4.5 g/bag) and stored.

4.3.2. Heating Oils at Simulated Frying Temperature

About 250 mL of each oil was placed into the stainless steel cooking pot. The oils were heated on laboratory hot plates with electric stirrers to a temperature of 190±2°C. The oils were heated continuously without cooling for 72 hours. The samples were taken at 0 hour, 5 hours, 12 hours, 24 hours, 48 hours, and 72 hours of heating. The oil samples were flushed with nitrogen and stored at -30°C until analysed.

4.3.3. Storage of Potato Chips.

The storage of potato chips performed by Petukhov (1996). Briefly, the potato chips collected from first and fifth day of frying were stored under accelerated storage conditions at 60 °C in the dark (Przybylski, *et al.*, 1993). The sealed potato chip bags were removed after 1, 2, 4, 8, 16 days and placed in the freezer at -30°C until analysed.

4.3.4. Method of Analysis:

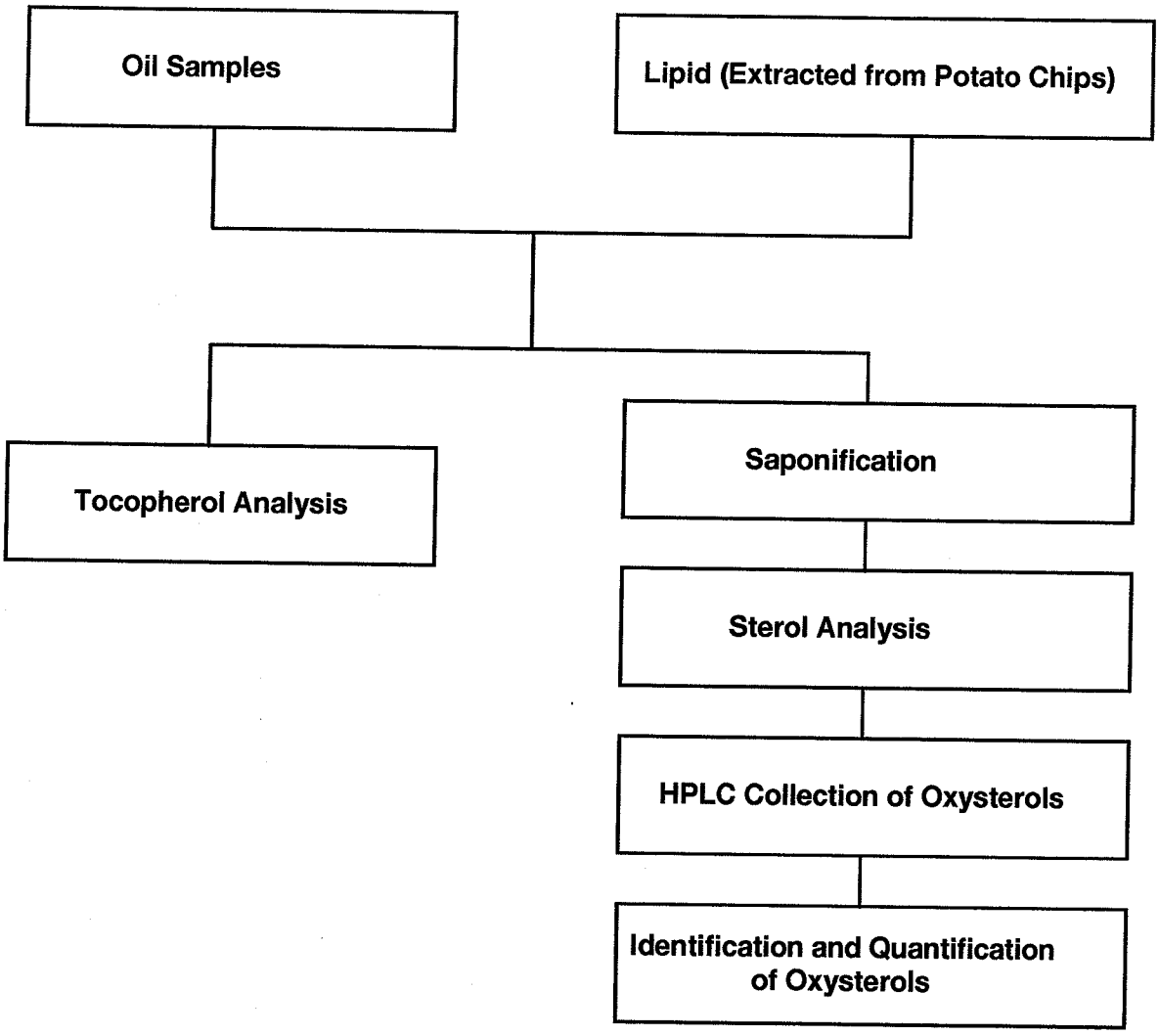
4.3.4.1. Lipid Extraction.

The procedure of lipid extraction of potato chips was based on the method described by Lee *et al.* (1985). Briefly, 8 g of potato chips were blended with 50 mL of chloroform/methanol (2:1) two times in a homogenizer. The combined extracts were filtered through a filter paper into separatory funnels; then 20 mL of water and 20 mL of chloroform were added. The mixtures were allowed to separate, the bottom layer was collected, and the solvent was removed under a stream of nitrogen. The extracted lipids from potato chips were weighed, flushed with nitrogen and stored at -30°C until analysed.

4.3.4.2. Saponification.

The saponification method was based on the procedure described by Lozano (1993). The procedures were as follows: 300-500 mg of oil was placed in threaded tubes and accurately weighed. The fat hydrolysis was carried out in 10 mL 1N methanolic KOH at room temperature for 16-18 hours. Cholesterol and 5α-cholestane were added into the sample as internal standards before saponification. The unsaponifiable components were extracted 3 times with 10 mL diethyl ether after addition of 10 mL distilled water. The organic layers were combined and washed two times with 10 mL water, then dried under nitrogen. Residues were dissolved in absolute ethanol and analysed by HPLC.

Fig. 4.1. Scheme of Sterol and Tocopherol Analysis



4.3.4.3. Determination of Sterols

The separation of sterols is based on the method described by Holen (1985). Shimadzu HPLC system with a UV detector set at 205 nm was used. The unsaponifiables were separated on a reversed phase C18 column (Beckman, 250 mm x 4.6 mm i.d., 5µm). The mobile phase used was 100% methanol or methanol with 0.5-1.0% of water, whichever gave better separations. Flow rate was kept at 1.0 mL/min. The sterols were quantified by external calibration using individual calibration curves.

4.3.4.4. HPLC Fraction Collection and Identification of Sterol Oxides.

During the separation of unsaponifiables, the first 12 minutes of solvent was collected using a Shimadzu fraction collector. The collected fraction was evaporated to dryness and then silylated with Sylon-BTZ and analysed on GC-MS to identify phytosterol oxidation products.

4.3.4.5. Identification and Quantification of Phytosterol Oxides

The identification of phytosterols was performed on 7070E VG Analytical Organic Mass Spectrometry System and Hewlett Packard GC model 5890. A fused silica capillary SPB-20 (0.25 mm x 30 m with a film thickness 0.15 µm, Supelco Co.,Oakville, ON, Canada) column was used. The chromatographic conditions were based on the method described by Przybylski and Eskin (1991). Briefly, the injector temperature was at 260°C. The column temperature was programmed from 90°C to 210°C at a rate of 30°C/min, then held for 1 minute and further programmed to 280°C at the rate of 5°C/min. The initial and final temperatures of the column were held for 8 and 40 min, respectively. Helium was used as a carrier gas. Mass spectra were

scanned within a mass range from 50 to 650 amu. The oil samples were saponified and unsaponifiables were evaporated to dryness, transferred into trimethylsilyl ethers by Sylon-BTZ and then loaded into GC-MS for identification. Component identification was based on the fragmentation and the comparison with spectra of authentic compounds provided by Dutta and Appelqvist (1995). For quantification, a Hewlett Packard model 5890 GC was used. The separation was performed on a capillary column (Resteck, Rtx-5, 30 m x 0.25 mm i.d. Bellefonte, PA, USA). The same chromatographic conditions were used with hydrogen as a carrier gas. The detector temperature was set at 280°C.

4.3.4.6. Tocopherol Analysis.

Tocopherol analysis was based on AOCS method (Ce 8-89). Modifications were made because relatively small amounts of samples were available in this study. The analysis procedure was as follows: 400- 500 mg of oils were weighed into 5 mL volumetric flask with hexane making up the remaining volume. 20-30 µL of the solutions were injected into HPLC. A Hewlett Packard fluorescence detector (Model HP 1046A) was employed for detection of tocopherols with the excitation at 290 nm and the emission at 330 nm. Tocopherols were separated on a normal phase silica column (LiChrosorb Si60, 5 µm, 250 x 4 mm i.d., Merck, Dormstadt, Germany) with hexane/2-propanol (98.5/1.5, v/v) as a mobile phase at a flow rate 1 mL/min. All tocopherols were quantified using a α-tocopherol calibration curve.

4.3.4.7. Analysis of Fatty Acids.

The fatty acid analysis was based on the methods described by Przybylski (1993). Briefly, 100-

500 mg of oils were weighed into threaded tubes, then 1 mL of petroleum ether was added, mixed with fat to obtain a monophase system and 12 mL 0.5N methanolic HCl solution was added. The mixture was heated to 70°C and held at this temperature for 1 hour with occasionally mixing until the solution was monophase. The mixture was then cooled to room temperature, and 6 mL of water and 5 mL of *iso*-octane were added. The clear *iso*-octane layer was removed and injected into GC for fatty acid analysis. Supelcowax 10 column (30 m x 0.25 mm i.d., Supelco, Oakville, ON, Canada) was used. The injector and detector temperatures were held at 250°C and 280°C, respectively. The column temperature was programmed as follows: from 190°C to 235°C at a rate of 3.0°C/min and then held for 7 minutes.

Chapter V

Results

5.1 Kinetics of Phytosterol Oxidation

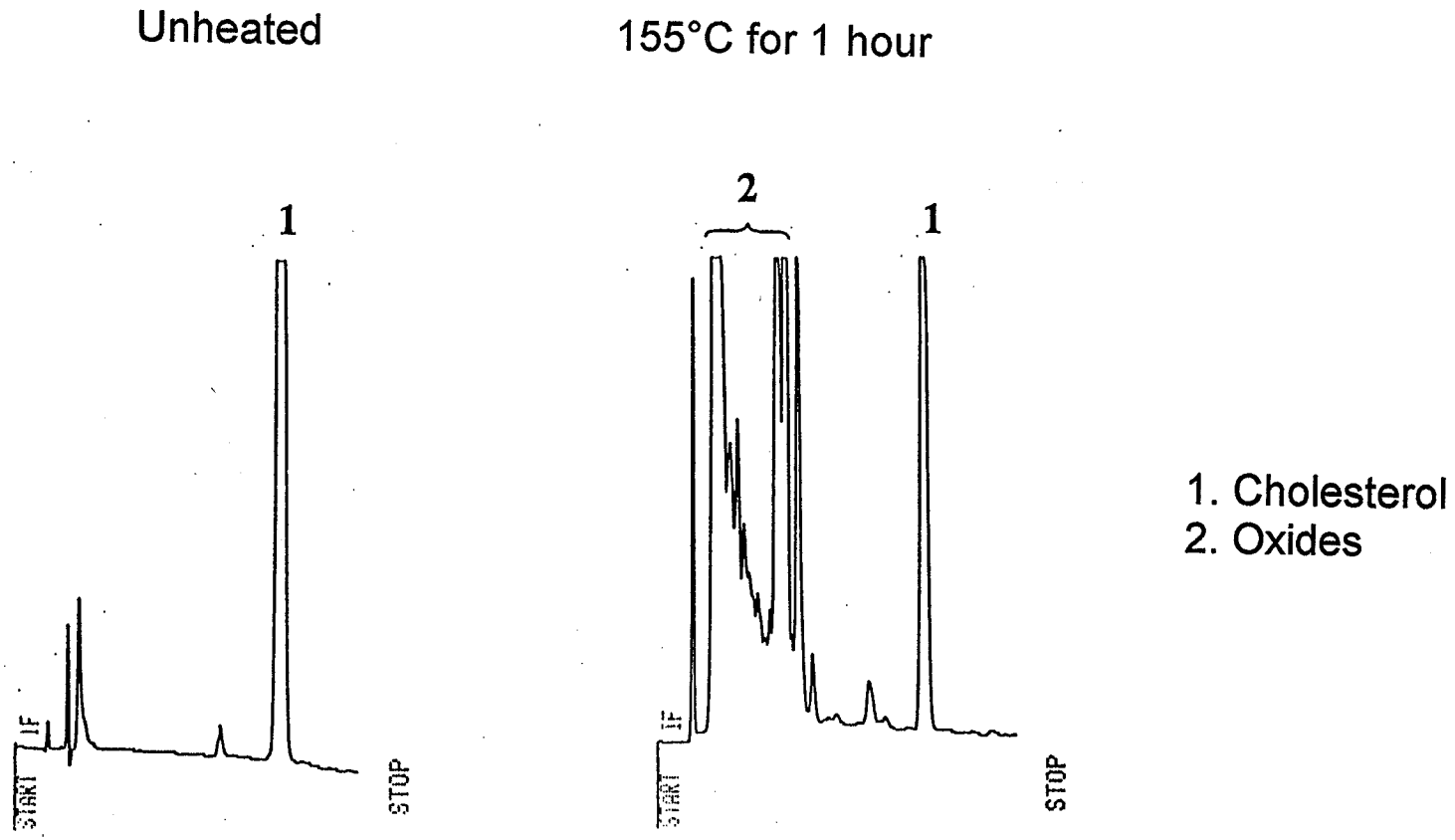
5.1.1. Determination of Sterols and Sterol Oxides.

The separation of cholesterol before and after heating is shown in Fig 5.1. The chromatogram showed that unheated cholesterol had only one major peak. When cholesterol was heated at 155°C for 1 hour, three major groups of peaks appeared with a retention time of up to 5 min. The area of the cholesterol peak decreased accordingly. In order to verify the position of cholesterol oxides in the chromatogram, standards of cholesterol oxides, such as: 7-ketocholesterol, 25-hydroxycholesterol, 7α-hydroxycholesterol, were injected into the system using the same operating condition. Cholesterol oxide standards appeared in the same band as the peaks from the oxidized samples (Fig 5.1, 5.2, 5.3). As cholesterol oxidation is a very complex reaction and more than 70 oxidation products have been identified (Smith, 1981), it is difficult to separate all individual cholesterol oxides on the HPLC. The peaks appeared in the oxides band, therefore, only representing groups of oxidized products. The identification of these peaks needed to be further investigated by GC, because of its higher separation efficiency.

ß-Sitosterol standard contained approximately 60% of ß-sitosterol and 20% of campesterol (Fig. 5.2). The chromatogram of the phytosterol mixture before heating showed two major peaks representing ß-sitosterol and campesterol. After heating at 155°C for 1 hour, two major

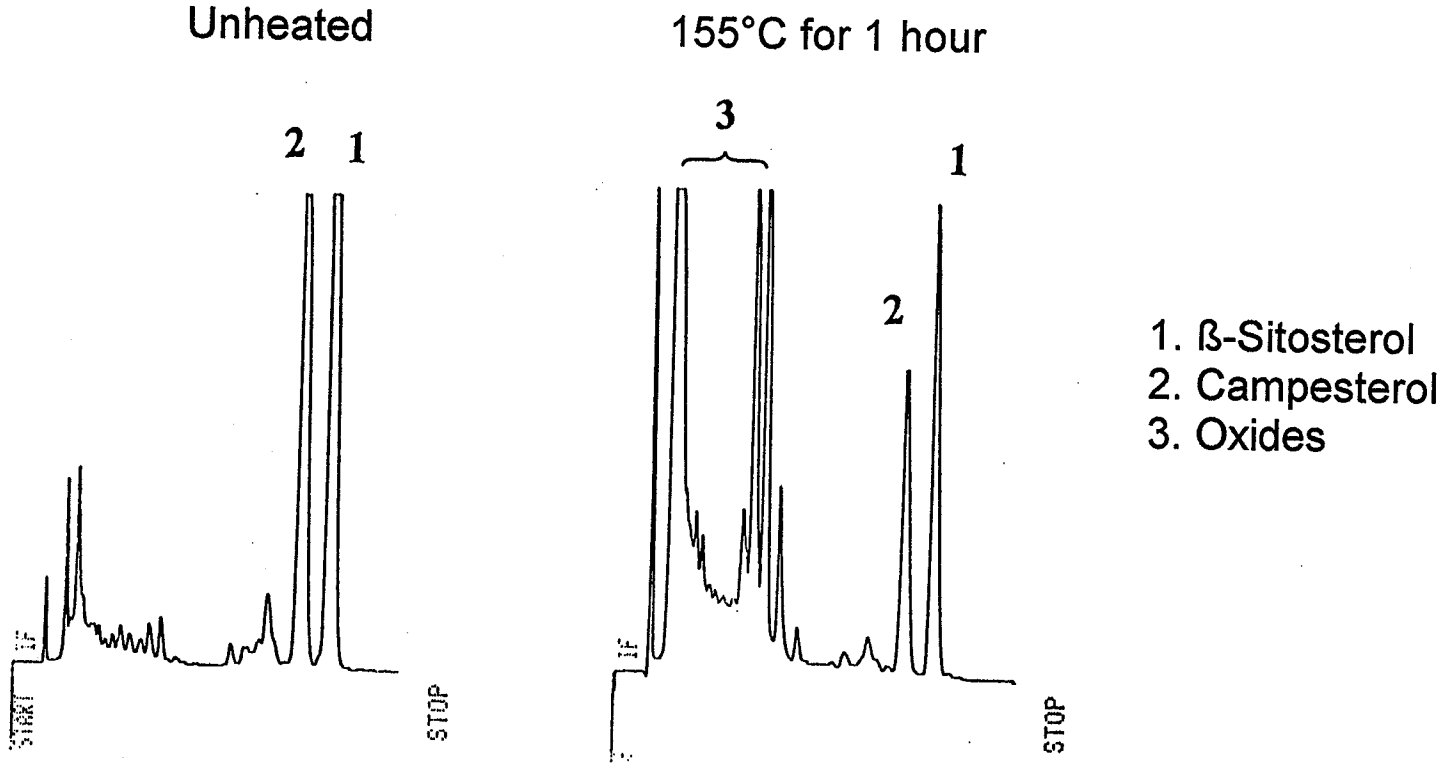
groups of peaks appeared with a retention time of up to 5 min. The peaks of unaltered sterols decreased accordingly. These new peaks appeared in the same band as cholesterol oxides, suggesting that the products formed from ß-sitosterol and campesterol might have similar chromatographic properties. The separation of the stigmasterol standard is shown in Fig. 5.3. However, relatively small amounts of stigmasterol oxidized products were observed after heating at the same condition.

Fig. 5.1. HPLC Separation of Cholesterol Oxidation Products



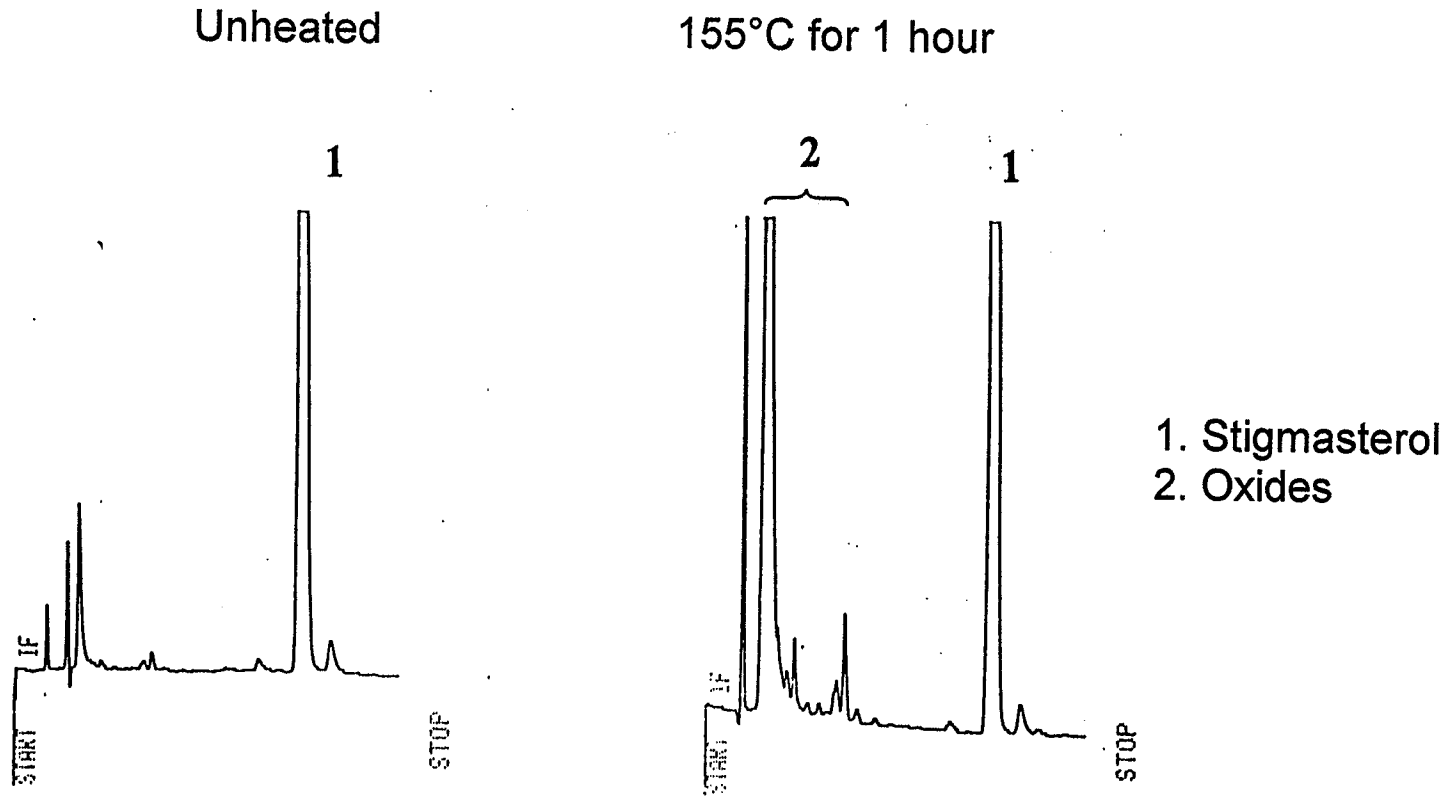
Chromatography conditions: Separation was performed on Shimadzu HPLC system with a RP-C18 column (SPE, 3µm, 250 mm x 4.0 mm i.d.). The mobile phase was methanol/acetonitrile (50/50, v/v) at flow rate 0.8 mL/min. UV detector was used with the wavelength set at 210nm.

Fig. 5.2. HPLC Separation of ß-Sitosterol and Campesterol Oxidation Products



Chromatography conditions: Separation was performed on Shimadzu HPLC system with a RP-C18 column (SPE, 3µm, 250 mm x 4.0 mm i.d.). The mobile phase was methanol/acetonitrile (50/50, v/v) at flow rate 0.8 mL/min. UV detector was used with the wavelength set at 210nm.

Fig. 5.3 HPLC Separation of Stigmasterol Oxidation Products

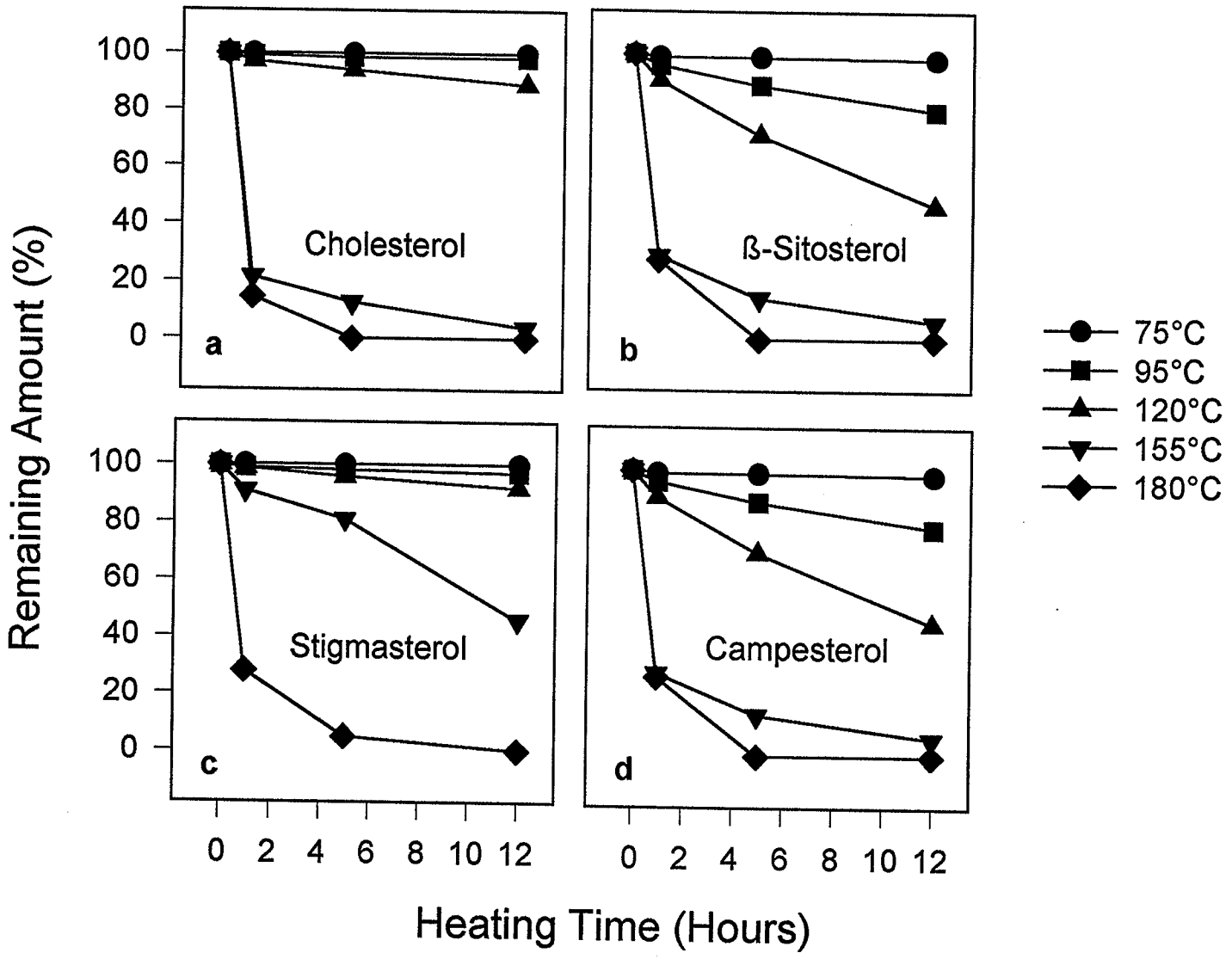


Chromatography conditions: Separation was performed on Shimadzu HPLC system with a RP-C18 column (SPE, 3µm, 250 mm x 4.6 mm i.d.). The mobile phase was methanol/acetonitrile (50/50, v/v) at flow rate 0.8 mL/min. UV detector was used with the wavelength set at 210nm.

5.1.2. Phytosterol Stability During Heating.

The changes to cholesterol, ß-sitosterol, campesterol and stigmasterol during heating are shown in Fig. 5.4. Cholesterol was quite stable with minimal changes occurring when heated at 75°C and 95°C up to 12 hours. When the temperature was increased to 120°C about 10% of cholesterol disappeared after 12 hours of heating (Fig. 5.4a). ß-Sitosterol and campesterol were less stable during heating than cholesterol. ß-Sitosterol was stable at 75°C but when it was heated to 95°C for 12 hours, about 20% of ß-sitosterol disappeared (Fig. 5.4b and c). The oxidation rate became faster when higher temperatures were applied. At 155°C and 180°C, the degradation rates of ß-sitosterol was similar to that of cholesterol and 80-85% of these sterols disappeared during the first hour of heating. The stability of campesterol was similar to that of ß-sitosterol (Fig. 5.4d). Stigmasterol showed the highest resistance to oxidation. It was very stable at 75°C, 95°C and 120°C with only very small changes being observed (Fig. 5.4). When heated to 155°C for 1 hour, only 10% of the stigmasterol disappeared as compared to 80% for all other sterols analysed. At 180°C, stigmasterol disappeared at a much faster rate; about 75% of this sterol was transformed after the first hour of heating, whereas the other three sterols disappeared totally under these conditions.

Fig. 5.4 Oxidative Stability of Sterols During Heating



5.1.3. The Degradation Products Produced by Sterols During Heating.

The formation of oxidation products from ß-sitosterol, campesterol and stigmasterol during heating is presented in Fig. 5.5 and 5.6 respectively. The total oxidation products produced by sterols were estimated by summing up the areas of all new peaks after heating and expressed as the percentage of the initial amounts of parent sterols. It was observed that higher temperatures caused a faster formation of oxidation products. When ß-sitosterol and campesterol were heated at 155°C for 1 hour, the production of total oxidation products was about 8 times faster than at 120°C for 1 hour. Longer heating time at high temperatures caused a rapid decrease of sterol oxides and the formation of further degradation compounds such as polymers. The amounts of polymers were estimated by the following formula:

$$\text{Polymers (\%)} = \frac{\text{total peak area of unheated sterols - total peak area of heated sterols}}{\text{total peak area of unheated sterols}}$$

These polymers were observed to be brown in colour the vials and were not soluble in methanol. The behaviour of stigmasterol was different. Stigmasterol showed higher resistance to oxidation during heating and produced lower amounts of oxides and higher amounts of further degradation products than other sterols. The maximum amounts of stigmasterol oxidation products were produced at 180°C when heated for 1 hour.

Fig. 5.5. Formation of ß-Sitosterol and Campesterol Oxidation and Polymer Products

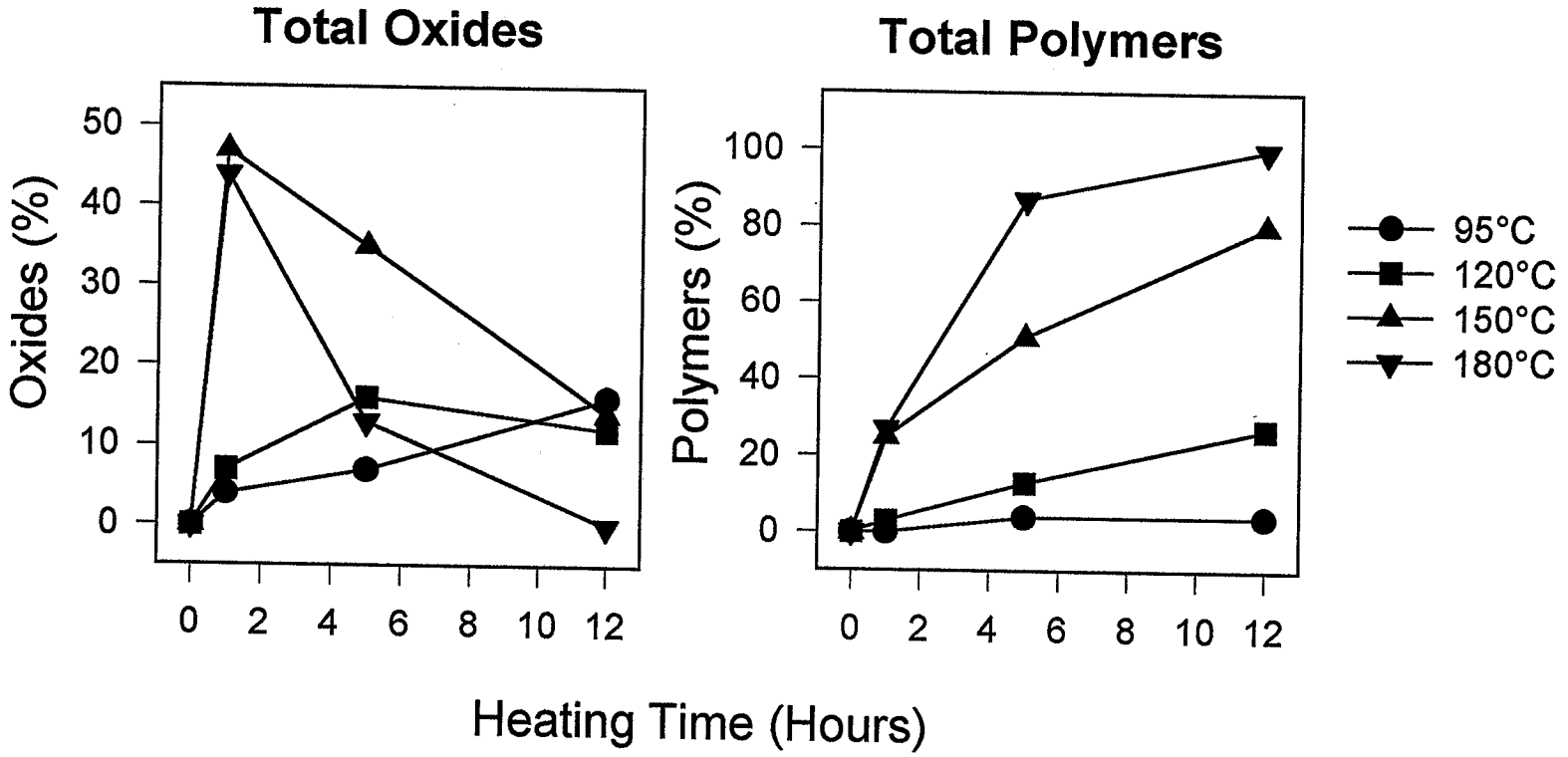
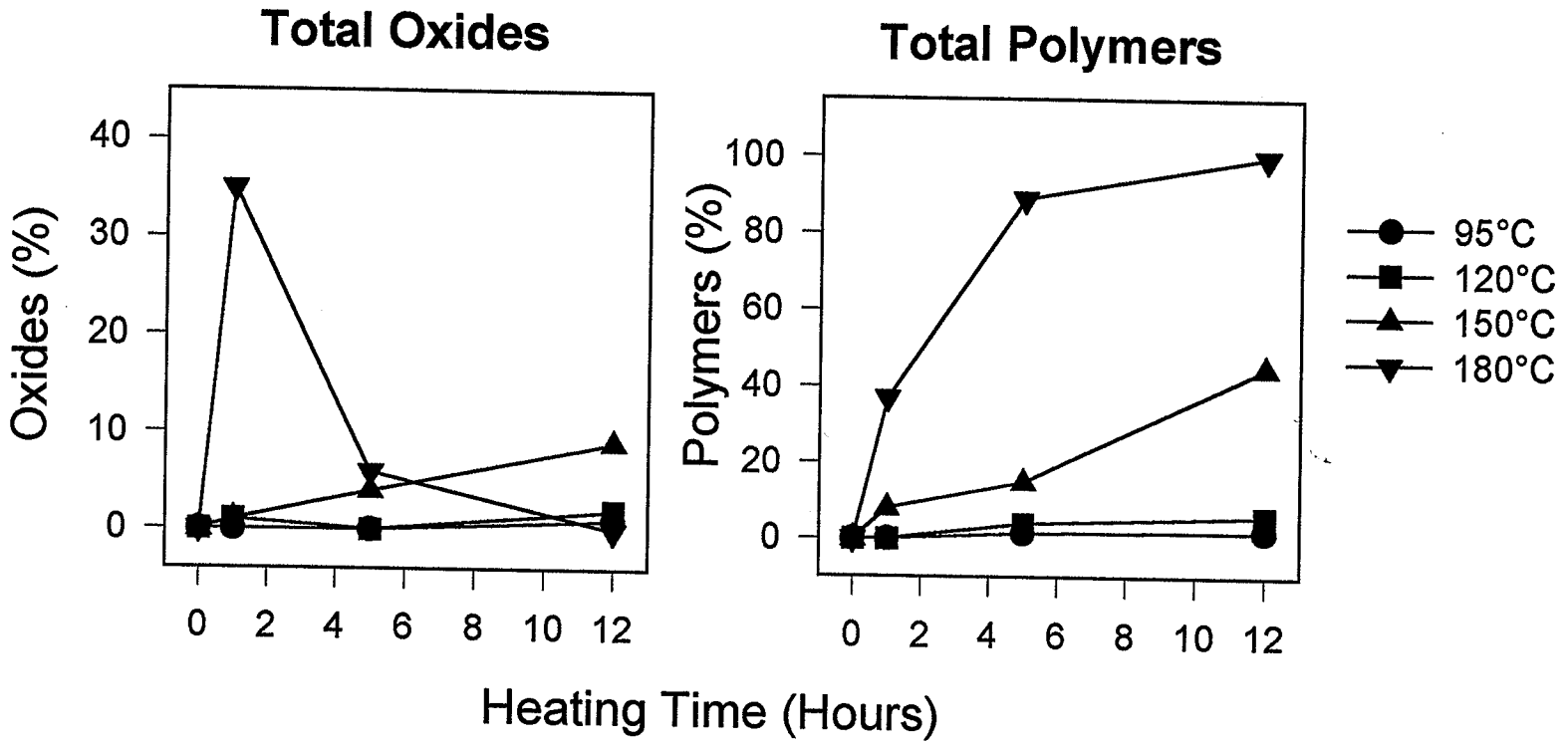


Fig. 5.6. Formation of Stigmasterol Oxidation and Polymer Products



5.1.4. Identification of Oxidized Phytosterol Products.

The sterol oxidation products were analysed on GC-MS as their trimethylsilyl ethers. A number of oxidation products formed from cholesterol and phytosterols were identified as follows:

Cholesterol oxidation products:

epimeric 7-hydroxycholesterols, 7-ketocholesterol, α or ß-epoxycholesterol.

ß-Sitosterol oxidation products:

7α- and 7ß-hydroxysitosterols, 22 or 25-hydroxysitosterol, α- and ß-epoxysitosterols, 7-ketositosterol,

Campesterol oxidation products:

epimeric 7-hydroxycampesterols, 7-ketocampesterol,

Stigmasterol oxidation products:

7α- and 7ß-hydroxystigmasterols, 5α- and 5ß-epoxystigmasterols, 7-ketostigmasterol.

The identification of sterol oxidation products was based on their fragmentation:

Sterol Oxidation Products	AMU	Fragment
7-Hydroxycholesterol	546	$[M^+]$,
	531	$[M^a - CH_3{}^b]$,
	456	[M - TMS],
	441	[M - TMS - CH_3],
	366	[M - 2TMS],
	351	[M -2TMS -CH_3];
7-Ketocholesterol:	472	$[M^+]$,
	452	$[M^+-CH_3]$,
	382	[M-TMS],
	367	[M-TMS-CH_3],
5α or ß-Epoxycholesterol:	474	$[M^+]$,
	456	[M-CH_3],
	384	[M-TMS],
	366	[M-TMS-CH_3].

[a] - Parent molecule, [b] - removed fragment, TMS - trimethylsilyl

Sterol Oxidation Products	AMU	Fragment
7α- or 7ß-Hydroxysitosterol:	574	$[M^+]$,
	559	$[M -CH_3]$,
	484	[M -TMS],
	469	$[M -TMS -CH_3]$,
	394	[M -2TMS],
	379	$[M -2TMS-CH_3]$;
22 or 25-Hydroxysitosterol:	574	$[M^+]$,
	559	$[M -CH_3]$,
	484	[M -TMS],
	470	$[M -TMS -CH_2]$,
	455	$[M -TMS -C_2H_5]$,
	394	[M -2TMS],
	379	$[M -2TMS -CH_3]$;
7ß- or 7α-Hydroxysitosterol:	574	$[M^+]$,
	559	$[M -CH_3]$,
	484	[M -TMS],
	470	[M -TMS -CH],
	394	[M -2TMS],
	379	$[M -2TMS -CH_3]$;

[a] - Parent molecule, [b] - removed fragment, TMS - trimethylsilyl

Names	AMU	Fragment
α-, or ß-, Epoxysitosterol:	502	$[M^+]$,
	487	$[M -CH_3]$,
	473	$[M -C_2H_5]$,
	412	[M -TMS],
	397	$[M -TMS -CH_3]$,
	369	$[M -TMS -CH_3 -C_2H_5]$;
7-Ketositosterol:	500	$[M^+]$,
	485	$[M -CH_3]$,
	410	[M -TMS],
	395	$[M -TMS -CH_3]$,
	377	$[M -TMS -CH_3 -HO_2]$;
7-Hydroxycampesterol:	560	$[M^+]$,
	470	[M -TMS],
	380	[M - 2TMS],
	268	$[M -2TMS -CH_2]$;
7-Ketocampesterol:	486	$[M^+]$,
	471	$[M -CH_3]$,
	396	[M -TMS],
	381	$[M -TMS -CH_3]$.

[a] - Parent molecule, [b] - removed fragment, TMS - trimethylsilyl

Sterol Oxidation Products	AMU	Fragment
7α- or 7ß- Hydroxystigmasterol:	572	$[M^+]$,
	482	[M -TMS],
	467	[M -TMS $-CH_3$],
	392	[M -2TMS],
	377	[M -2TMS $-CH_3$];
5α- or 5ß- Epoxystigmasterol:	500	$[M^+]$,
	482	[M $-H_2O$],
	469	[M $-H_2O$ $-CH_3$],
	410	[M -TMS],
	392	[M $-H_2O$ -TMS];
7-Ketostigmasterol:	498	$[M^+]$,
	483	[M $-CH_3$],
	469	[M $-C_2H_5$],
	408	[M -TMS].

[a] - Parent molecule, [b] - removed fragment, TMS - trimethylsilyl

5.1.5. Kinetics of Sterol Oxidation

The oxidation of sterols is similar to the oxidation of lipids and enhanced by contact with oxygen at elevated temperatures. It has been understood that the unsaturation in the B ring and tertiary carbons at 22 and 25 positions can produce free radicals and lead to sterol oxidation (Mearker, 1987). However, the mechanisms of sterol oxidation have not been fully understood. Kinetic studies of sterol oxidation may help to gain a better understanding of the mechanism of sterol oxidation.

The behaviour of a reaction can be classified in terms of the concept of orders. The velocity of the reaction (Swinborne, 1971) can be applied to the oxidation of sterols:

$$-\frac{dC_{st}}{dt}=KC_{st}^{\ n}C_{O2}^{\ m} \qquad [1]$$

Where C_{st} = concentration of sterols, C_{O2} = concentration of O_2, K= reaction constant, n,m = reaction orders, t = time.

Since the sterols were heated in the air, the concentration of oxygen C_{O2} is constant. The equation [1] can be simplified to:

$$-\frac{dC_{st}}{dt}=K_1C_{st}^{\ n} \qquad (C_{O2}\text{= constant}) \qquad [2]$$

Where K_1= new reaction constant.

The integrated form of equation [2] will be:

$$Kt = [1/(n-1)][1/C^{n-1} - 1/C_o^{n-1}] \quad [3]$$

Where n = reaction order, C = concentration of sterols at time t, and C_O=initial concentration of sterols, K= reaction constant, and t= time.

If the reaction is zero order i.e. n=0, the equation [3] becomes

$$Kt = C_o + C \quad [4]$$

Since initial concentration C_o is constant, the linear relationship will be observed between changes in concentration of sterols C and reaction time t.

If the reaction is of the first order, the equation [3] becomes

$$Kt = lnC_o + lnC \quad [5]$$

The *ln* of concentration C have a linear relationship with reaction time t.

If the reaction is of the second order, the equation [3] becomes

$$Kt = 1/C - 1/Co \quad [6]$$

A linear relationship will be observed between the reaction time t and the reciprocal of concentration $(1/C)$.

Applying these relationships to sterol changes during heating, zero order reactions were observed when cholesterol, stigmasterol, ß-sitosterol and campesterol were heated to 75°C, 95°C and 120°C (Fig. 5.7). First order reactions were observed for campesterol, ß-sitosterol,

stigmasterol during heating at 95°C, 120°C and 155°C (Fig. 5.8). Second order reactions were observed when most of the analysed sterols were heated to 155°C and 180°C (Fig.5.9). The order of reaction indicates how many reactants are involved in reaction(s). The higher order, the more components react at the same time.

Fig. 5.7. Kinetics of Sterol Oxidation - Zero Order Reactions

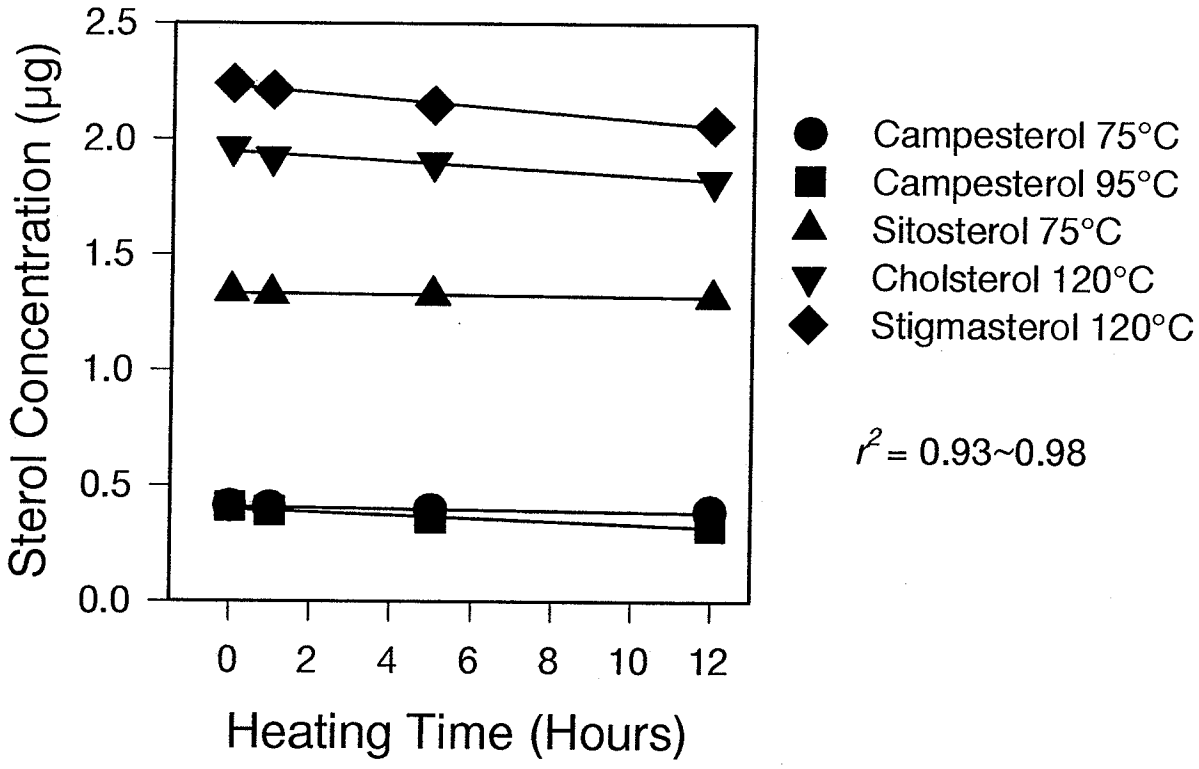


Fig. 5.8. Kinetics of Sterol Oxidation - First Order Reactions

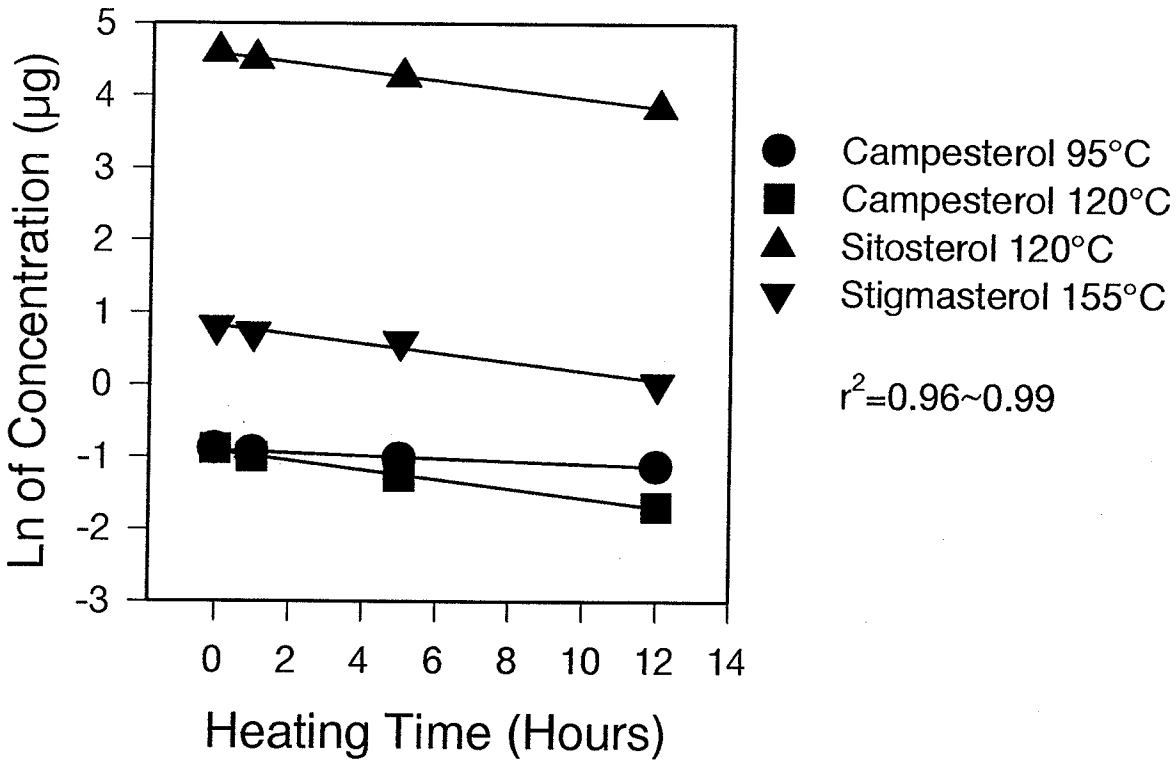
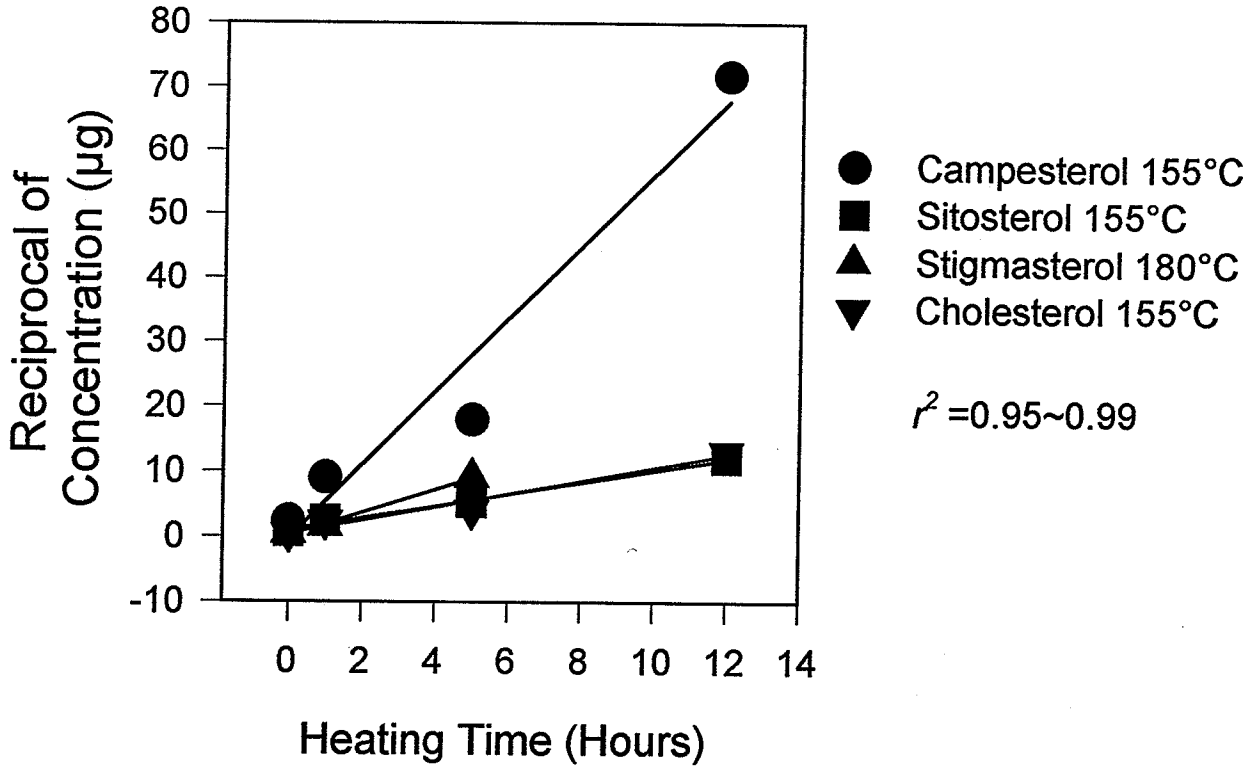


Fig. 5.9. Kinetics of Sterol Oxidation - Second Order Reactions



5.2. Oxidation of Sterols During Frying and Storage of Fried Products

5.2.1. Fatty Acid Compositions of the Oils

The fatty acid composition of four canola oils used in the study is presented in Table 5.1. The contribution of linolenic acid (18:3) was 10% in RCO, 3% in LLCO and 6.7% in HOCO. Linoleic acid content (18:2) was 21% in RCO, 27% LLCO and 8% in HOCO. HYCO did not contain linolenic acid and only 6% linoleic acid was present. Fatty acid isomers were found in some oils, including *trans*-linolenic fatty acids, as indicated in Table 5.1. The amounts of *trans* linolenic fatty acid isomers found in HOCOwas 3.8% of the total fatty acids and were higher than expected. The presence of *trans* isomers of linolenic acid in HOCO indicate that deodorization was probably performed at higher temperature than normal.

Table 5.1 Fatty Acid Composition of Canola Oils (%)

Oils	16:0	18:0	18:1	18:2	18:3	18:3*t**	20:0	20:1	22:0
RCO	3.98	2.19	58.88	21.28	9.89	1.36	0.89	1.84	0.51
LLCO	4.01	2.26	59.85	27.29	3.14	0.77	0.82	1.71	0.48
HOCO	3.20	2.43	76.50	7.76	6.75	3.84	0.95	1.80	0.62
HYCO	3.96	12.26	74.00	6.11	0.00	0.00	0.93	1.79	0.24

*18:3*t* = trans linolenic acid(s)

5.2.2. Phytosterol Changes During Potato Chip Frying

Three major phytosterols were found in canola oils: ß-sitosterol, campesterol and

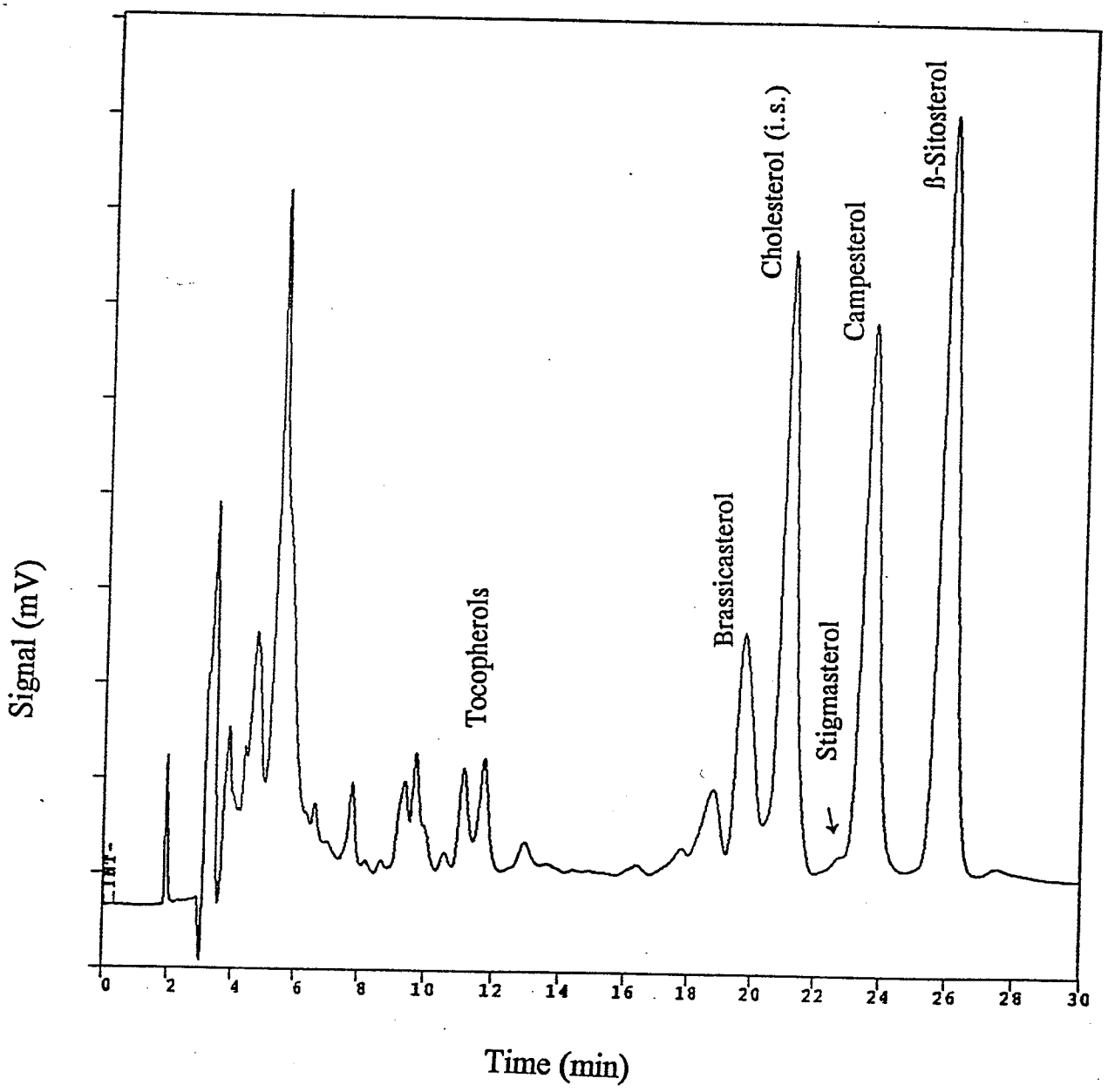
brassicasterol. The separation of phytosterols in canola oils by HPLC is shown in Fig. 5.10. The initial contents of the three major sterols in analysed canola oils were different, possibly due to the processing and/or genetical modification (Table 5.2). The total initial phytosterol contents were 7912 ppm, 7597 ppm 5549 ppm and 7288 ppm for RCO, LLCO, HOCO and HYCO, respectively. The lower level of total sterol contents in HOCO may be related to the high deodorization temperatures and/or genetic modification.

Table 5. 2. Phytosterol Contents in Canola Oils (ppm)

Oil	Brassicasterol	Campesterol	Sitosterol	Total Sterols
RCO	814	3250	3848	7912
LLCO	712	3189	3696	7597
HOCO	432	2179	2938	5549
HYCO	817	3080	3391	7288

The changes of phytosterol content during frying are shown in Tables 5.3 to 5.6. The contents of phytosterol in all frying oils decreased throughout the frying process. Because 10-15% fresh oil was added prior to frying each day, 10-15% of fresh sterols were added at the same time. At the end of 5 days of frying, the losses of sterol contents were about 55%, 58%, 51% and 56% for RCO, LLCO, HOCO and HYCO, respectively. The losses of phytosterols were attributed to the absorption of frying oils by the potato chips and the oxidation of the phytosterols during the frying process.

Fig. 5.10 Separation of Phytosterols in RCO by HPLC



Chromatography conditions: Separation was performed on Shimadzu HPLC system with a RP-C18 column (Beckman, 5µm, 250 mm x 4.5 mm i.d.). The mobile phase was methanol/acetonitrile (99.5/0.5 v/v) at flow rate 1.0 mL/min. UV detector was used with the wavelength set at 205nm.

Table 5. 3. Changes in Phytosterol Content in RCO During Frying of Potato Chips (ppm)

Time (days)	Brassica-sterol	Campe-sterol	Sito-sterol	Total Sterols	Measured Loss	Amount Added[a]	Total Loss (%)
0[b]	814	3250	3848	7912	-	-	-
1	758	3134	3878	7770	142	-	2
2	740	3130	3726	7596	316	949	16
3	731	3168	3804	7703	209	1899	27
4	685	3057	3812	7554	358	2848	41
5	700	3019	3643	7362	550	3798	55

[a] - Calculation based on an average of 12% fresh oils added daily.
[b]- The oil heated for 30 minutes was label as day 0.

Table 5. 4. Changes in Phytosterol Content in LLCO During Frying of Potato Chips (ppm)

Time (days)	Brassica-sterol	Campe-sterol	Sito-sterol	Total Sterols	Measured Loss	Amount Added[a]	Total Loss (%)
0[b]	712	3189	3696	7597	-	-	-
1	710	3134	3578	7422	175	-	2
2	690	3113	3550	7353	244	912	15
3	667	3086	3581	7334	263	1823	27
4	656	2928	3522	7106	491	2735	42
5	631	2826	3360	6817	780	3647	58

[a] - Calculation based on an average of 12% fresh oils added daily.
[b]- The oil heated for 30 minutes was label as day 0.

Table 5. 5. Changes in Phytosterol Content in HOCO During Frying of Potato Chips (ppm)

Time (days)	Brassica-sterol	Campe-sterol	Sito-sterol	Total Sterols	Measured Loss	Amount Added [a]	Total Loss (%)
0[b]	432	2179	2938	5549	-	-	-
1	440	2170	2899	5509	40	-	1
2	442	2163	2867	5472	77	666	13
3	393	2135	2870	5398	151	1332	27
4	456	2087	2789	5332	217	1998	40
5	419	2124	2844	5387	162	2664	51

[a] - Calculation based on an average of 12% fresh oils added daily.
[b]- The oil heated for 30 minutes was label as day 0.

Table 5. 6. Changes in Phytosterol Content in HYCO During Frying of Potato Chips (ppm)

Time (days)	Brassica-sterol	Campe-sterol	Sito-sterol	Total Sterols	Measured Loss	Amount Added [a]	Total Loss (%)
0[b]	817	3080	3391	7288	-	-	-
1	820	3010	3385	7215	73	-	1
2	824	2922	3383	7129	159	875	14
3	794	2842	3399	7035	253	1749	27
4	782	2828	3383	6993	295	2624	40
5	763	2782	3194	6739	549	3498	56

[a] - Calculation based on an average of 12% fresh oils added daily.
[b]- The oil heated for 30 minutes was label as day 0.

5.2.3. Phytosterol Changes During Storage of Potato Chips

The lipid content in potato chips was 30-35% (w/w). The phytosterol changes during the 16 days of storage at 60°C are shown in Tables 5.7 to 5.14. At the end of 16 days of storage, the total losses of phytosterols in fried potato chips from the first and the fifth day of frying were as follows: 3.2% and 3.9% for RCO, 5.4% and 4.8% for LLCO, 7.6% and 3.9% for HOCO, and 3.6% and 9.7% for HYCO.

The changes of individual sterols in the oils were found to be different during the storage. In most cases, the losses of ß-sitosterol and campesterol were about 3% to 7% at the end of the storage period whereas the losses of brassicasterol were at about 7% to 12% in most of cases indicating lower stability.

Table 5.7. Phytosterol Changes in Potato Chip During Storage (RCO Day 1 of Frying, ppm in Extracted Lipids)

Time (days)	Brassica-sterol	Campe-sterol	Sitosterol	Total Sterols	Total Loss in Amounts (ppm)
0	829	2928	4241	7998	-
2	813	2927	4217	7957	41
4	801	2884	4149	7834	164
8	784	2927	4200	7911	87
16	735	2899	4110	7744	254
Loss* (%)	**11.33**	**1.01**	**3.08**	**3.18**	

* Losses at the end of storage.

Table 5. 8. Phytosterol Changes in Potato Chip During Storage (RCO Day 5 of Frying, ppm in Extracted Lipids)

Time (days)	Brassica-sterol	Campe-sterol	Sitosterol	Total Sterols	Total Losses in Amounts (ppm)
0	787	2958	4315	8060	-
2	805	2897	4228	7930	130
4	785	2911	4265	7961	99
8	764	2897	4198	7859	201
16	724	2877	4147	7748	312
Losses* (%)	**8.01**	**2.78**	**3.89**	**3.87**	

* Losses at the end of storage.

Table 5. 9. Phytosterol Changes in Potato Chip During Storage (LLCO Day 1 of Frying, ppm in Extracted Lipids)

Time (days)	Brassica-sterol	Campe-sterol	Sitosterol	Total Sterols	Total Losses in Amounts (ppm)
0	832	3156	4119	8107	-
2	826	3101	4064	7991	116
4	807	3061	4051	7919	188
8	765	3056	3975	7796	311
16	729	3045	3895	7669	438
Losses* (%)	**12.37**	**3.52**	**5.43**	**5.40**	

* Losses at the end of storage.

Table 5. 10. Phytosterol Changes in Potato Chip During Storage (RCO Day 5 of Frying, ppm in Extracted Lipids)

Time (days)	Brassica-sterol	Campe-sterol	Sitosterol	Total Sterols	Total Loses in Amounts (ppm)
0	754	3161	3923	7838	-
2	759	3088	3870	7717	121
4	736	3026	3791	7553	285
8	752	3082	3871	7705	133
16	701	2953	3812	7466	372
Losses* (%)	**7.03**	**5.65**	**2.83**	**4.75**	

* Losses at the end of storage.

Table 5. 11. Phytosterol Changes in Potato Chip During Storage (HOCO Day 1 of Frying, ppm in Extracted Lipids)

Time (days)	Brassica-sterol	Campe-sterol	Sitosterol	Total Sterols	Total Losses in Amounts (ppm)
0	479	2295	3131	5905	-
2	473	2282	3126	5881	24
4	468	2256	3096	5820	85
8	442	2114	2823	5379	526
16	430	2157	2901	5488	417
Losses* (%)	**10.23**	**6.00**	**7.35**	**7.06**	

* Losses at the end of storage.

Table 5. 12. Phytosterol Changes in Potato Chip During Storage (HOCO Day 5 of Frying, ppm in Extracted Lipids)

Time (days)	Brassica-sterol	Campe-sterol	Sitosterol	Total Sterols	Total Loses in Amounts (ppm)
0	460	2190	2944	5594	-
2	459	2172	2901	5532	62
4	428	2173	2896	5497	97
8	423	2188	2876	5487	107
16	409	2137	2828	5374	220
Losses* (%)	**11.10**	**3.43**	**3.94**	**3.93**	

* Losses at the end of storage.

Table 5. 13. Phytosterol Changes in Potato Chip During Storage (HYCO Day 1 of Frying, ppm in Extracted Lipids)

Time (days)	Brassica-sterol	Campe-sterol	Sitosterol	Total Sterols	Total Losses in Amounts (ppm)
0	939	3016	3823	7778	-
2	889	2951	3777	7617	161
4	921	2996	3815	7732	46
8	922	2994	3818	7734	44
16	899	2917	3682	7498	280
Losses* (%)	**4.25**	**3.28**	**3.68**	**3.60**	

* Losses at the end of the storage.

Table 5. 14. Phytosterol Changes in Potato Chip During Storage (HYCO day 5 Frying, ppm of Extracted Lipids)

Time (days)	Brassica-sterol	Campe-sterol	Sitosterol	Total Sterols	Total Losses in Amounts (ppm)
0	854	2817	3682	7353	-
2	806	2722	3488	7016	337
4	832	2838	3673	7343	10
8	797	2722	3541	7060	293
16	745	2640	3252	6637	716
Losses* (%)	**12.76**	**6.28**	**9.68**	**9.74**	

Losses at the end of the storage.

5.2.4. Phytosterol Changes During Heating at Simulated Frying Temperature

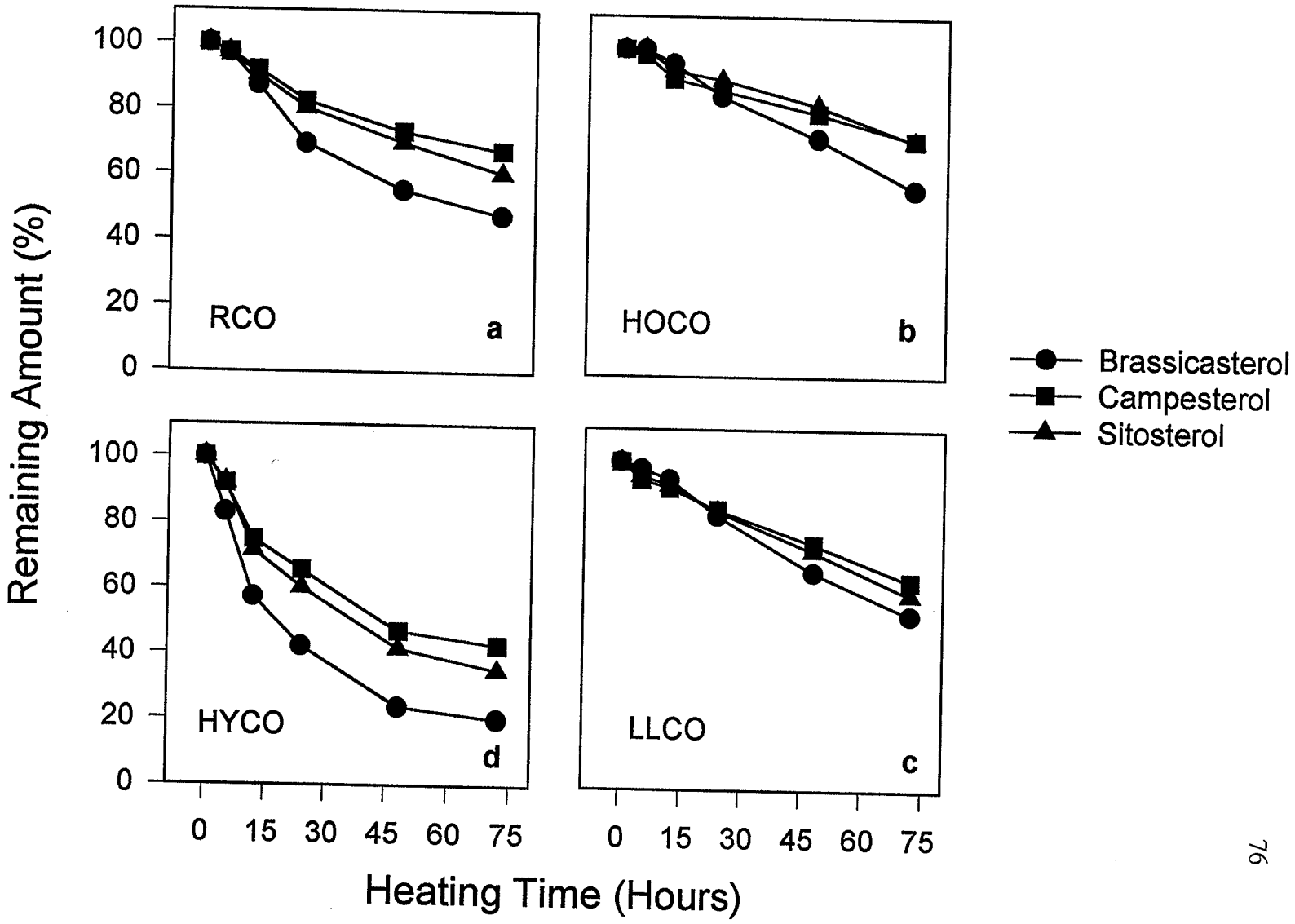
In order to verify the pattern of phytosterol changes at frying temperatures and to eliminate the addition of fresh oils during frying, a simulated frying experiment was performed. The changes to the phytosterols during the heating of canola oils at simulated frying temperature are shown in Fig.5.11.

In RCO, all three major phytosterols decreased continuously throughout the heating. At the end of heating, 47%, 67% and 60% of brassicasterol, campesterol and ß-sitosterol remained, respectively. Brassicasterol showed the lowest stability among the three phytosterols. The ß-sitosterol and campesterol were similar in stability (Fig. 5.11a).

Similar patterns of change in phytosterols were observed for LLCO and HOCO (Fig. 5.11b and c). At the end of heating, the remaining of brassicasterol, campesterol and ß-sitosterol were found at levels of 57%, 72%, 72% for LLCO and 53%, 64%, 60% for HOCO, respectively.

The pattern of phytosterol changes in HYCO was different from the other three canola oils (Fig. 5.11d). All phytosterols disappeared at a faster rate than in other canola oils. After 24 hours of heating 60%, 40% and 35% of brassicasterol, campesterol and sitosterol disappeared, respectively, in this oil. Again, brassicasterol was the least stable among all the phytosterols.

Fig. 5.11. Sterol Changes During Heating at Simulated Frying Temperature



5.2.5. Identification and Quantification of Phytosterol Oxidation Products During Heating at Simulated Frying Temperature

The separation of phytosterols and their oxidation products as trimethylsilyl ethers isolated from HYCO at the end of heating is shown in Fig. 5.12. The peaks a, b, c represent the three major phytosterols, brassicasterol, campesterol and ß-sitosterol, respectively. Their mass spectra are presented in Fig. 5.13 to Fig 5.15, respectively. The compounds were identified by matching with mass spectra of their authentic products and by their fragmentation patterns. A number of oxidation products were produced during the heating period. These oxidation products were tentatively identified by mass spectrometry. Peak d and h were identified as epimeric 7-hydroxycampesterols (Fig. 5.16 and Fig.5.18). Park and Addis, (1986) reported that 7α-hydroxycholesterol and 7ß-hydroxycholesterol, α-epoxycholesterol and ß-epoxycholesterol had similar mass spectra. Mass spectra of 7α-hydroxysitosterol and 7ß-hydroxysitosterol, 7α-hydroxycampesterol and 7ß-hydroxycampesterol, α-epoxysitosterol and ß-epoxysitosterol, α-epoxycampesterol and ß-epoxycampesterol were reported by Dutta and Appelqvist (1995). Based on the retention data and the separation pattern, peak d was identified as 7α-hydroxycampesterol, while peak h was identified as 7ß-hydroxycampesterol (Fig. 5.16 and 5.18). Peak g and I was identified as 7α-hydroxysitosterol and 7ß-hydroxysitosterol, respectively (Fig. 5.17 and 5.19). Peak j and k were identified as ß-epoxycampesterol and α-epoxycampesterol, respectively (Fig. 5.20 and 5.21). Peak l was found to be either α- or ß- epoxybrassicasterol (Fig, 5.22). Peak n and p were identified as ß-epoxysitosterol and α-epoxysitosterol, respectively (Fig. 5.23 and 5.25). Peak o, q, r were identified as 7-ketobrassicasterol, 7-ketocampesterol and 7-ketositosterol, respectively (Fig.

5.24, 5.26, 5.27). Some components were not identified, mainly because their spectra were not complete due to the low concentration of components. These spectra are presented in Fig. 5.28 to Fig.5.30.

The levels of these oxidation products that accumulated in frying oils during the heating at simulated frying temperature are in Tables 5.15 to 5.18. The fresh canola oils contained very little or no sterol oxidation products. The amounts of oxidation products increased when the heating time increased. In fresh HYCO, the levels of sterol oxidation products were just within detection limits. When the oil was heated for 72 hours, the total amount of oxidation products detected was 923 ppm. The most abundant oxidation products were 7-ketones of ß-sitosterol, campesterol and brassicasterol. At the end of the heating the total amounts of 7-ketositosterol, 7-ketocampesterol and 7-ketobrassicasterol in HYCO were 235 ppm, 200 ppm and 97 ppm, respectively. In the other analysed canola oils, the amounts of oxidation products formed was different. The amounts of phytosterol oxidation products accumulated in HYCO was the highest among the four canola oils evaluated. The total amounts of oxidation products produced at the end of heating at simulated frying temperature were 923 ppm, 363 ppm, 224 ppm and 346 ppm for HYCO, RCO, LLCO and HOCO, respectively.

Fig. 5.12. GC Separation of Phytosterol Oxides Isolated from HYCO after 72 Hours of Heating at Simulated Frying Temperature (190°C)

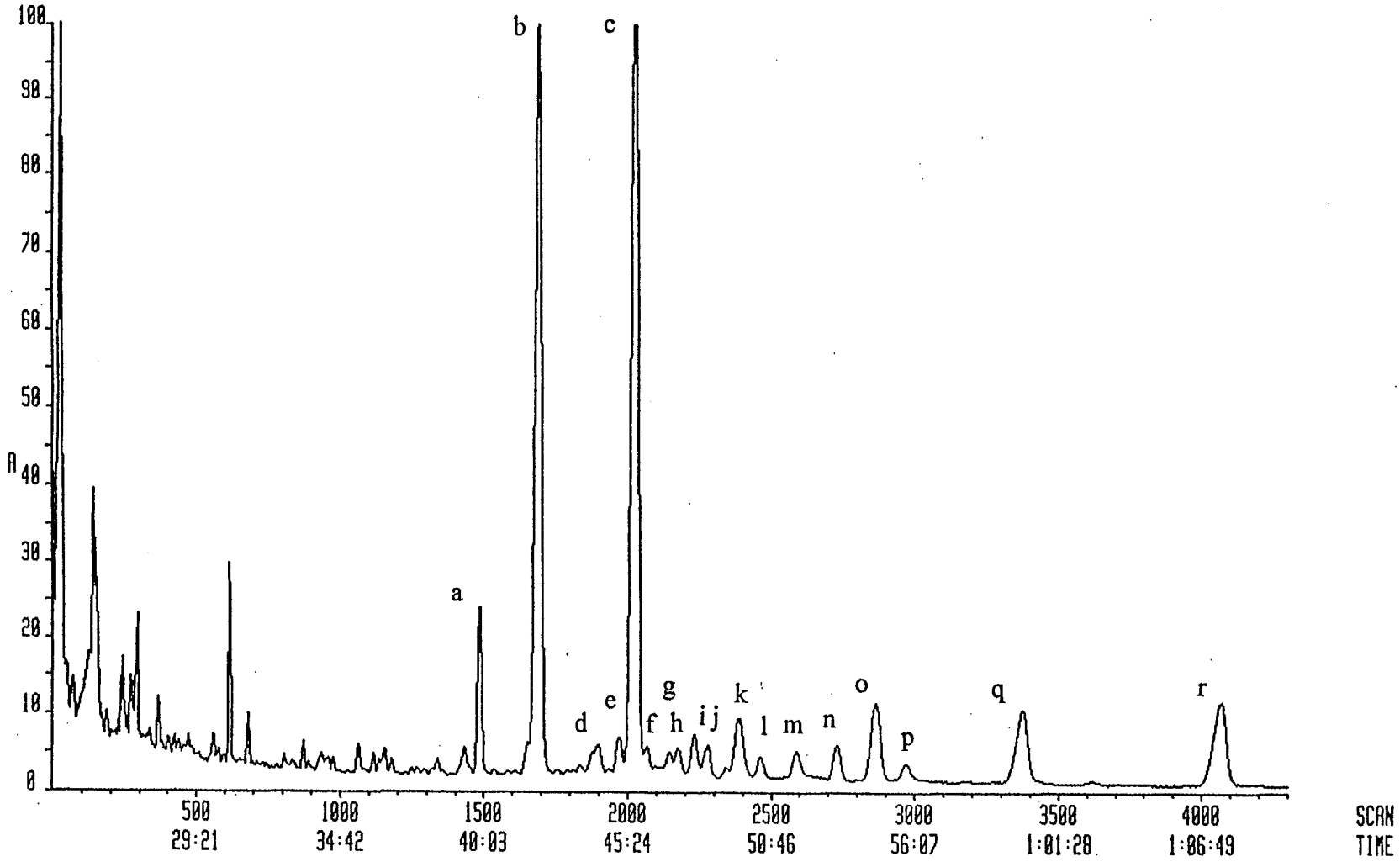
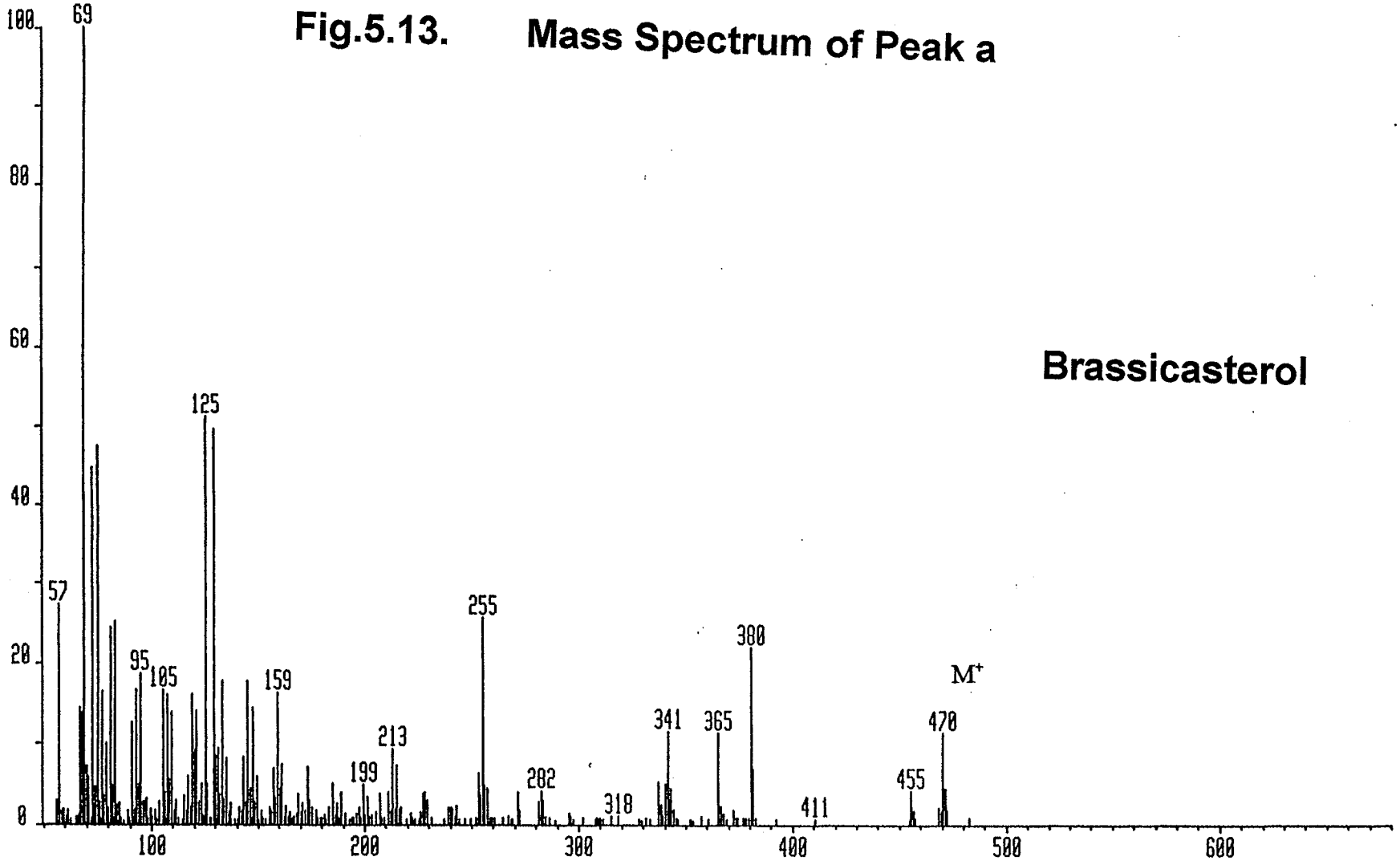


Fig.5.13. Mass Spectrum of Peak a



Brassicasterol

Fig.5.14. Mass Spectrum of Peak b

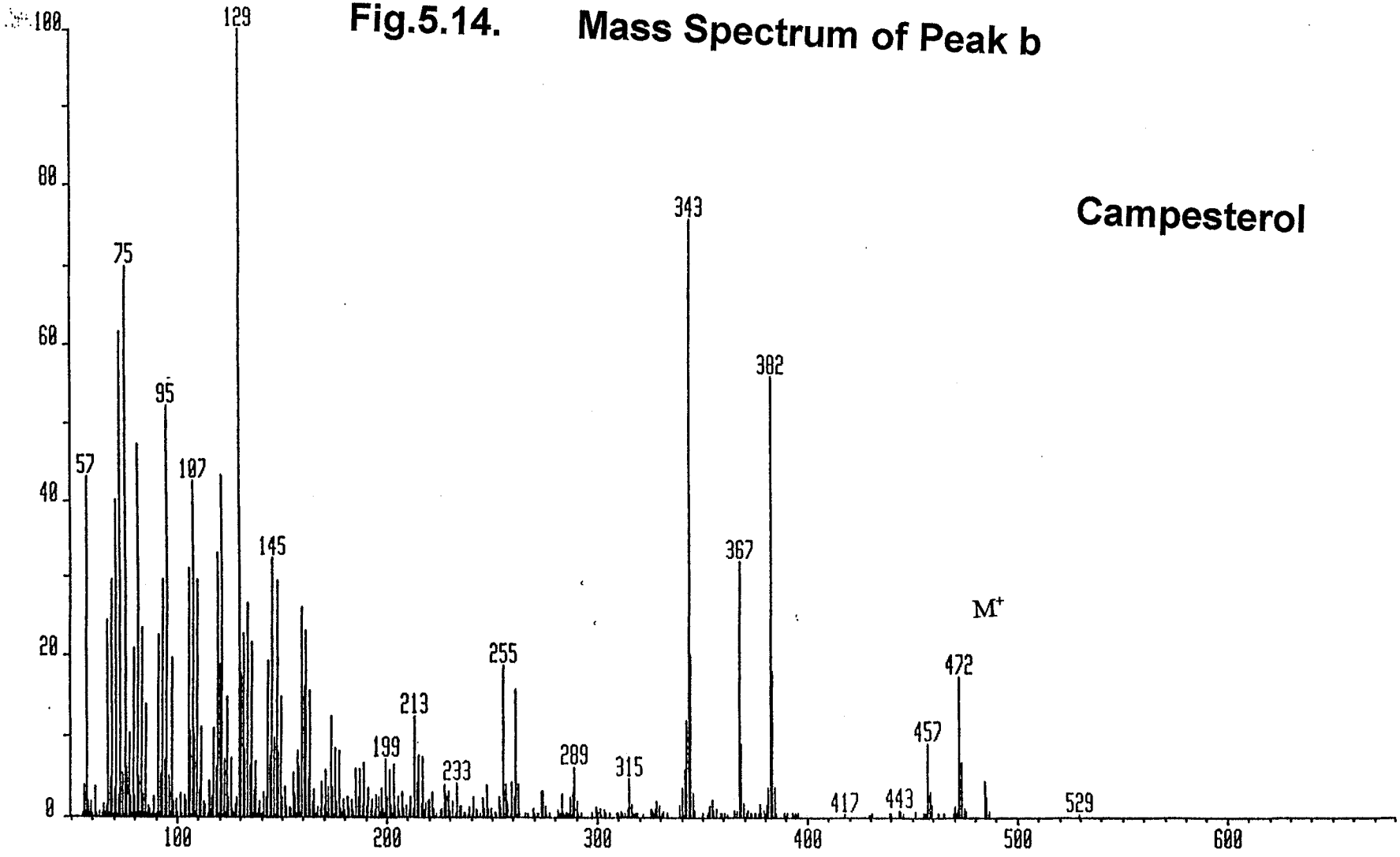


Fig.5.15. Mass Spectrum of Peak c

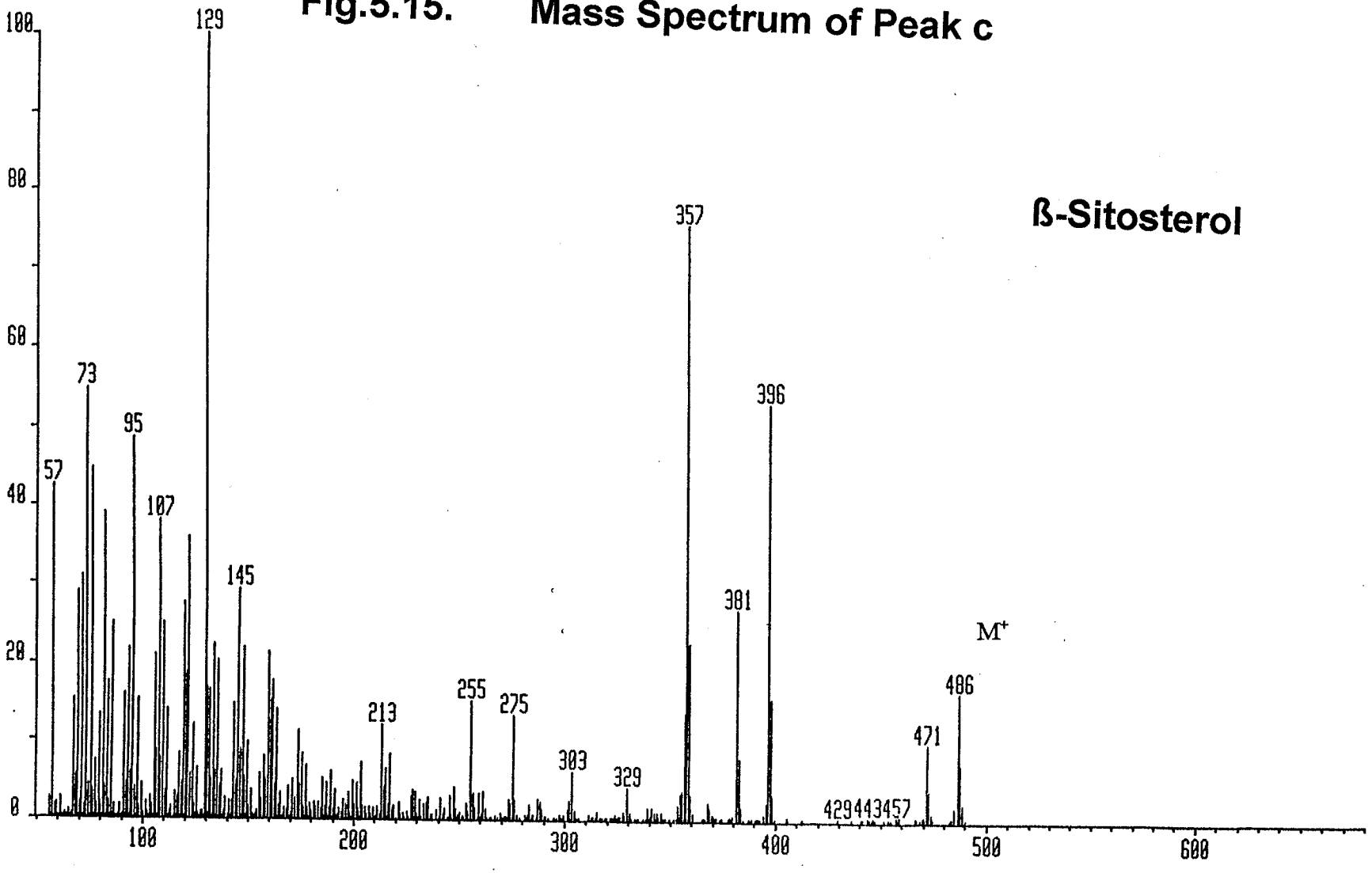
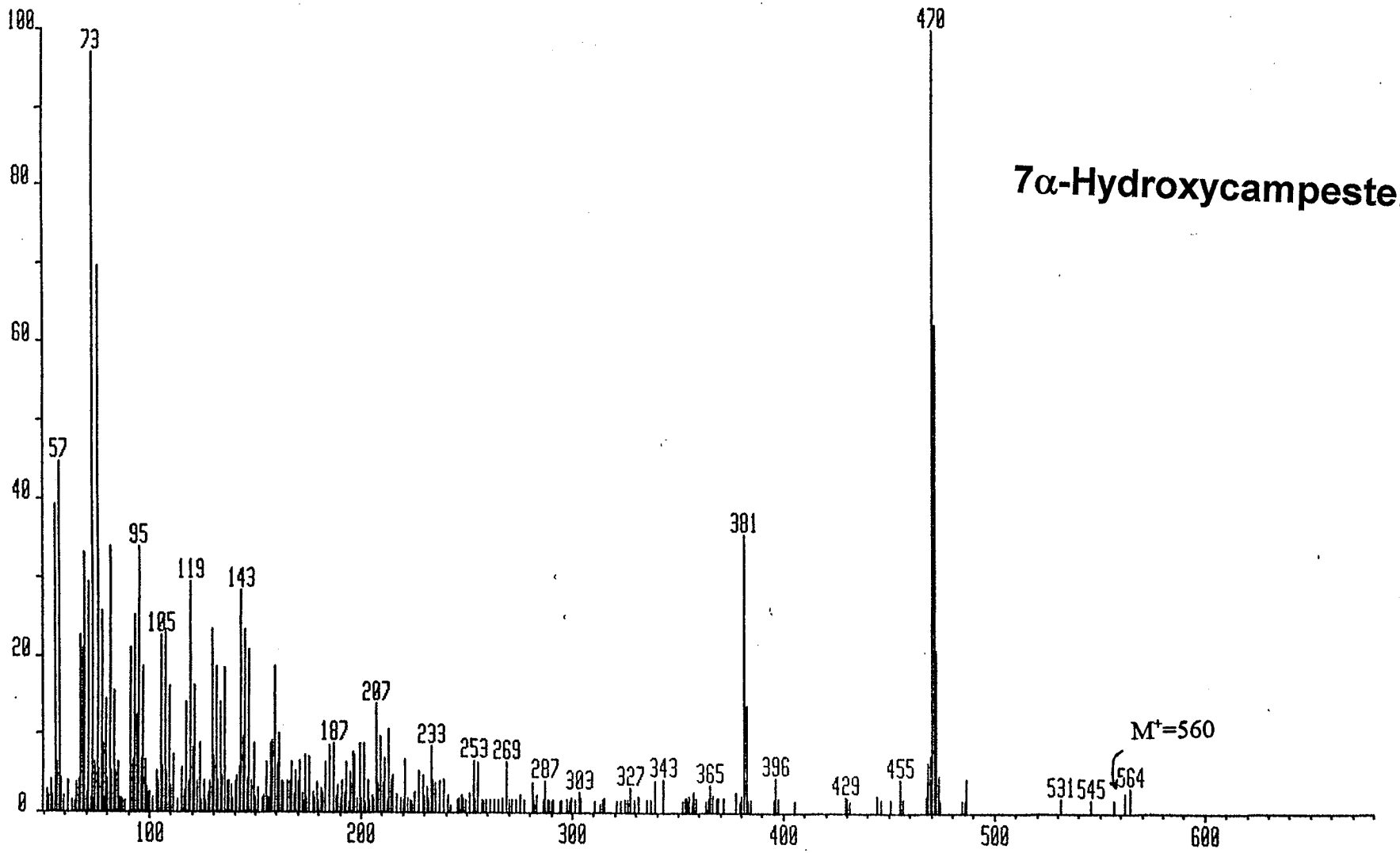
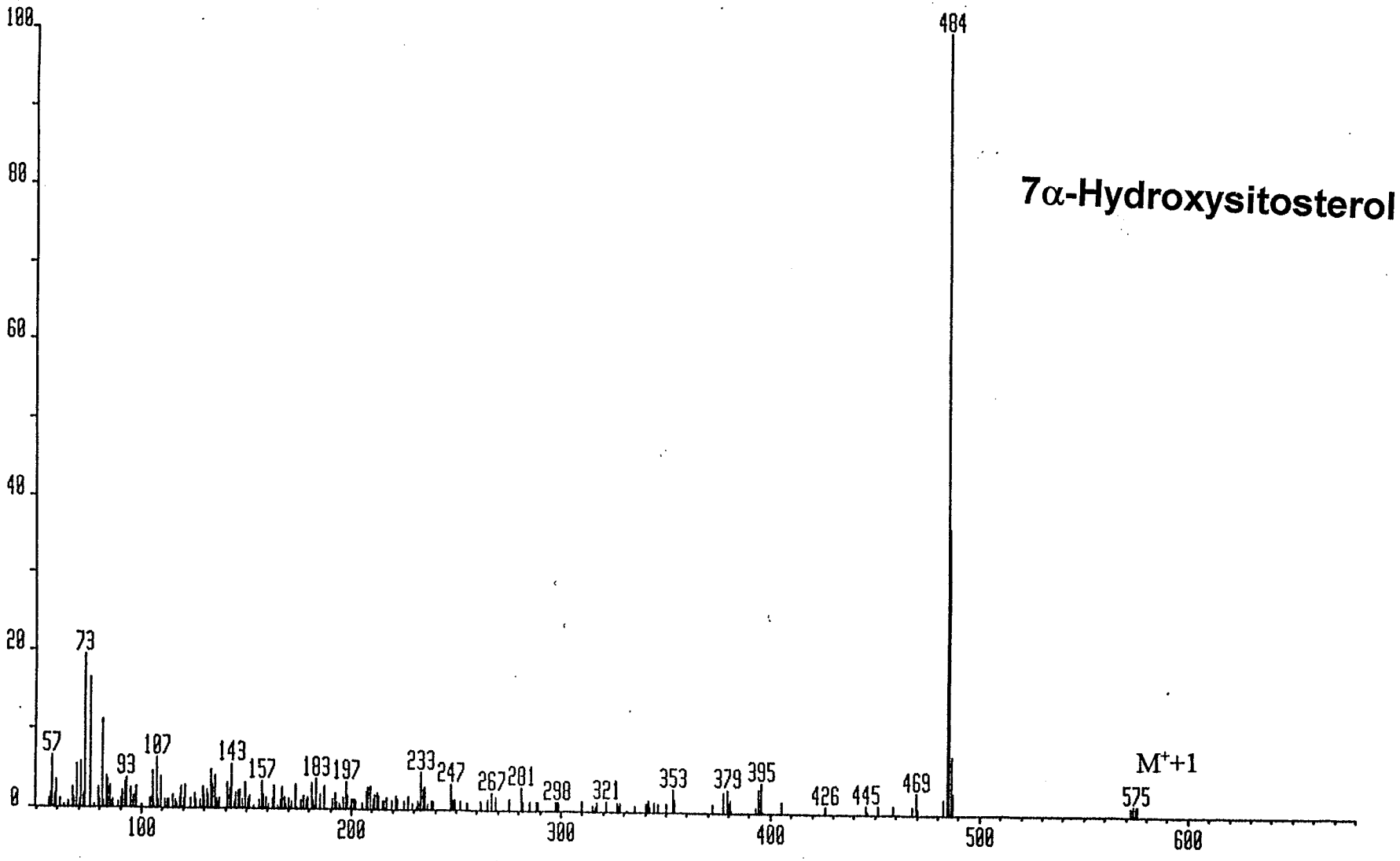


Fig.5.16. Mass Spectrum of Peak d



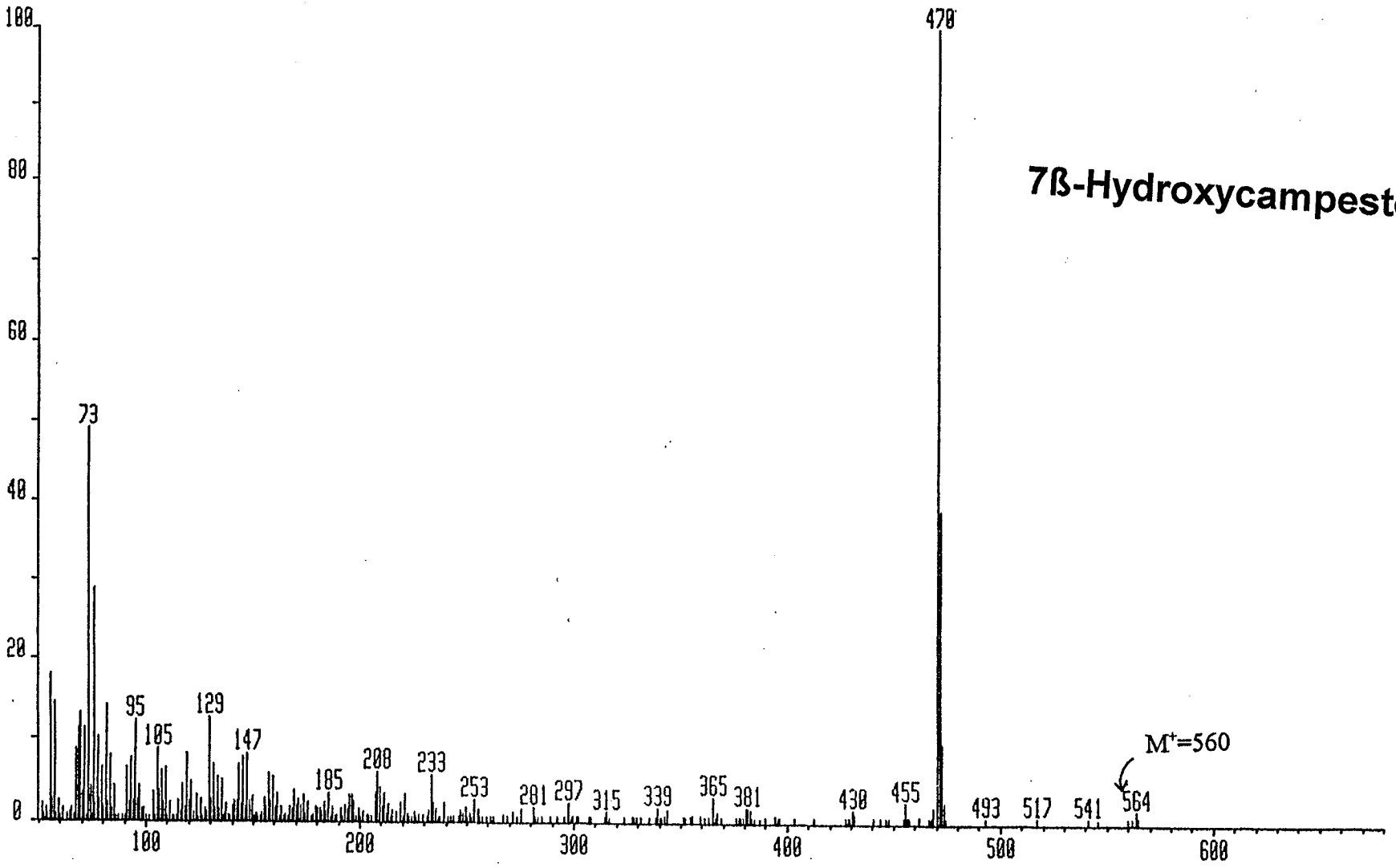
7α-Hydroxycampesterol

Fig.5.17. Mass Spectrum of Peak g



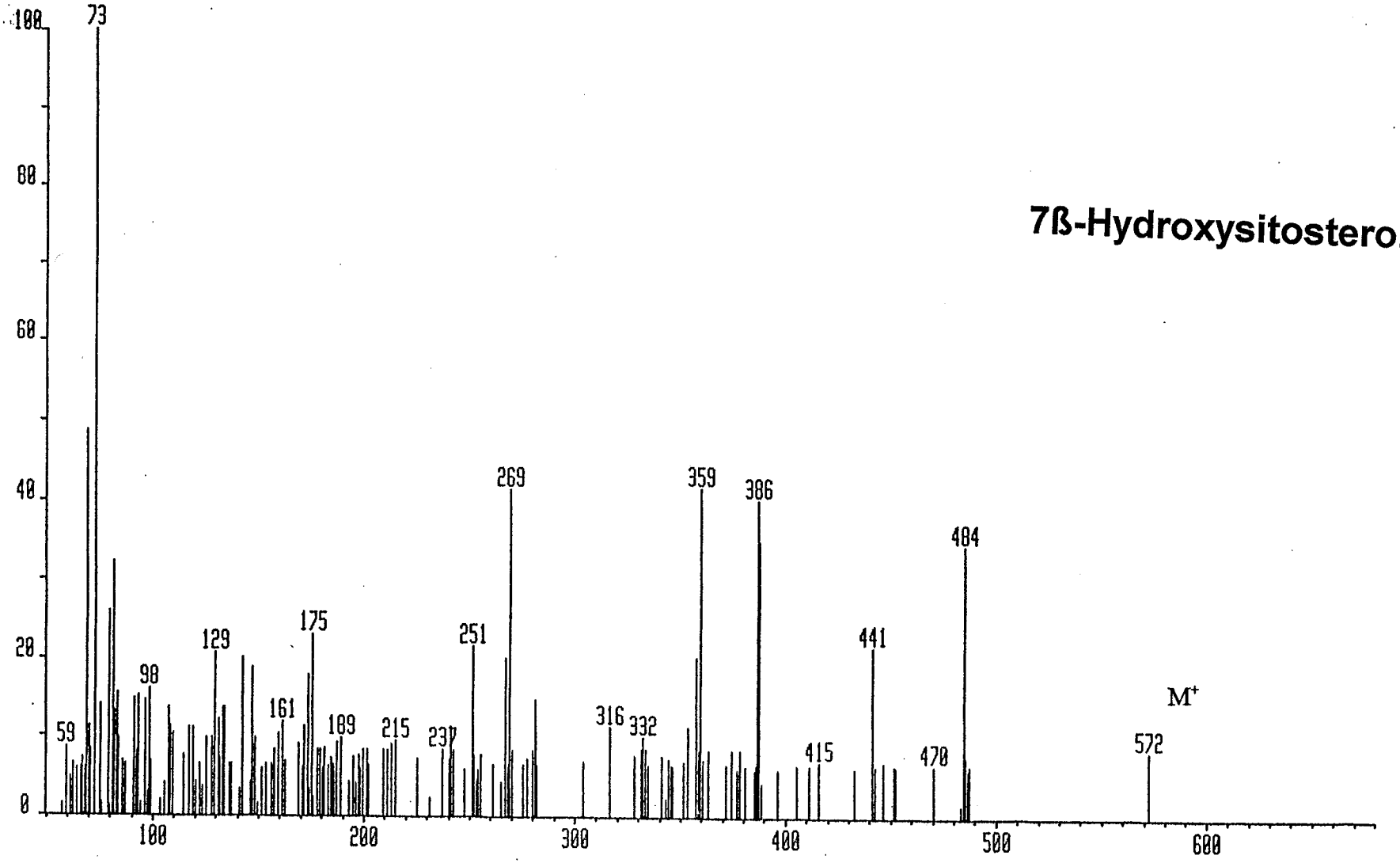
7α-Hydroxysitosterol

Fig.5.18. Mass Spectrum of Peak h



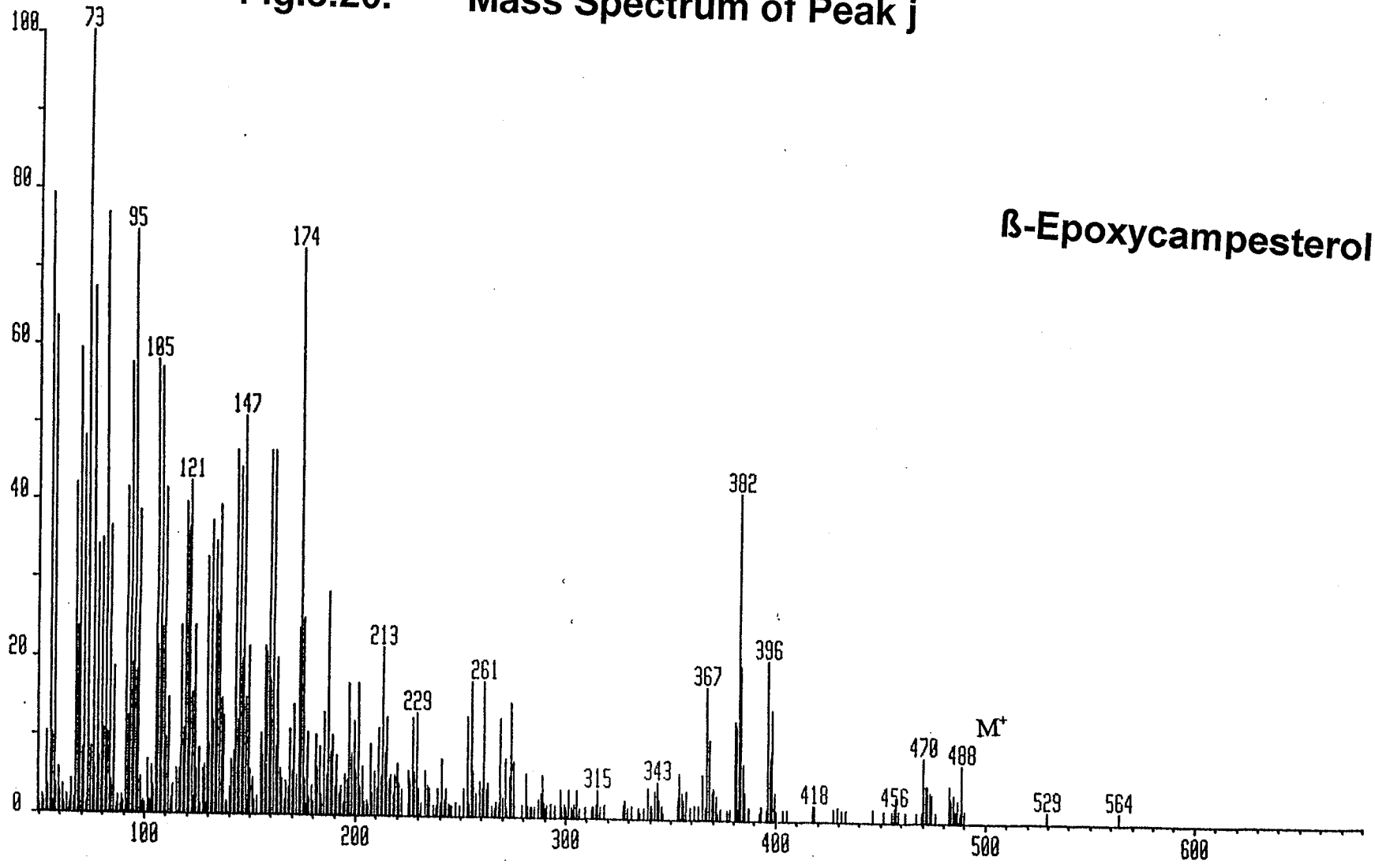
7ß-Hydroxycampesterol

Fig.5.19. Mass Spectrum of Peak I



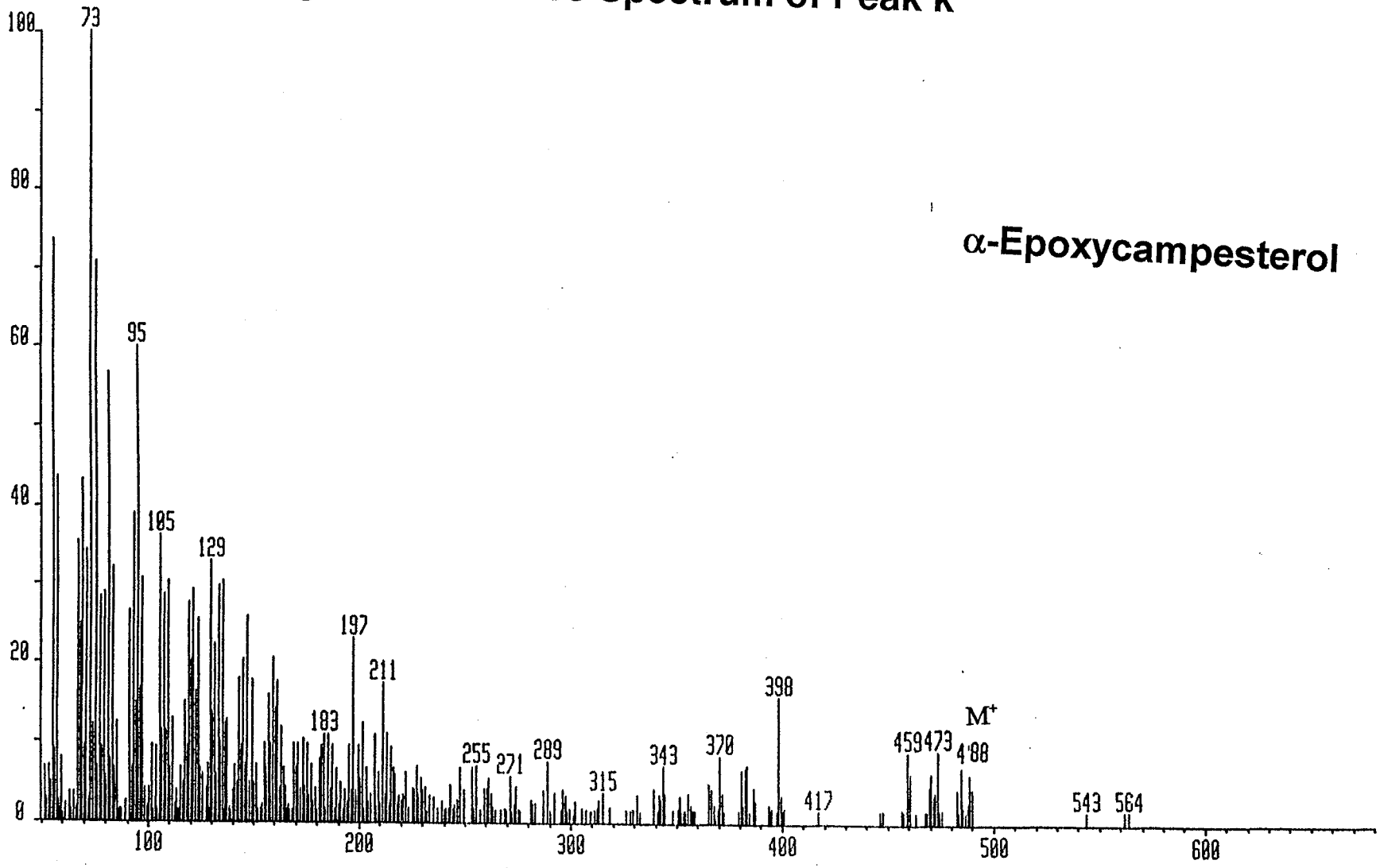
7ß-Hydroxysitosterol

Fig.5.20. Mass Spectrum of Peak j



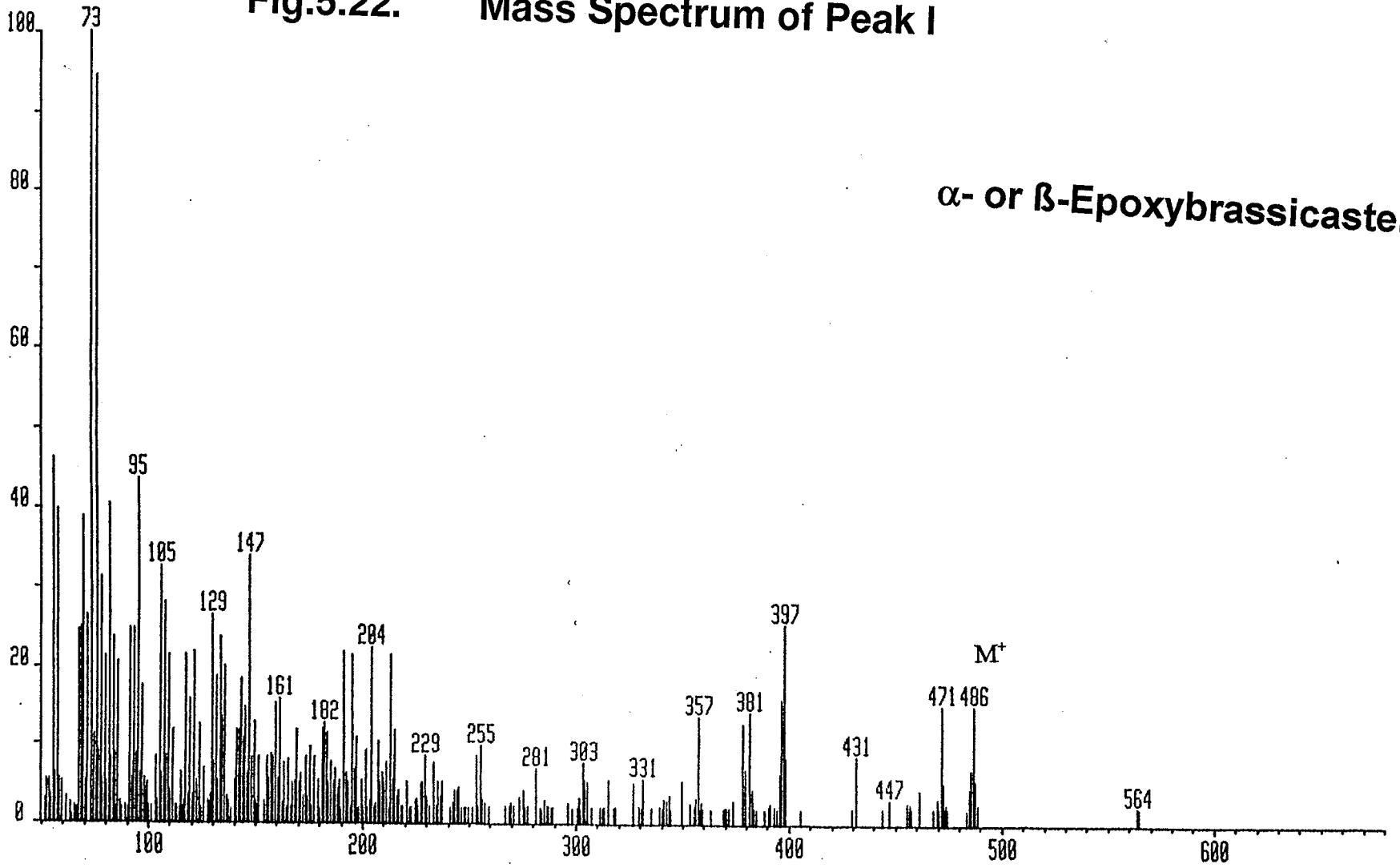
ß-Epoxycampesterol

Fig.5.21. Mass Spectrum of Peak k



α-Epoxycampesterol

Fig.5.22. Mass Spectrum of Peak I



α- or ß-Epoxybrassicasterol

Fig.5.23. Mass Spectrum of Peak n

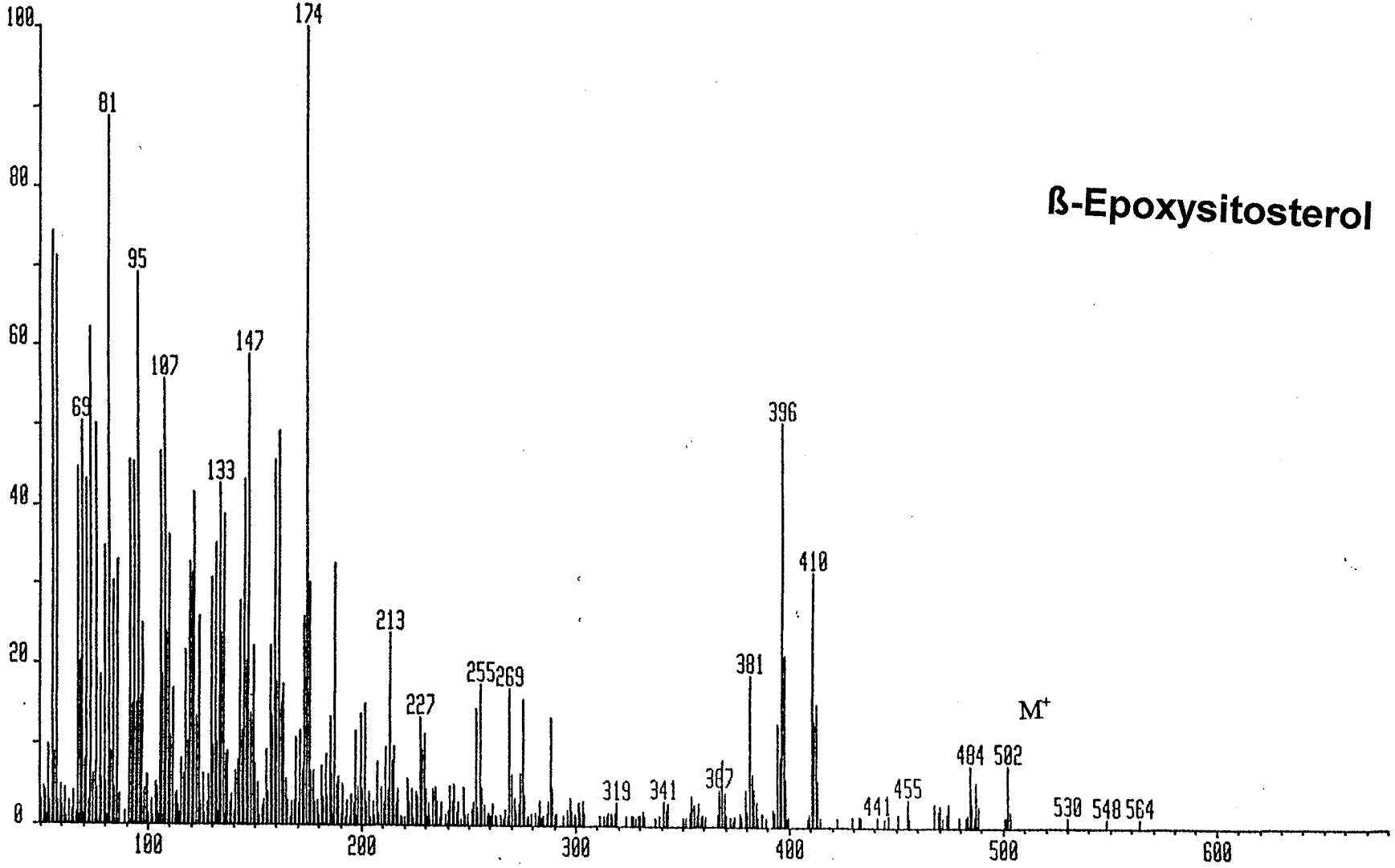


Fig.5.24. Mass Spectrum of Peak o

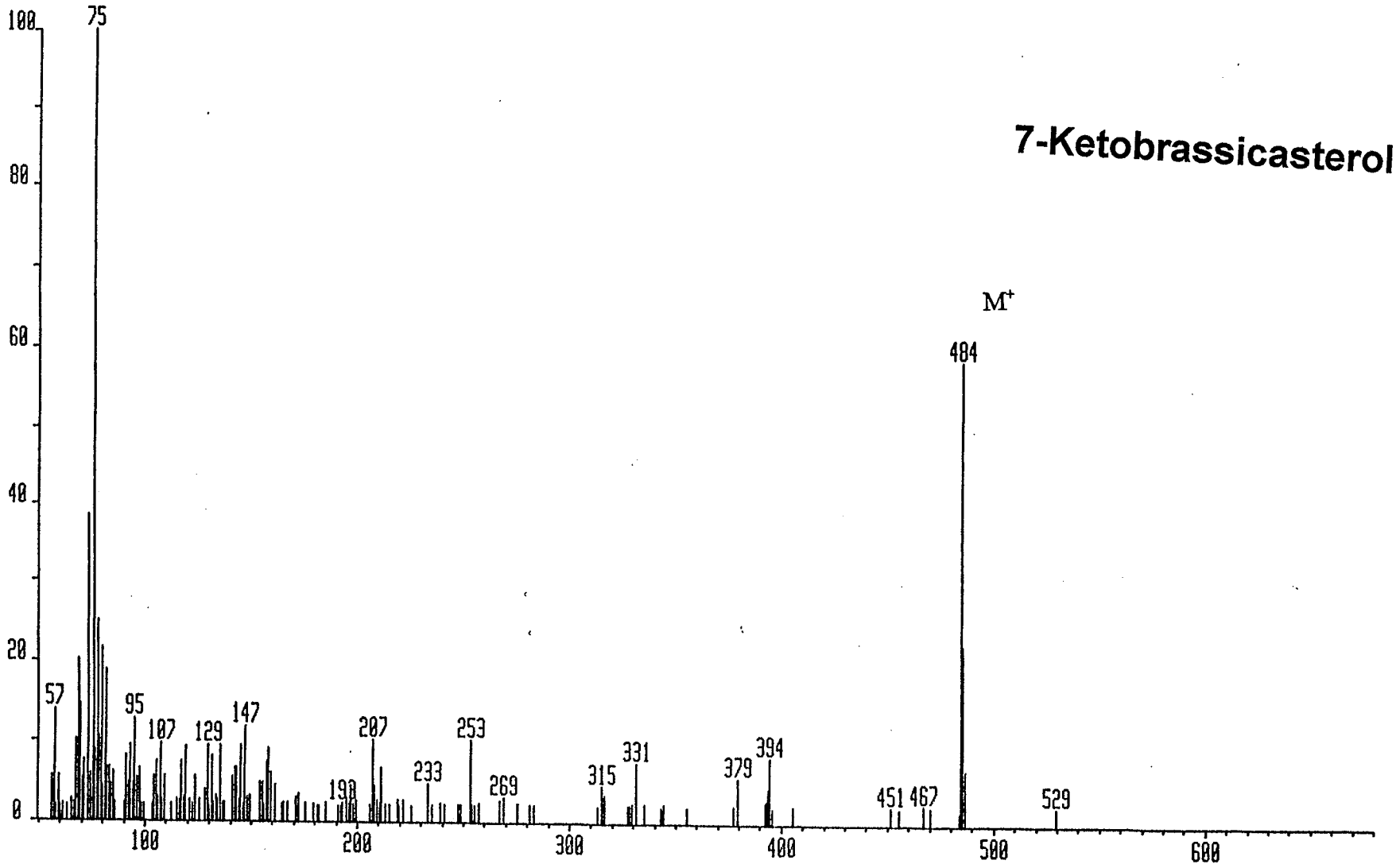


Fig.5.25. Mass Spectrum of Peak p

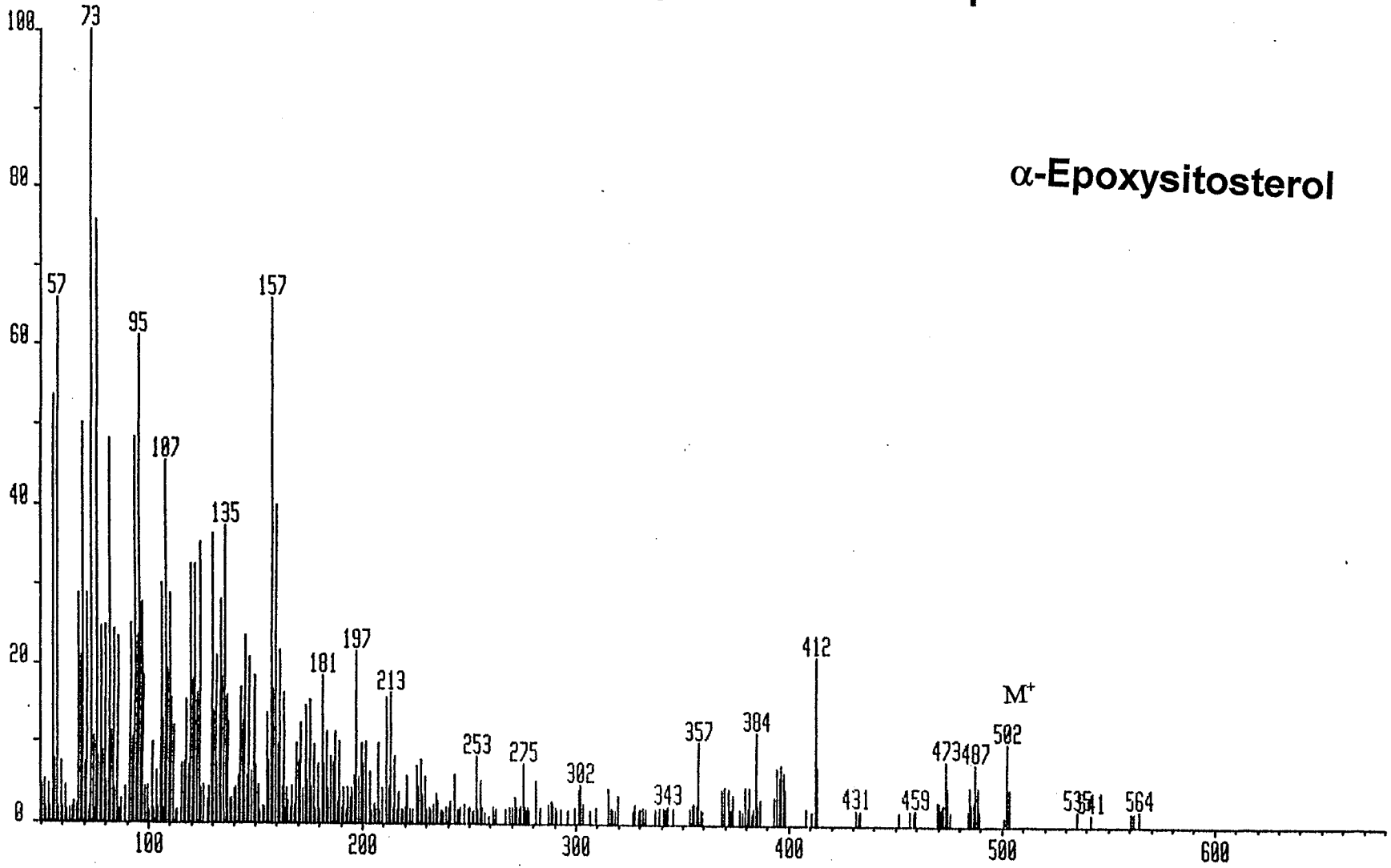


Fig.5.26. Mass Spectrum of Peak q

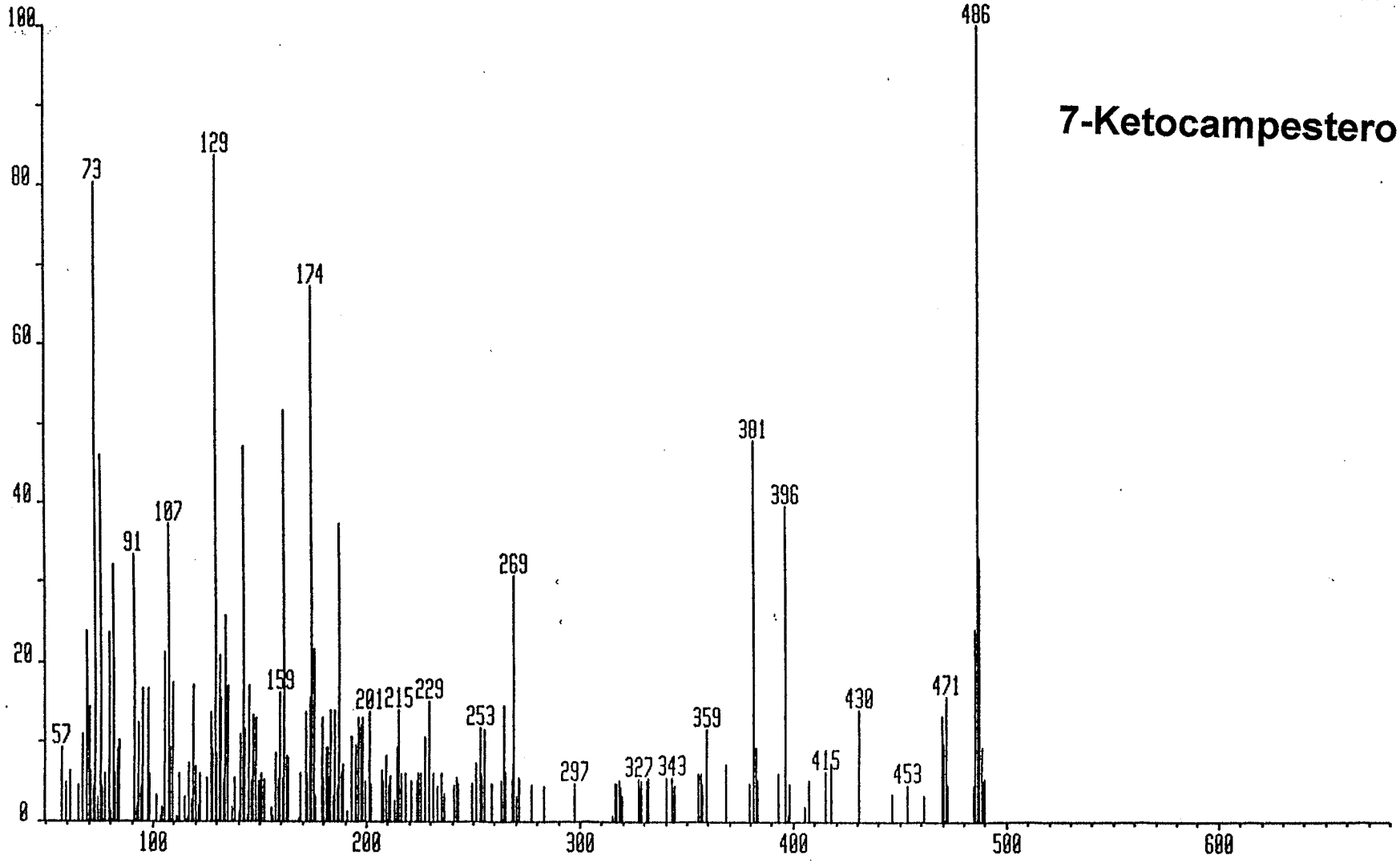


Fig.5.27. Mass Spectrum of Peak r

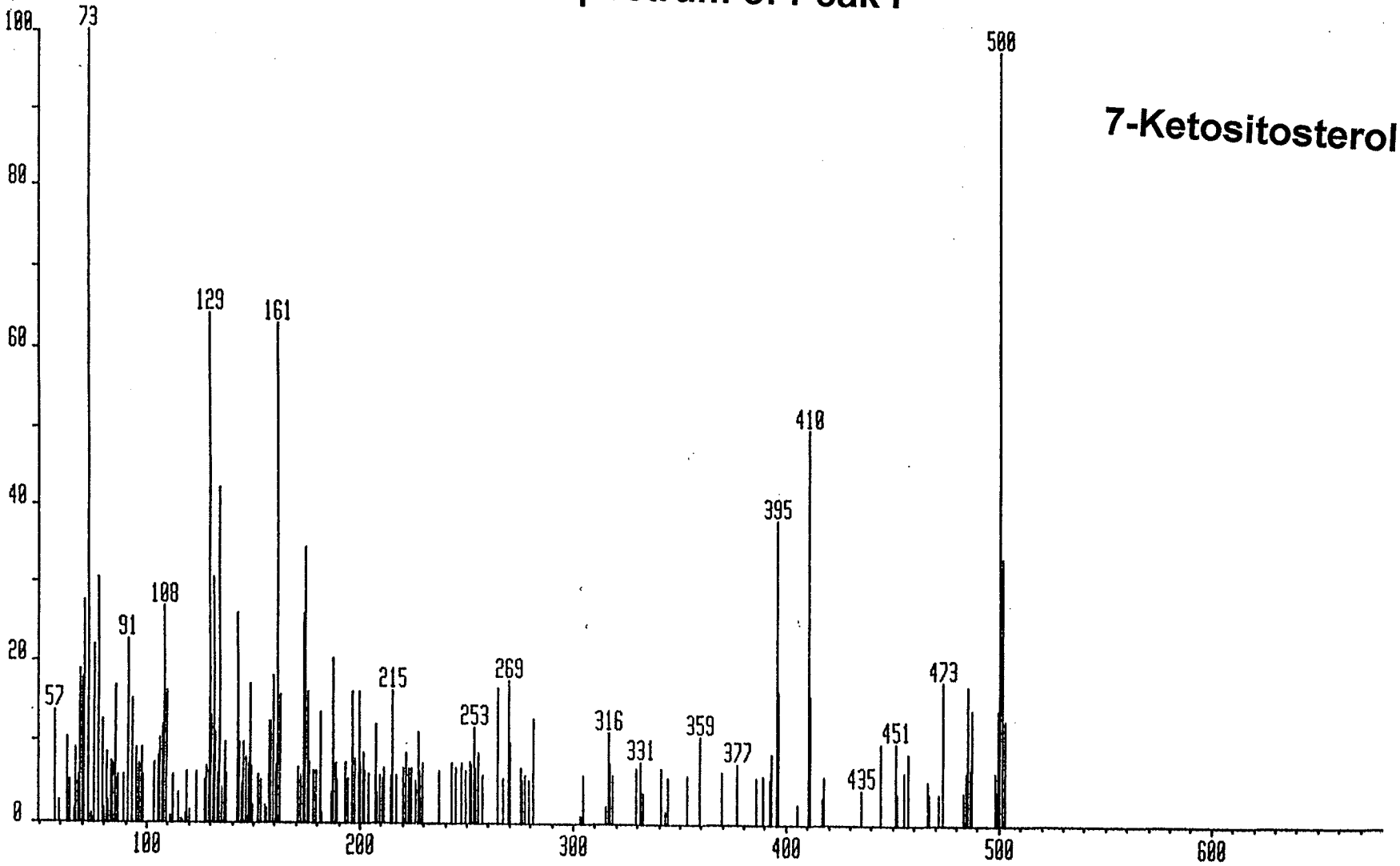


Fig.5.28. Mass Spectrum of Peak e

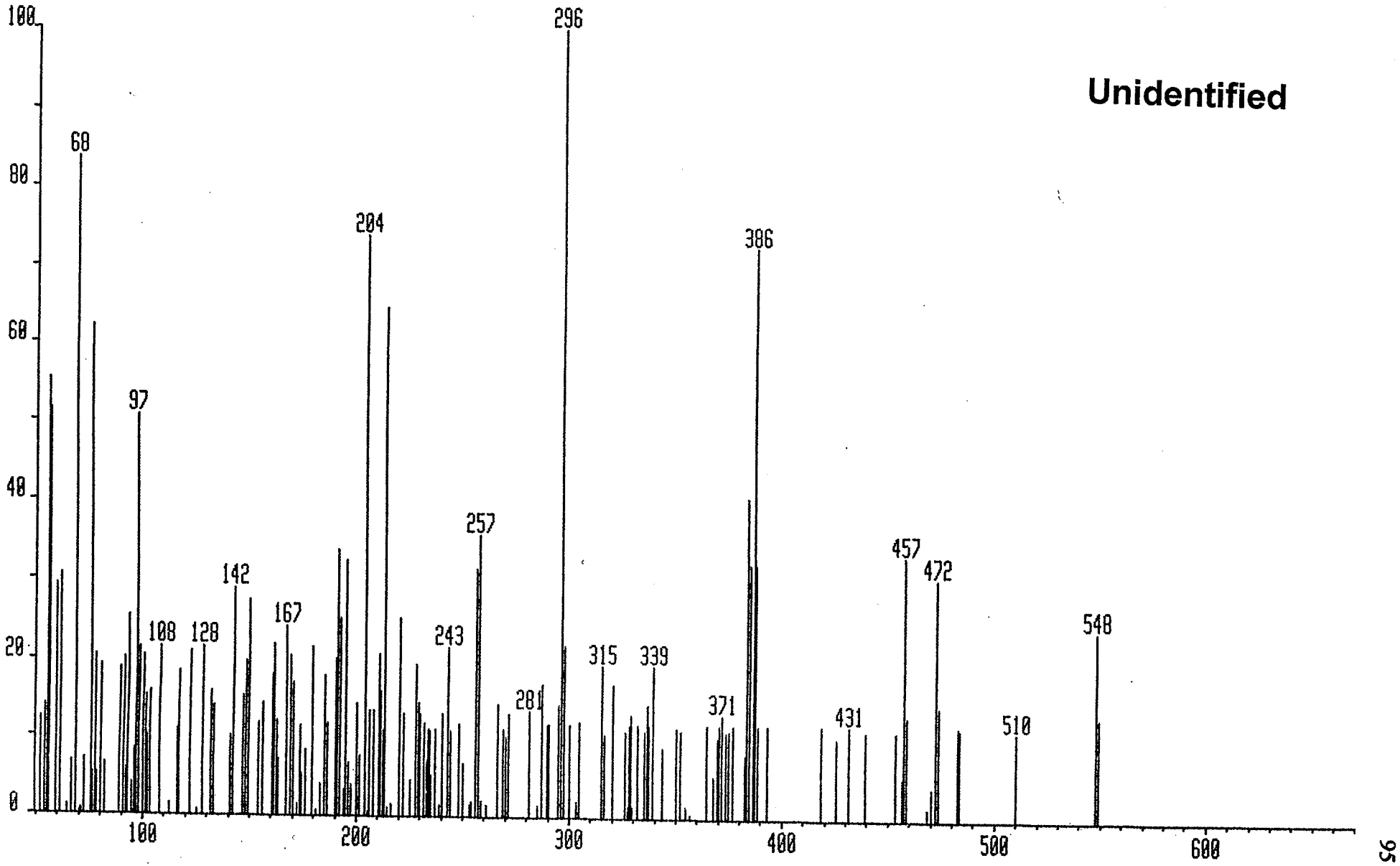


Fig.5.29. Mass Spectrum of Peak f

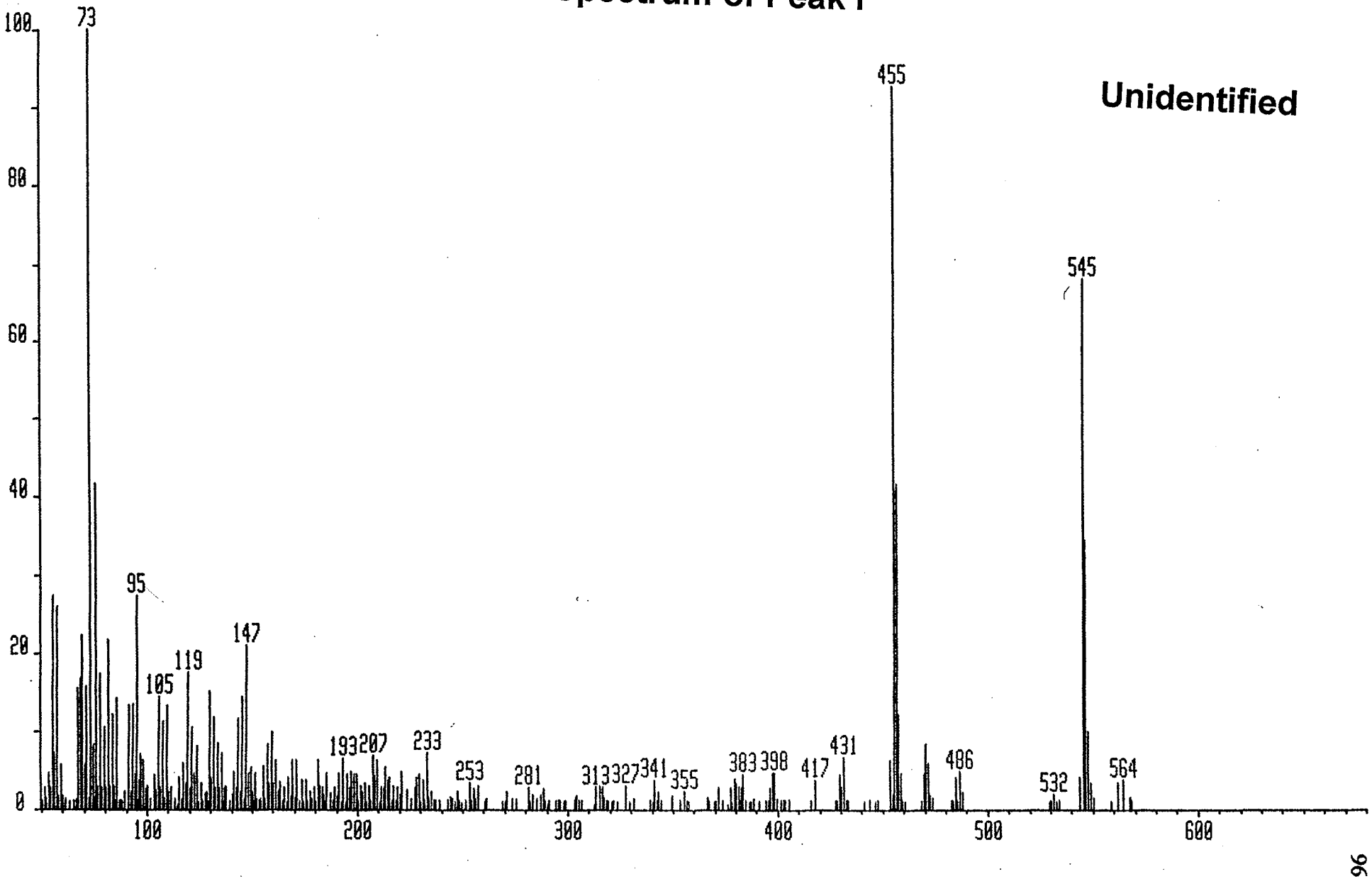


Fig.5.30. Mass Spectrum of Peak m

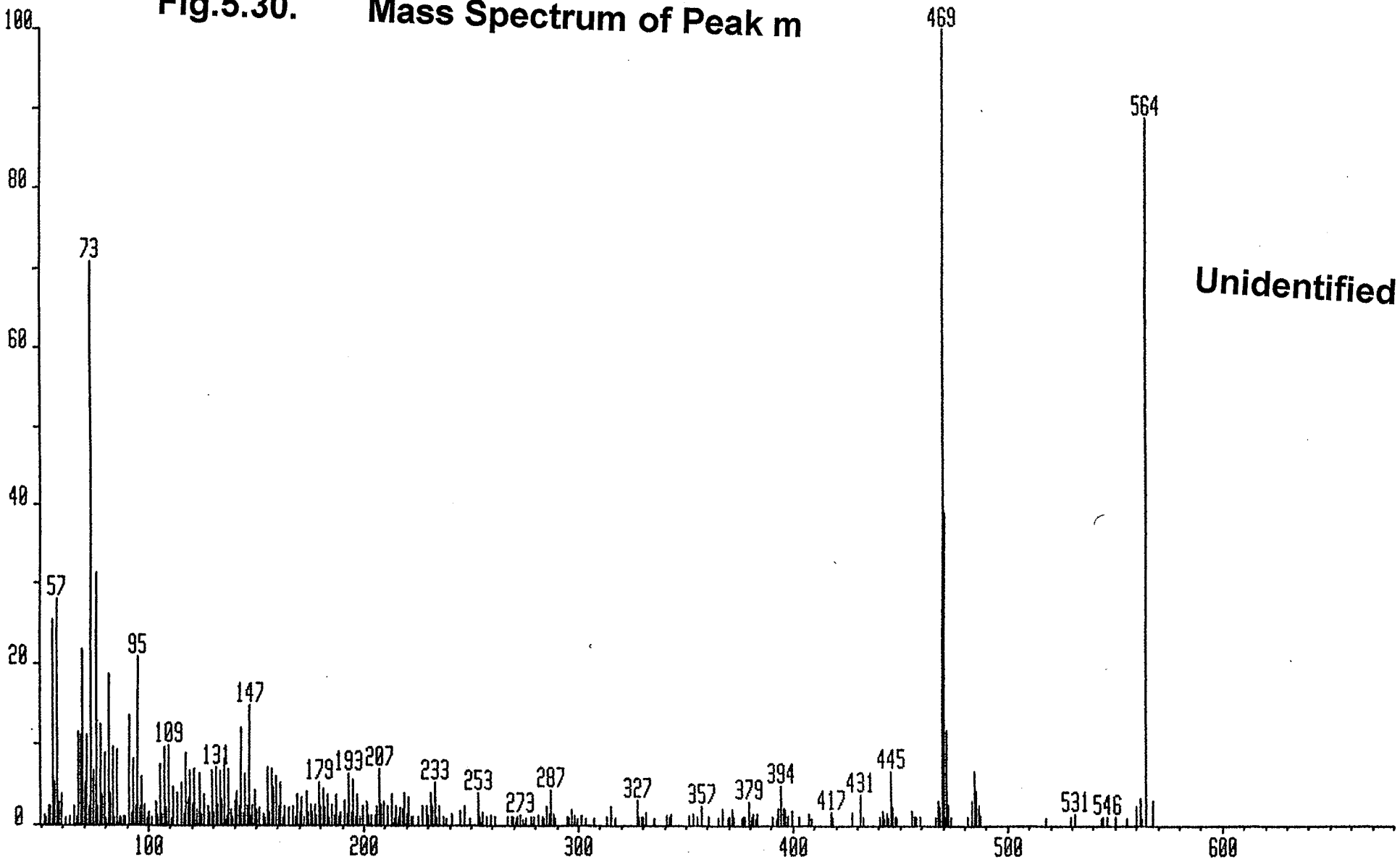


Table 5. 15. Levels (ppm) of Phytosterol Oxidation Products Formed in HYCO During Heating at Simulated Frying Temperature

	Heating Time (Hours)					
	0	**5**	**12**	**24**	**48**	**72**
ß-Sitosterol						
7α-OH	3.87	17.56	28.88	44.87	67.76	58.27
7ß-OH	0	0	55.27	71.35	98.96	94.96
α-Epoxy	0	2.62	12.72	22.99	35.69	17.43
ß-Epoxy	0	12.48	11.28	12.66	11.25	7.70
7-Keto	0	0	52.55	116.03	197.87	234.92
Total	**3.87**	**32.66**	**160.7**	**267.9**	**411.53**	**413.28**
Campesterol						
7α-OH	0	0	24.35	25.19	27.83	32.37
7ß-OH	0	0	37.61	50.24	47.05	44.74
α-Epoxy	0	13.64	37.50	57.71	74.92	61.14
ß-Epoxy	3.87	6.75	29.81	48.82	74.05	61.37
7-Keto	3.96	9.86	52.86	105.90	167.88	200.36
Total	**7.83**	**30.25**	**182.13**	**287.86**	**391.73**	**399.98**
Brassicasterol						
Epoxy	0	0	2.48	5.42	18.47	12.29
7-Keto	0	0	0	62.50	94.35	97.58
Total	**0**	**0**	**2.48**	**67.92**	**112.82**	**109.87**
Grand Total:						***923.13***

Table 5.16. Levels (ppm) of Phytosterol Oxidation Products Formed in RCO During Heating at Simulated Frying Temperature

	Heating Time (Hours)					
	0	**5**	**12**	**24**	**48**	**72**
ß-Sitosterol						
7α-OH	0	5.49	7.23	13.01	17.62	38.43
7ß-OH	0	tr.	5.26	26.76	38.23	62.75
α-Epoxy	0	tr.	3.51	8.25	9.05	14.15
ß-Epoxy	0	tr.	tr.	14.39	9.39	7.61
7-Keto	0	5.02	12.66	16.98	36.47	62.63
Total	**0**	**10.51**	**28.66**	**79.39**	**110.76**	**185.57**
Campesterol						
7α-OH	0	tr.	tr.	1.51	20.42	11.05
7ß-OH	0	0	0	12.23	32.74	25.63
α-Epoxy	0	0	10.07	16.00	13.92	31.52
ß-Epoxy	0	1.28	1.01	2.13	18.39	41.48
7-Keto	0	25.26	30.49	13.21	39.09	58.19
Total	**0**	**26.54**	**41.57**	**45.08**	**124.56**	**167.87**
Brassicasterol						
ß-Epoxy	0	0	0	1.15	11.05	10.21
7-Keto	0	tr.	17.3	49.47	47.76	57.65
Total	**0**	**0**	**17.3**	**50.62**	**58.81**	**67.86**
Grand Total:						***363.65***

Table 5. 17. Levels (ppm) of Phytosterol Oxidation Products Formed in LLCO During Heating at Simulated Frying Temperature

	Heating Time (Hours)					
	0	**5**	**12**	**24**	**48**	**72**
ß-Sitosterol						
7α-OH	0	0	7.30	15.80	17.92	17.02
7ß-OH	0	0	tr.	2.56	3.12	6.65
α-Epoxy	0	0	tr.	tr.	2.77	11.35
ß-Epoxy	0	0	tr.	tr.	7.70	16.23
7-Keto	0	0	1.28	2.99	11.18	29.71
Total	**0**	**0**	**8.58**	**21.35**	**42.69**	**80.96**
Campesterol						
7α-OH	0	tr.	4.95	3.90	22.22	27.87
7ß-OH	0	0	tr.	2.98	22.25	28.99
α-Epoxy	0	0	tr.	9.62	10.00	6.00
ß-Epoxy	0	tr.	17.30	15.80	17.92	17.02
7-Keto	0	0	5.26	13.57	25.39	36.79
Total	**0**	**0**	**27.51**	**45.87**	**97.78**	**116.67**
Brassicasterol						
ß-Epoxy	0	0	0	0.43	1.07	1.05
7-Keto	0	tr.	9.25	31.03	45.98	25.70
Total	**0**	**0**	**9.25**	**31.46**	**47.05**	**26.75**
Grand total:						***224.38***

Table 5.18. Levels (ppm) of Phytosterol Oxidation Products Formed in HOCO During Heating at Simulated Frying Temperature

	Heating Time (Hours)					
	0	**5**	**12**	**24**	**48**	**72**
ß-Sitosterol						
7α-OH	5.56	9.96	12.36	16.12	16.09	13.35
7ß-OH	4.86	5.08	9.57	21.27	23.66	20.67
α-Epoxy	0	3.56	7.10	9.22	21.56	33.60
ß-Epoxy	0	8.05	11.22	14.55	23.32	29.19
7-Keto	0	1.98	2.81	8.40	25.02	41.47
Total	**10.42**	**28.63**	**43.06**	**69.56**	**109.65**	**138.28**
Campesterol						
7α-OH	0	0	tr.	5.21	20.14	39.08
7ß-OH	0	0	tr.	5.74	16.09	29.95
α-Epoxy	0	tr.	5.61	9.57	23.66	20.67
ß-Epoxy	tr.	2.92	18.28	15.31	16.09	25.27
7-Keto	0	tr.	2.81	7.20	26.73	41.49
Total	**0**	**2.92**	**26.7**	**43.03**	**102.71**	**156.46**
Brassicasterol						
ß-Epoxy	0	0	0	7.14	10.84	14.46
7-Keto	0	0	5.63	12.52	25.09	36.82
Total	**0**	**0**	**5.63**	**19.66**	**35.93**	**51.28**
Grand Total:						***346.02***

5.3. Tocopherol Changes During Frying and Storage of Potato Chips

5.3.1. Tocopherol Compositions in Canola Oils

The tocopherol contents in fresh canola oils are presented in Table 5.19. Three tocopherol isomers were found in canola oils: α-, γ- and δ-tocopherols. α- and γ- Tocopherols comprised of 97% to 99% of total the tocopherols present in the oils. δ-Tocopherol was found at a level of 1-2 mg/100g in the analysed canola oils. Since the amounts of δ-tocopherol were close to the detection limit, the change of δ-tocopherol during the frying and storage was not studied. The total tocopherol levels were similar in RCO and HYCO. Genetically modified canola oils, LLCO and HOCO, had lower amounts of tocopherols than unmodified canola oils, RCO and HYCO.

Table 5. 19. Composition of Tocopherols (mg/100g) for Canola Oils

Name	α-Tocopherol	γ-Tocopherol	δ-Tocopherol	Total tocopherol
RCO	21.18	42.65	1.94	65.77
LLCO	12.75	26.13	1.75	40.62
HOCO	7.70	14.70	-	22.40
HYCO	19.76	40.39	2.03	62.18

5.3.2. Tocopherol Changes in Oils During Frying of Potato Chips

The changes to α- and γ-tocopherols in canola oils during the frying of potato chips are presented in Fig. 5.31. Frying reduced the level of tocopherol in all frying oils over time, but differed among the oils. The α-tocopherol amounts decreased faster in HYCO than in other canola oils evaluated. At the end of frying, about 80% of α-tocopherol disappeared in the HYCO, while only 20% in the RCO. The loss of α-tocopherol in LLCO and HOCO was 82% and 70%, respectively. Similar pattern of changes was observed for γ-tocopherol (Fig.5.38). After the 5 days of frying, 90% of γ-tocopherol disappeared in the HYCO. The losses of γ-tocopherol were 40% in LLCO, 50% in RCO and 65% in HOCO. Since 10-15% of fresh oils were added daily during the frying, 10-15% of the fresh tocopherols were added daily as well. At the end of frying, 50-75% of the tocopherols were added along with the fresh oils. The results suggested that if fresh oils had not been added, all the tocopherols would have been consumed at the end of frying.

5.3.3. Tocopherol Changes in Canola Oils During Heating at Simulated Frying Temperature

The patterns of α- and γ-tocopherol changes in canola oils under heating at simulated frying conditions are shown in Fig. 5.32. After 24 hours of heating, α- and γ- tocopherols were not present in HYCO, HOCO and RCO. The tocopherols in LLCO were still present until 48 hours of heating. There are two reasons that tocopherols disappeared faster in the heating at simulated frying temperature than in regular frying: a) in regular frying, fresh oils were added daily, and therefore fresh tocopherols were added daily at the same time and b) the frying temperature of regular frying was 180±5°C whereas in the heating at simulated frying

temperature it was 190±2°C.

5.3.4. Changes of Tocopherols in Fried Potato Chips During Storage

The tocopherol levels in the lipids extracted from the potato chips prior to storage are shown in Table 5.20. Total tocopherol level of the potato chips collected from day 1 of frying was found the highest in RCO followed by HYCO. The genetically modified canola oils, LLCO and HOCO contained lower level of tocopherols than RCO and HYCO. The results indicate that genetic modification of the oils may be one of the factor that lower the level of tocopherols in the oils.

Fig. 5.31. Tocopherol Changes in Canola Oils During Potato Chip Frying

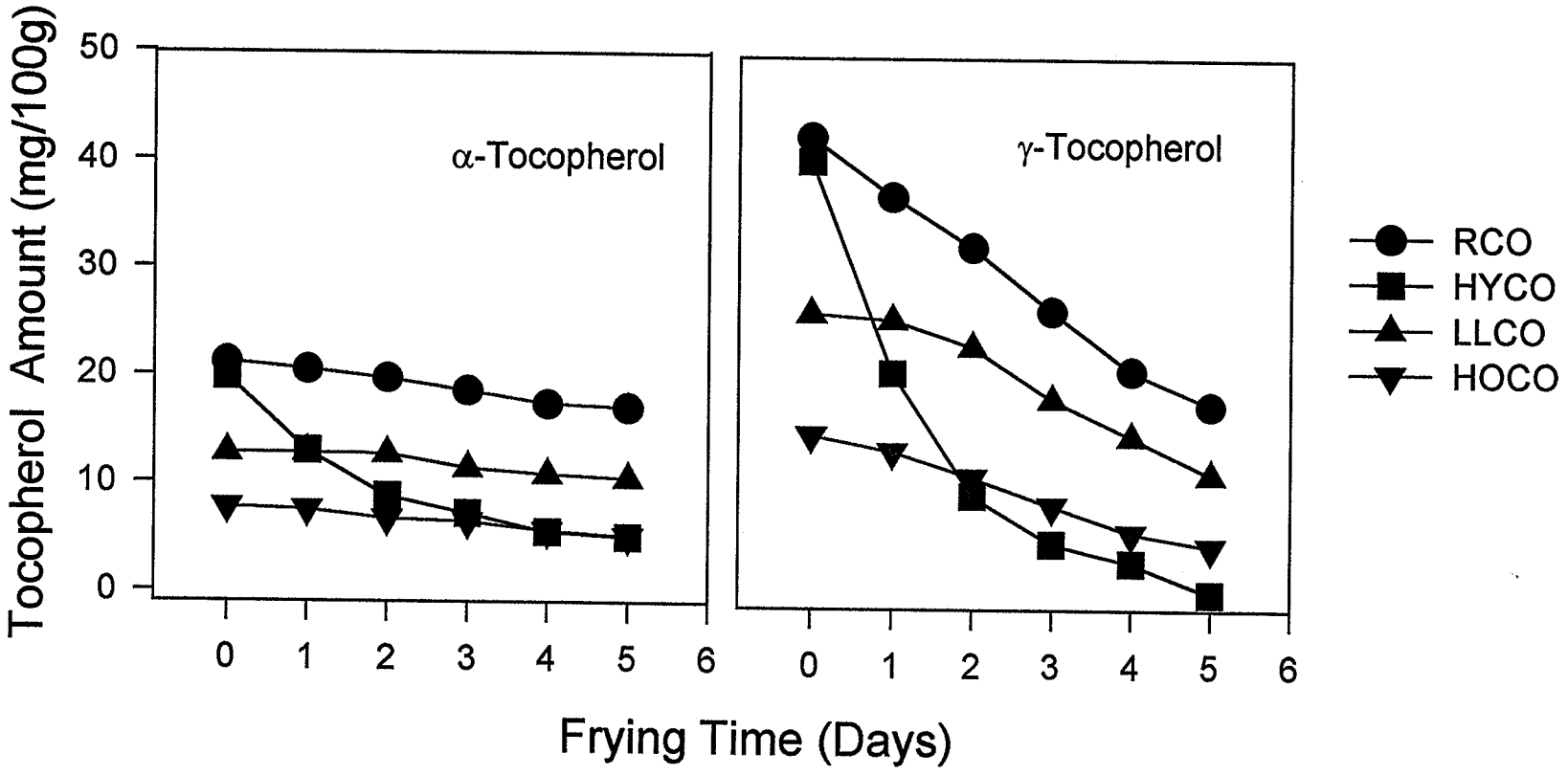


Fig. 5.32. Tocopherol Changes in Canola Oils at Simulated Frying Temperature

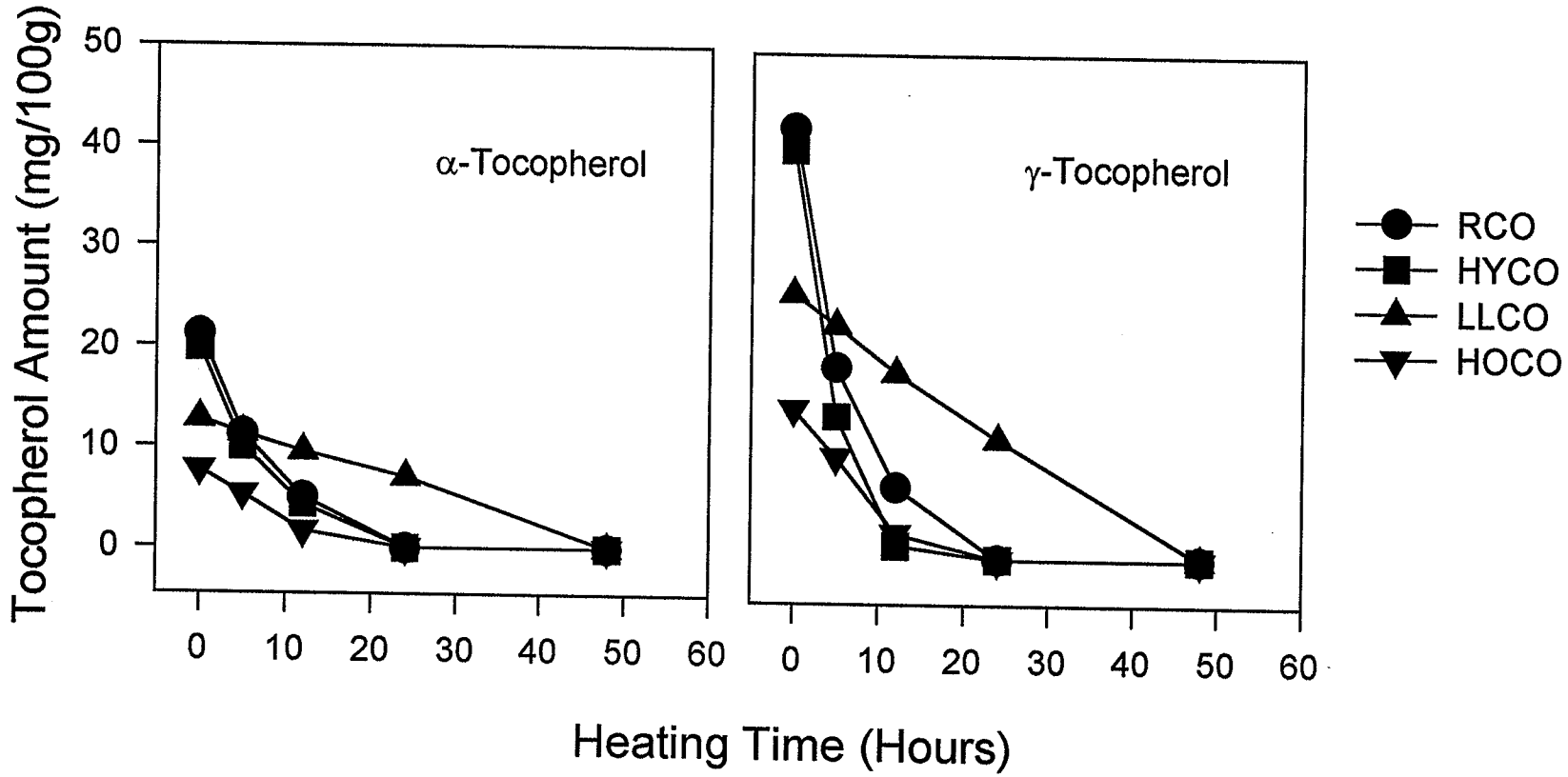


Table 5. 20. Tocopherol Amount in Potato Chips Before Storage (mg/100g of Lipids)

Oil		α-Tocopherol	γ-Tocopherol	δ-Tocopherol	Total
RCO	*day 1*[a]	21.0	41.8	1.7	64.5
	day 5[b]	16.1	24.7	1.4	42.2
LLCO	*day 1*	11.7	26.8	1.2	39.7
	day 5	9.2	13.8	1.5	24.5
HOCO	*day 1*	6.9	14.6	1.2	22.7
	day 5	2.6	4.7	1.0	8.3
HYCO	*day 1*	13.5	29.2	2.0	44.7
	day 5	6.3	3.0	1.6	10.9

[a]- Potato chips collected during first day of frying.
[b] -Potato chips collected during fifth day of frying

The changes of α- and γ-tocopherols in stored potato chips collected during the first day of frying and then stored at 60°C for 16 days are shown in Fig. 5.33. The tocopherol in potato chips decreased in the following order: HYCO > HOCO ≈ LLCO > RCO. Tocopherols in potato chips fried in HYCO showed the best retention during storage among all the oils evaluated. The tocopherol amounts decreased at the fastest rate in the potato chips fried in RCO. At the end of storage 100% of α-tocopherol and 93% of γ-tocopherol disappeared in potato chips fried in RCO. γ-Tocopherol disappeared at a lower rate, this could indicate lower effectiveness in preventing lipid oxidation.

The changes of α- and γ-tocopherols in potato chips collected during the fifth day of frying

are presented in Fig. 5.34. Their pattern of tocopherol changes were similar to that of the potato chips collected during the first day of frying. However, lower initial amounts of tocopherols were found in the potato chips collected during the fifth day of frying. Again, tocopherols decreased faster in potato chips fried in RCO, which had the highest level of polyunsaturated fatty acids. The retention of α- and γ-tocopherols in the two genetically modified canola oils was found better than RCO. At the end of storage, low concentration of tocopherols were observed in the stored potato chips from frying day 5 compared to day 1, due to the low initial amounts of tocopherols in the chips. The overall α-tocopherol decreased at faster rate than γ-tocopherol in the stored potato chips collected during the fifth day of frying.

Fig. 5.33. Tocopherol Changes in Potato Chips During Storage (First Day of Frying)

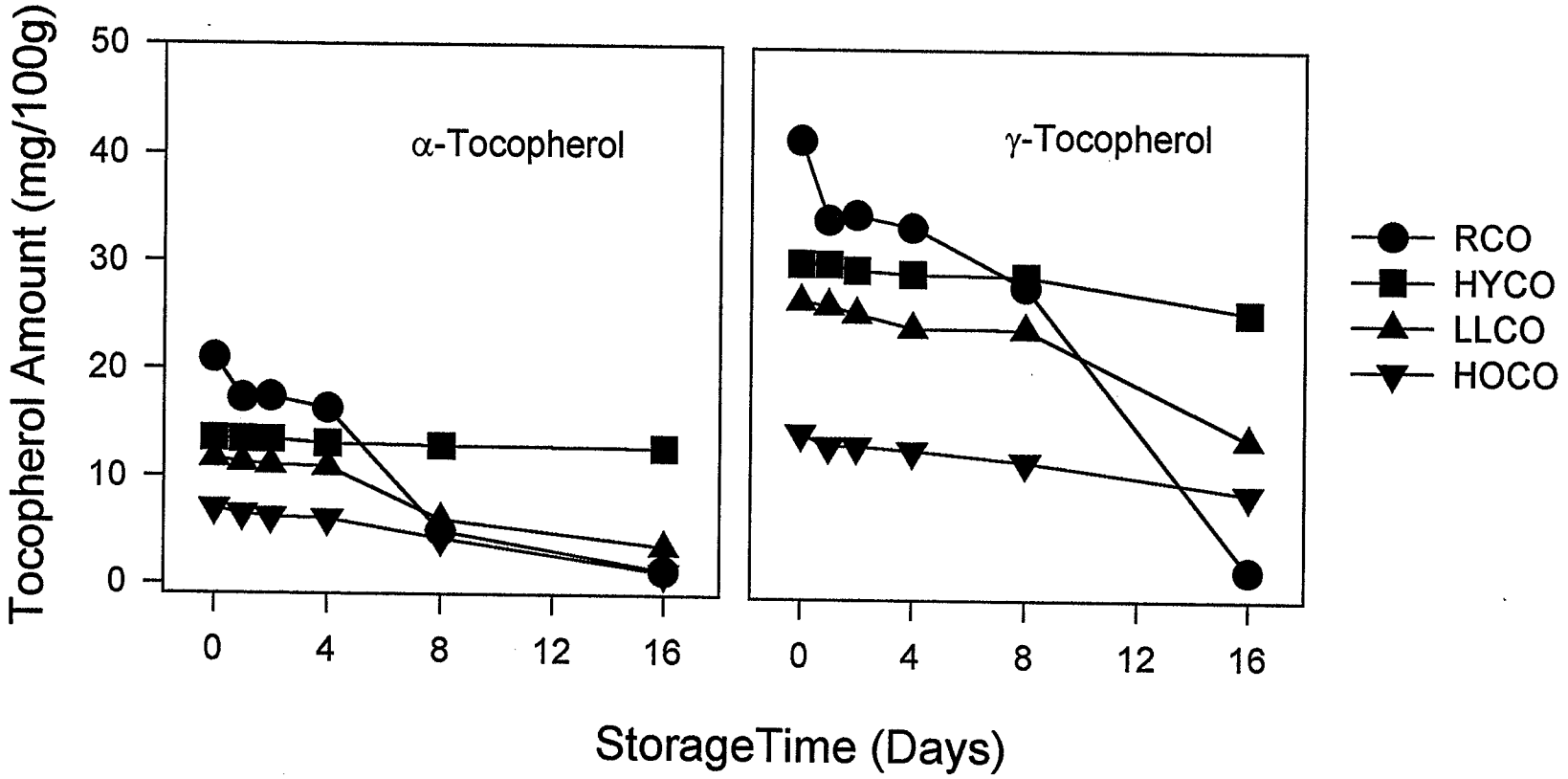
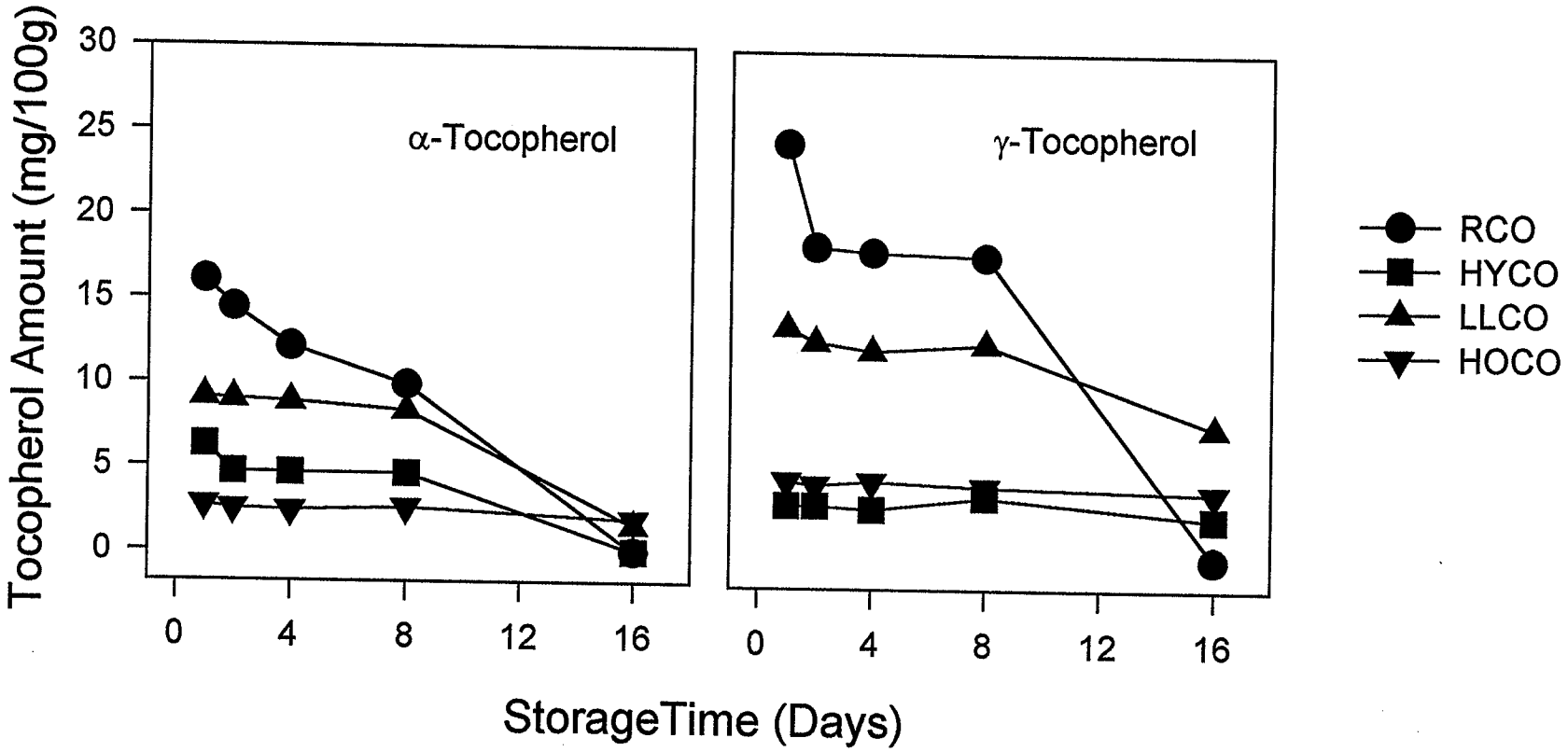


Fig. 5.34. Tocopherol Changes in Potato Chips During Storage (Fifth Day of Frying)



Chapter VI

Discussion

6.1. Sterols Oxidation:

6.1.1. Sterol Stability During Heating

The present study on the stability of phytosterols and cholesterol showed that the degree of oxidation of phytosterols and cholesterol was strongly related to the temperature, length of heating time and the storage time. These observations were consistent with early reports (Finocchiaro *et al.*, 1984, Nourooz-Zadeh and Appelqvist, 1987, Morgan and Armstrong, 1992, Dutta and Appelqvist, 1995, Daly *et al*, 1983).

The present study on cholesterol stability during heating showed that cholesterol was stable when heated at 95°C for 12 hours and oxidized when heating temperatures increased above 100°C. These findings are in agreement with those of Osada *et al.*, 1993a, Kim and Nawar, 1993. Osada *et al* (1993a) heated the cholesterol in its pure form to temperatures ranging from 100-200°C for 24 hours. They found that cholesterol was stable at 100°C for 24 hours and oxidized at a faster rate when the temperatures were above 100°C. Similar results have been observed by Kim and Nawar (1993). These authors suggested that conventional cooking at 100°C for a short time might not be a concern for cholesterol oxidation, whereas the frying process where temperature greater than 100°C are achieved are of the great concern. A few studies have focused on phytosterol oxidation at elevated temperatures (Daly *et al.*, 1983, Blekas and Boskou, 1989). However, systematic study to investigate phytosterol stability on

various temperatures has not been conducted so far. The present study investigated the stability of phytosterols at 75°C, 95°C, 120°C, 155°C and 180°C temperatures. ß-Sitosterol and campesterol were found to have lower stability during heating than cholesterol. These sterols oxidized when heating temperatures were below 100°C and had similar thermal stability with each other. During the heating at simulated frying temperatures, brassicasterol was found to have lower thermal stability than ß-sitosterol and campesterol. Stigmasterol, on the other hand, showed the greatest stability during heating among all the evaluated sterols.

A number of oxidation products have been identified when phytosterols were heated. Daly *et al.*, (1983) identified some of the oxidation products formed from pure sitosterol heated at 100°C. They characterized 7α- and 7ß-hydroxysitosterols, 7-ketositosterol, epimeric epoxysitosterols, Δ^4-sitosterol-3,6-dione, Δ^4-sitosterol-3-one and Δ^5 -sitosterol-3-one using TLC and GC-MS. In the present study, when standard of ß-sitosterol was heated, 7α- and 7ß-hydroxysitosterols, 7-ketositosterol, epimeric epoxysitosterols were identified. Other oxidation products such as Δ^4-sitosterol-3,6-dione, Δ^4-sitosterol-3-one and Δ^5-sitosterol-3-one were not detected in the present study. Blekas and Boskou (1989) isolated oxidation products when 5% stigmasterol was heated with purified triacylglycerol at 180°C. Using TLC, IR spectroscopy and GC-MS, these authors tentatively identified epimeric 7-hydroxystigmasterols, epoxides, stigmast-22-en-3,5,6-triol, stigmasta-4,22-diene-3-one, stigmasta-3,5,22-triene-7-one and stigmasta-3,5,22-triene. In the present study, only epimeric 7-hydroxystigmasterols, epimeric 5,6-epoxystigmasterols and 7-ketostigmasterol were identified. This was probably because of two reasons: (1) different pathways of sterol oxidations may exist and therefore different

products might be formed when pure sterols were heated with triglycerides (Maerker, 1987), and (2) the amounts of some oxidation products were too small to be detected in the present study. A number of small peaks was observed, but it was not possible to interpret the spectra.

ß-Sitosterol, campesterol and cholesterol produced high amounts of oxidation products at the beginning of heating, but these oxidation products were quickly transferred into large molecular polymers when time of heating was prolonged. Osada *et al.* (1993a) observed the same pattern of changes during heating of cholesterol. Stigmasterol behaved differently than other sterols evaluated. Stigmasterol produced less oxidation products and more large molecular compounds when heated at various temperatures.

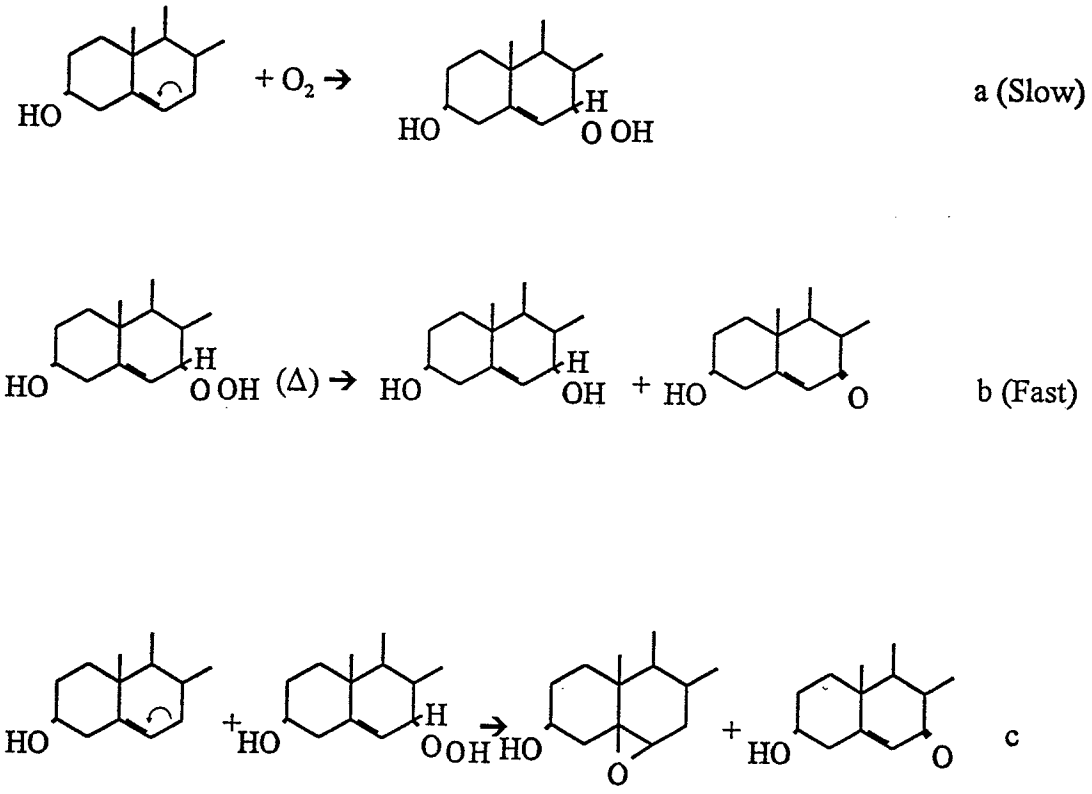
The mechanism of phytosterol oxidation was studied in the present study in terms of reaction orders. It is believed that cholesterol oxidation proceeds with a free radical mechanism (Smith, 1981, 1987). According to Maerker (1987) and Yanishlieva (1980) the unsaturated double bond in the ring of cholesterol molecule is sensitive to free radical oxidation by oxygen attack, and form intermediate oxidation products, such as hydroperoxides (Fig. 6.1a). The hydroperoxides are not stable and converted to 7α and 7ß-hydroxycholesterols, and 7-ketocholesterol as the principal oxidation products of cholesterol (Fig. 6.1b). The formation of the hydroperoxides is relatively slow and determined the whole reaction rate. Intermediate oxidation products such as hydroperoxides could react with cholesterol radicals to form cholesterol epoxides and hydroxycholesterols (Fig.6.1 c). At low temperature such as 75°C, 95°C and 100°C the rate of the oxidation of sterols was zero or first order reaction because

reaction a was the rate determine process. When the samples were heated at 150°C and 180°C, mechanism of the oxidation reaction was changed. In this situation, large amounts of intermediate oxidation products, such as sterol hydroperoxides, were formed in the system within a short period of time. These intermediate products then participated in the free radical oxidation reaction (reaction c). A second order reaction was observed. The observation of the sterol oxidation mechanism complied with the current knowledge of cholesterol and phytosterol oxidation (Paniangvait, 1995, Smith, 1981, 1987, Maerker, 1987, Yanishlieva and Marinova, 1980).

6.1.2. Sterol Stability During Frying

The present study investigated the phytosterol oxidation during the frying in two different conditions (1) the actual frying of potato chips and (2) heating at simulated frying temperature. In both studies, the amounts of phytosterols continuously decreased throughout the frying process. During the actual frying of potato chips, the total amounts of sterol losses at the end of frying were from 50% to 58%. The losses of phytosterols in actual frying can be partially explained by the absorption of the frying oil by the potato chips. During the heating at simulated frying temperature the losses of phytosterols ranged from 40% to 50% in RCO, LLCO and HOCO, and 70% to 80% in HYCO. These results agreed with the previous studies

Fig. 6.1.Mechanism of Cholesterol Oxidation



Adapted from Mearker (1987), and Yanishieva and Marinova, (1980)

by Park and Addis (1986) where beef tallow was heated to 155°C and 190°C for up to 400 hours and the changes of cholesterol were recorded. They observed that 40% to 45% of cholesterol amounts disappeared at the end of heating. Bascoul *et al.*(1986) reported that 25% of cholesterol was destroyed after 60 hours of commercial frying. Ghavami and Morton (1984) heated soybean oil at 180°C for 96 hours and observed that 13% to 32% of the total amounts of phytosterols were lost by the end of heating.

Phytosterol oxidation was found to be affected by the fatty acid composition of oil. The rate of decomposition of total sterols was calculated (Table 6.1). The total sterol decomposition rate was between 43 to 67 ppm/hour in RCO and 30 to 45 ppm/hour in LLCO and HOCO. The results suggested that the content of 18:3 and 18:2 fatty acid directly effected the rate of sterol decomposition and oxidation. This observation was consistent with early reports on the effect of different fatty acids on cholesterol oxidation (Osada, 1993b, Kim and Nawar, 1991, Ohshima *et al.*, 1993, Li *et al.*, 1994). These authors observed that when cholesterol was heated with saturated fats such as tristearin and beef tallow, oxidized cholesterol was produced at a low rate. When cholesterol was heated in polyunsaturated fat such as soybean, linseed, safflower, sardine oils and triolein, oxidation products were formed more rapidly. Smith (1981) suggested that the formation of hydroperoxides of polyunsaturated fatty acids during oxidation may be necessary to initiate cholesterol oxidation. It is conceivable that cholesterol oxidation may have proceeded in a analogous way to fatty acid oxidation. Smith (1981) postulated that cholesterol oxidation in food and biological systems maybe proceeded through intermolecular or intramolecular interaction.

Table 6.1. Phytosterol Decomposition Rate During Heating at Simulated Frying Temperature (ppm/hour)

Heating Time	RCO	HYCO	LLCO	HOCO
0	-	-	-	-
5	46.80	127.60	5.20	54.00
12	64.08	168.33	45.58	35.42
24	67.88	115.71	38.92	36.08
48	51.48	84.85	33.13	32.00
72	43.01	61.69	33.96	31.25

The present study also found that fatty acids were not the only factors that affected the decomposition of sterols. The rate of sterol losses in HYCO were at the highest and ranged from 61 to 168 ppm/hour. This suggested that other factors might have also played important roles on sterol decomposition during heating. The fast decrease of phytosterols in HYCO need further investigation and may be attributed to the possible presence of increased amounts of metals.

6.1.3. Phytosterol Oxidation Products During Heating at Simulated Frying Temperature

During the heating at simulated frying temperature, the major oxidation products formed were

7α and 7ß-hydroxycampesterols, 7α and 7ß-hydroxysitosterols, α- and ß-epoxycampesterols, α- and ß-epoxysitosterols, α- or ß-epoxybrassicasterol and 7-ketones of sitosterol, campesterol and brassicasterol. 7α and 7ß-Hydroxybrassicasterols were not detected in the study, possibly because they were either in a very small amounts, or degraded very fast into large molecular compounds. The mass spectra of these small peaks detected showed that they had sterol structures (Fig. 5.33 to 5.36). Two earlier studies investigated cholesterol oxidation during extensive heating (Yan and White, 1990, Park and Addis, 1986). Park and Addis (1986) investigated cholesterol oxidation in heated beef tallows at 150°C and 190°C up to 400 hours. They found that major cholesterol oxidation products formed were 7α-hydroxycholesterol, α-epoxycholesterol, 7β-hydroxycholesterol, β-epoxycholesterol and 7-ketocholesterol. Yan and White (1990) heated lard enriched with two and ten times higher amounts of cholesterol at 180°C up to 240 hours. Similarly, the major identified oxidation products were 7α-hydroxycholesterol, α-epoxycholesterol, 7β-hydroxycholesterol, β-epoxycholesterol and 7-ketocholesterol. Park and Addis (1986) observed no triol cholesterol derivative in the heated tallow throughout the heating process. Yan and White (1990) observed only trace amounts of this cholesterol derivative in the heated lard with cholesterol level at twice the normal level. The lard with 10 times the normal cholesterol level contained no detectable triol derivatives. Both studies also did not observed any presence of 25-hydroxycholesterol and cholesta-3,5-dien-7-one. Park and Addis (1986) suggested that the finding of cholesta-3,5-dien-7-one in chicken embryo by Pennock et al. (1962) and in anhydrous milk fat by Flanagan et al. (1975) should be suspected as artifacts formed during hot saponification. Comparing these two studies with the present study of phytosterol oxidation, phytosterols oxidized in a very similar way to

cholesterol. Similar oxidation products were formed such as epimeric phytosterol hydroxides, epoxides and phytosterol ketones in major quantity in the heated canola oils. There were reports that other phytosterol oxidation products such as 25-hydroxy phytosterols, Δ^4-sitosterol -3,6-dione, Δ^4-sitosterol-3-one and Δ^5-sitosterol-3-one were formed during the oxidation (Daly, *et al.*, 1983), but this study did not detect any. This maybe because (a) these oxidation products were in very small amounts or had degraded into further polymers and (b) the cold saponification and simplified analytical procedures used eliminated or minimized the artifact formation.

Similar to cholesterol, phytosterol 7-ketones were found to accumulate more extensively than other phytosterol oxidation products during the frying. 7-Ketositosterol, 7-ketocampesterol and 7-ketobrassicasterol were found at the highest amounts in all the heated oils and steadily increased when as heating time was increased. Park and Addis (1986) found that tallow heated to 150°C resulted in the formation of 7-ketocholesterol which was almost linearly related to the heating time, reaching about a 10% conversion level of the initial cholesterol content after 376 hours of heating. The epimeric 7-hydroxycholesterols did not increase proportionally to the heating time. α-Epoxides were formed in larger quantities than epimeric 7-hydroxycholesterols but did not increase with heating time. Yan and White (1990) also found that the accumulation of 7-ketocholesterol in the heated lard was the largest among all other oxidation products. In the present study, phytosterol oxidation products behaved similarly to the changes of cholesterol oxidation products described by Yan and White (1990) and Park and Addis (1986). In all four oils, 7-keto phytosterols were increased in amounts up to the end. Most of the other

oxides such as epimeric 7-hydroxy phytosterols, epimeric epoxy phytosterols increased in the amounts at the beginning of heating and then decreased when the heating was prolonged. According to recent knowledge of cholesterol oxidation, the autoxidation of cholesterol proceeds via an initial formation of the epimeric 7α- and 7ß-hydroxyperoxides (7-OOHs) which are later transferred into their corresponding alcohols and further into 7-ketones. Therefore, it was anticipated that at elevated temperatures and in the absence of water, the decomposition of 7-hydroperoxides is likely to prefer the formation of 7-ketocholesterol through dehydration to that of epimeric 7-hydroxycholesterols (Park and Addis, 1986).

In this study, the amounts of total phytosterol oxidation products formed were not equal to the amounts of phytosterol lost during heating. The total formation of oxidation products in the frying oils at the end of frying time were at 923 ppm, 363 ppm, 224 ppm and 346 ppm for HYCO, RCO, LLCO and HOCO respectively. The total losses of phytosterols at the end of the frying period were at 4442 ppm, 3097 ppm, 2445 ppm, 2250 ppm, respectively for HYCO, RCO, LLCO and HOCO. Yan and White (1990) observed that a total of 1550 ppm and 1470 ppm cholesterol disappeared at the end of heating of lards containing ten and two times higher level of cholesterol than normal, respectively. The total cholesterol oxidation products they detected were only 480 ppm and 320 ppm for these lard samples, respectively. They suggested that there might be other degradation products formed which were not analysed and/or there were losses through volatilization. Park and Addis (1986) suggested that at extremely high temperatures there may be a lesser chance for cholesterol to be oxidized but higher probability for cholesterol oxides to be quickly broken down right after formation and therefore making

them undetected.

6.1.3. Sterol Stability During Storage of Potato Chips

The present study investigated the sterol changes during the storage of potato chips fried in different oils. At the end of the storage total sterol losses of phytosterols were between 3% and 10% in the majority of analysed potato chips. As discussed in Chapter 5.1, phytosterols showed good stability when heated at 70°C to 80°C up to 12 hours. Cholesterol was reported to be stable at low temperatures for a short period of time although long term storage and food processing at low temperatures might have caused oxidation of cholesterol (Nourooz-Zadeh and Appelqvist, 1987, 1992, Osada *et al.*, 1993b). Since some phytosterols such as ß-sitosterol, campesterol and brassicasterol have lower stability than cholesterol, then oxidation is expected during storage. Nourooz-Zadeh and Appelqvist (1993) detected various levels of phytosterol oxidation products in stored soybean oil and wheat flour. They found that soybean oil stored for one year at 4°C h contained little amounts of phytosterol oxides, but wheat flour stored for 2, 8 and 36 months at room temperatures contained 0.2 ppm to 118 ppm of phytosterol oxides. The higher amounts of oxidation products in the flour might be due to the fact that flour has larger area exposed to air than oils. The present study confirmed their observations that oxidation of phytosterols in low temperatures is generally slow and small changes in phytosterols were observed.

6.2. Tocopherols

6.2.1. Tocopherol Changes During Frying.

In the present study, the amounts of tocopherols constantly decreased during both actual frying and the heating at simulated frying temperature. The losses of tocopherols were found to be related to fatty acid composition. The rate of decomposition of tocopherols was calculated and is shown in Table 6.2, and 6.3. The decomposition rates in RCO were 20.0 ppm/hr and 49.9 ppm/hr for α- and γ-tocopherols, respectively. In HOCO, tocopherols disappeared at the rate of 4.3 ppm/hr and 8.8 ppm/hr and in LLCO at 3.0 ppm/hr and 6.5 ppm/hr for α- and γ-tocopherols, respectively. These results suggested that the rate of losses of tocopherols were related to the amount of unsaturated fatty acid present in the oil.

Table 6.2. α-Tocopherol Decomposition Rate During the Heating at Simulated Frying Temperature (ppm/hour)

Time	RCO	HYCO	LLCO	HOCO
0				
5	20.00	16.50	3.00	4.30
12	13.50	10.90	2.70	4.60
24	-	-	2.40	-
48	-	-	-	-

Table 6.3. γ-Tocopherol Decomposition Rate During Heating at Simulated Frying Temperature (ppm/hour)

Time	RCO	HYCO	LLCO	HOCO
0				
5	49.90	47.20	6.50	8.80
12	31.20	29.20	6.80	9.50
24	-	-	6.30	-
48	-	-	-	-

In HYCO, the decomposition rate was 15.0 ppm/hr and 47.2 ppm/hr for α- and γ-tocopherols, respectively. This disappearance rate was close to that of RCO, suggesting that at frying temperatures, other factors, such as the possibly of trace metals may accelerate tocopherol oxidation.

Individual tocopherol isomers were found to decompose at different rates during heating. In the study γ-tocopherol decomposed at a faster rate than α-tocopherol (Table 6.2 and 6.3). It is generally known that α-tocopherol has better antioxidant activities than γ-tocopherol. Lea (1960) and Parkhurst *et al.* (1968) reported that the order of decomposition rates of tocopherols were γ>δ>α in the oils at frying temperatures. Yoshida *et al*, (1991) reported that the decomposition order of four tocopherol isomers during microwave heating was α>γ>β>δ. The differences in the order of tocopherol losses are probably due to different experimental conditions used, such as the type of oil and food used during frying and heating conditions (Miyagawa, *et al*, 1991).

6.2.2. Tocopherol Changes During Storage.

The study showed that tocopherol changes during the storage of potato chips were closely related to the fatty acid composition of the frying oils. In all cases, the tocopherols in potato chips fried in HYCO were the most stable. The tocopherols with the least stability were found in potato chips fried in RCO. This is because tocopherols act as antioxidants in a food system to prevent oxidation of fatty components by oxidizing themselves into tocopheroxyl oxides (Suarna, 1990, 1991). During storage, the fate of tocopherols was mainly determined by the reaction between tocopherol and peroxides of fatty acids. Polyunsaturated fatty acids are the most susceptible to oxidation. HYCO with 0% of linolenic and 6% of linoleic fatty acids, was the least susceptible to oxidation during storage, and therefore the lowest in tocopherol losses. RCO contained 10% of linolenic and 21% of linoleic acids, a rapid disappearance of tocopherols during storage was observed. In genetically modified LLCO and HOCO, the polyunsaturated fatty acids have been reduced and the retention of tocopherols in the potato chips fried in these oils was greatly improved compared to RCO.

The degradation rate of individual tocopherol isomers was different between storage and frying. α-Tocopherol degradation rate was higher than γ-tocopherol during the storage, suggesting that the mechanisms of tocopherol degradation during frying and storage were different.

Chapter VII

Conclusions

7.1. Phytosterols

The stability of major phytosterols including ß-sitosterol, campesterol, stigmasterol and brassicasterol were investigated at various temperatures in this study. The stability of these phytosterols was also compared with cholesterol. The results can be summarized as follows:

1). The oxidative stability of sterols decreased in following order: stigmasterol > cholesterol > ß-sitosterol ≈ campesterol > brassicasterol.

2). Temperatures mostly affected the rate of oxidation of sterols, major phytosterols showed good stability at temperatures below 100°C but were unstable above this temperature.

3). The oxidation rate of phytosterols during frying was affected by fatty acid composition of the oil; generally, higher unsaturation in the oil caused faster oxidation rate of the phytosterols in the oil.

4). During storage of potato chips at 60°C for 16 days small changes in phytosterols were observed.

5). All phytosterols produced high amounts of oxidation products at frying temperature, the major identified phytosterol oxidation products had similar chemical structure to cholesterol oxidation products.

7.2. Tocopherols

Tocopherol changes were investigated in the oils during frying and in the stored potato chips.

1). The amounts of all tocopherols decreased at a high rate in all frying oils.

2). Tocopherol losses were the highest during frying in HYCO; in genetically modified LLCO and HOCO the rate of tocopherol depletion was lower than in RCO.

3). The disappearance of γ-tocopherol was faster than α-tocopherol in canola oils during frying, while during storage of potato chips an opposite relation was observed.

4). Unsaturation was the main factor affecting the tocopherol amounts during storage of potato chips.

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Appendix

Mass Spectra of Authentic Phytosterol Oxidation Products

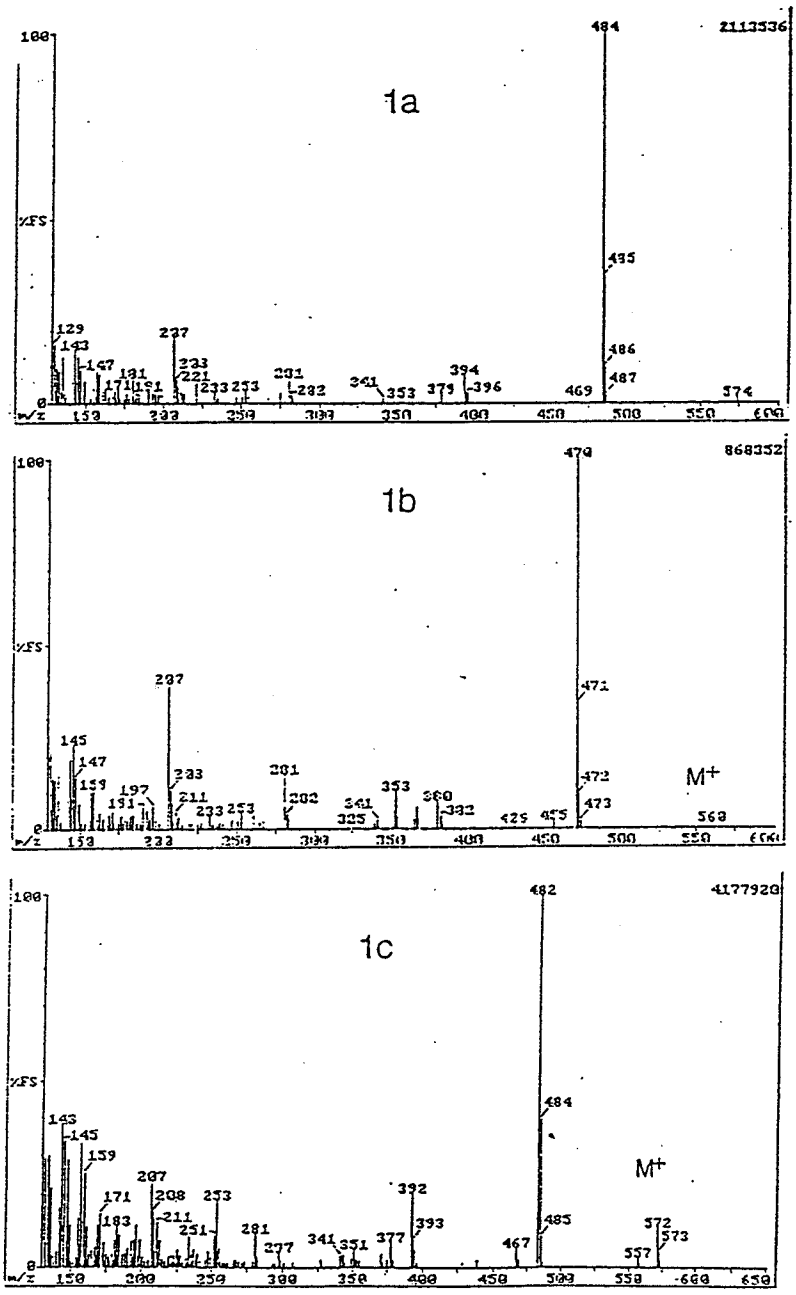
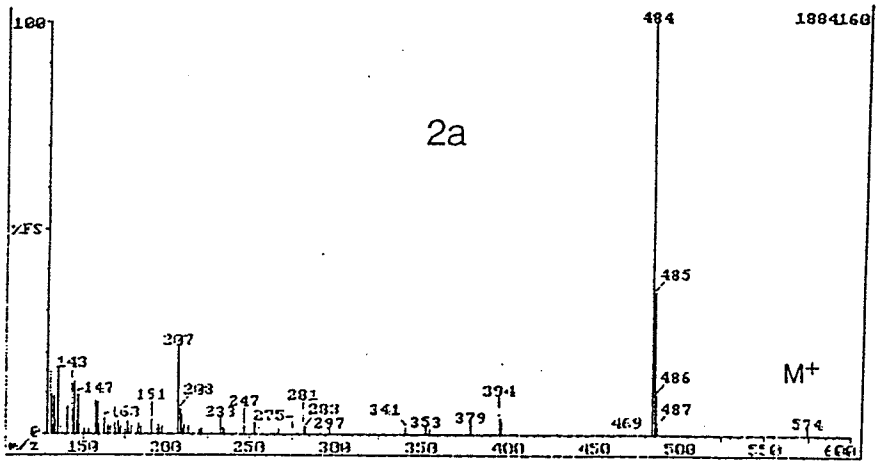


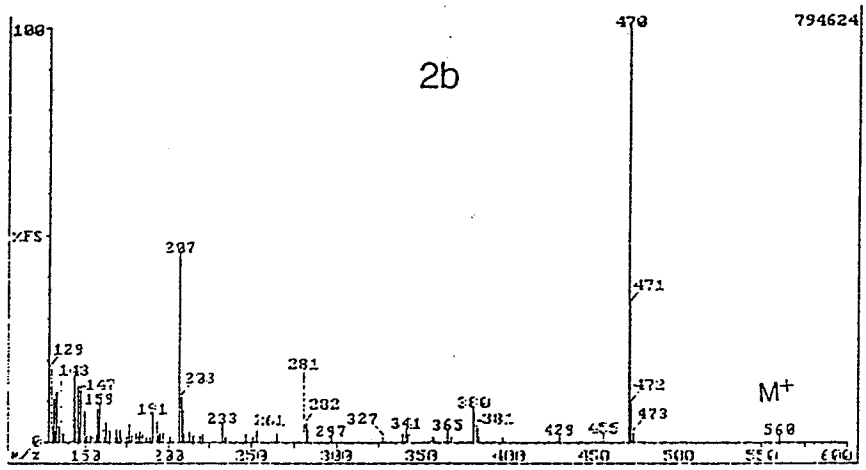
Fig. 1a: Mass spectrum of authentic sample of TMS-ether derivative of 7α-hydroxysitosterol M^+ 574 and a base peak at 484 (M^+- 90)

Fig.1b: Mass spectrum of authentic sample of TMS-ether derivative of 7α-hydroxycampesterol M^+ 560 and a base peak at 470 (M^+- 90)

Fig. 1c: Mass spectrum of authentic sample of TMS-ether derivative of 7α-hydroxystigmasterol M^+ 572 and a base peak at 482 (M^+- 90)

Adapted from Dutta and Appelqvist (1995)





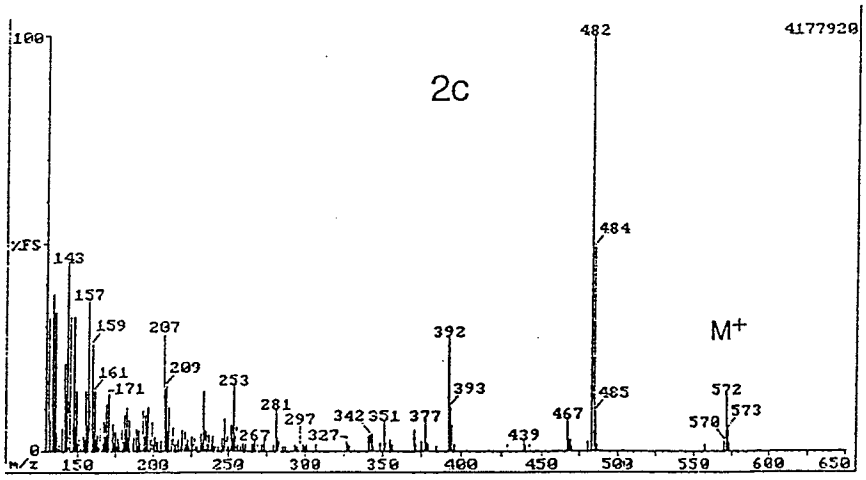


Fig. 2a: Mass spectrum of auyhentic sample of TMS-ether derivative of 7β-hydroxysitosterol M^+ 574 and a base peak at 484 (M^+- 90)

Fig. 2b: Mass spectrum of authentic sample of TMS-ether derivative of 7β-hydroxycampesterol M^+ 560 and a base peak at 470 (M^+- 90)

Fig. 2c: Mass spectrum of authentic sample of TMS-ether derivative of 7β-hydroxystigmasterol M^+ 572 and a base peak at 482 (M^+- 90)

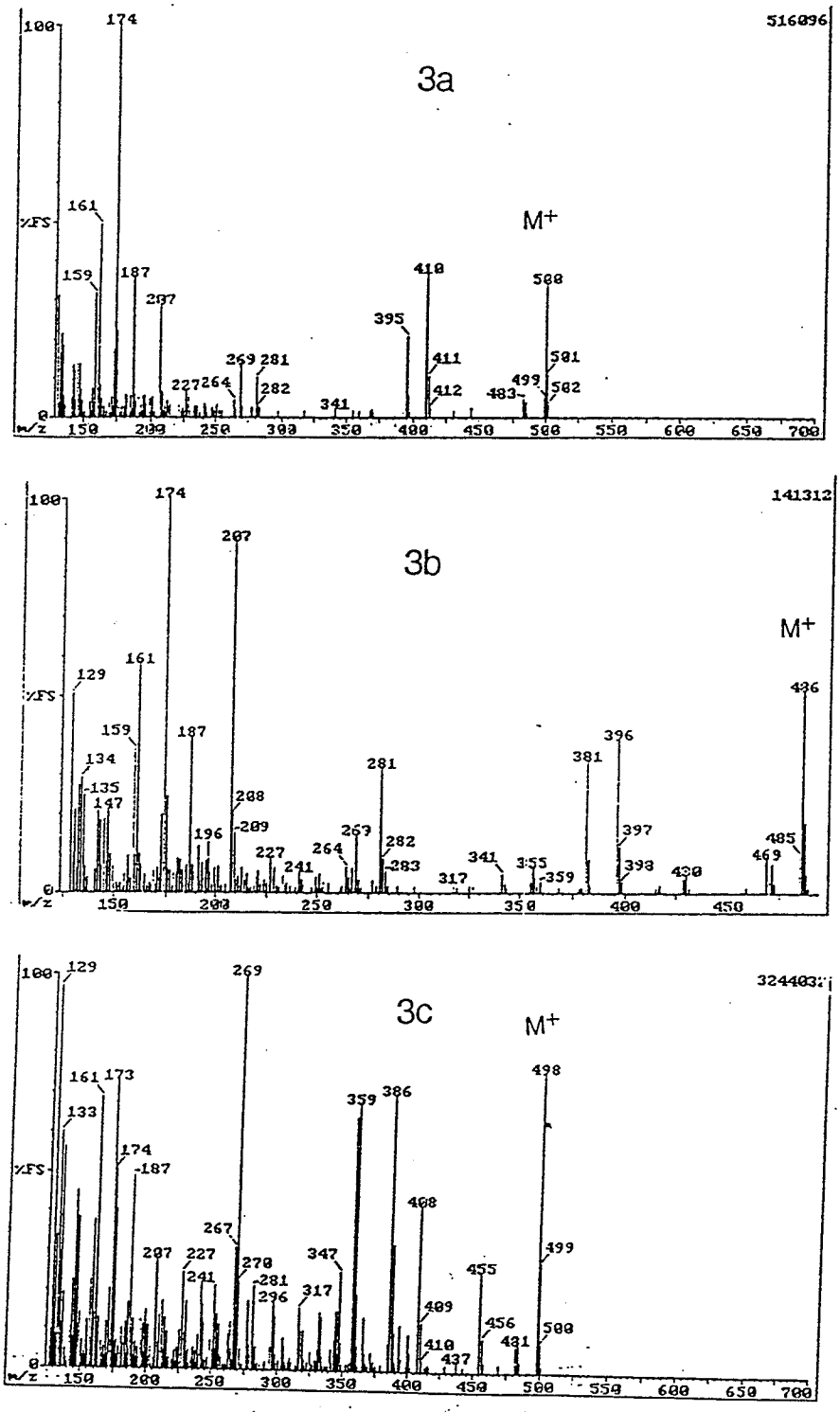


Fig. 3a: Mass spectrum of authentic sample of TMS-ether derivative of 7-ketositosterol M^+ 500

Fig. 3b: Mass spectrum of authentic sample of TMS-ether derivative of 7-ketocampesterol M^+ 486

Fig. 3c: Mass spectrum of authentic sample of TMS-ether derivative of 7-ketostigmasterol M^+ 498

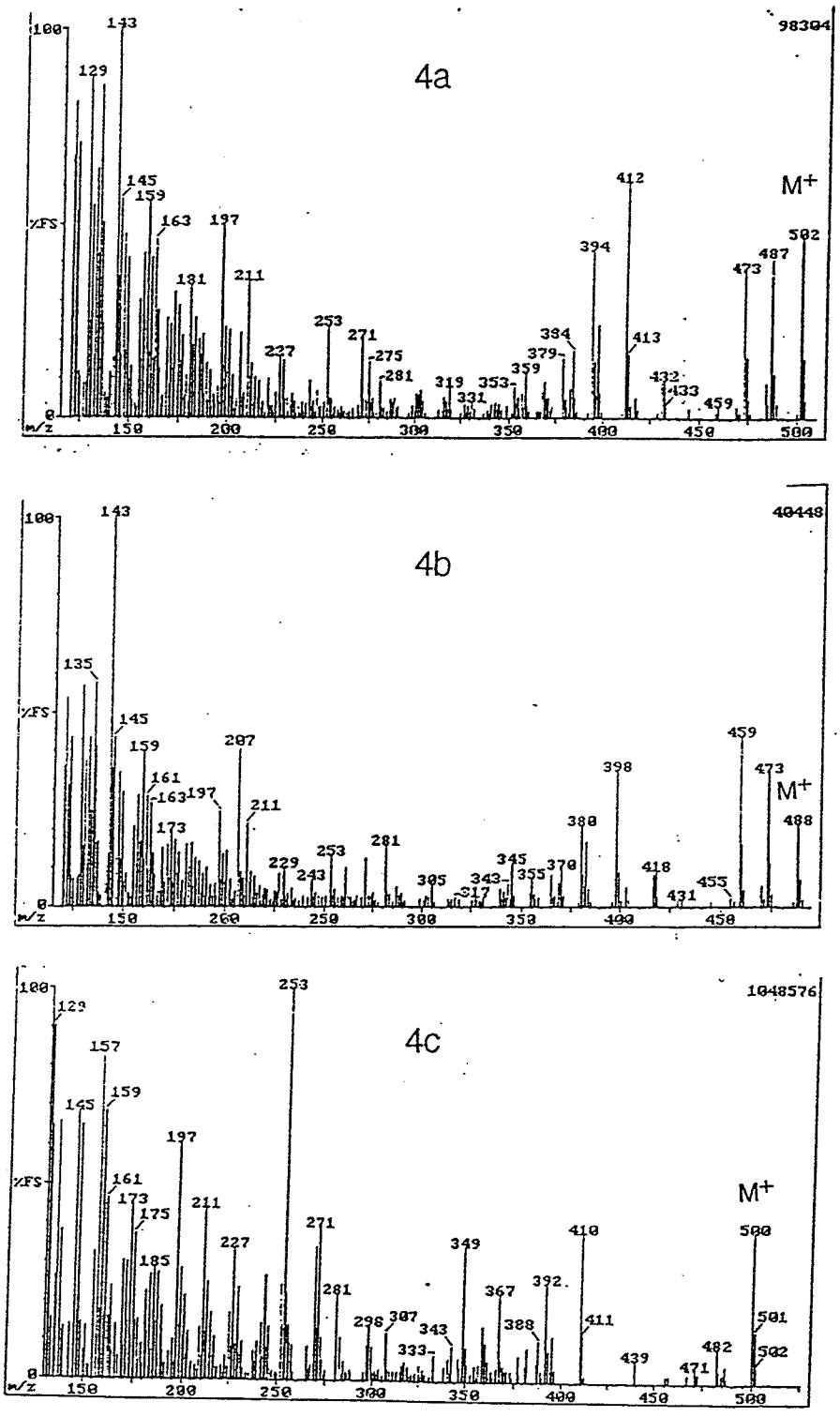
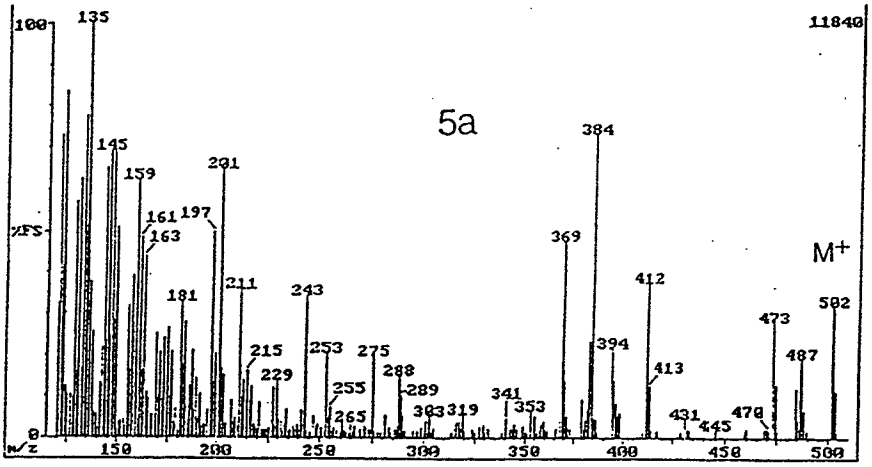
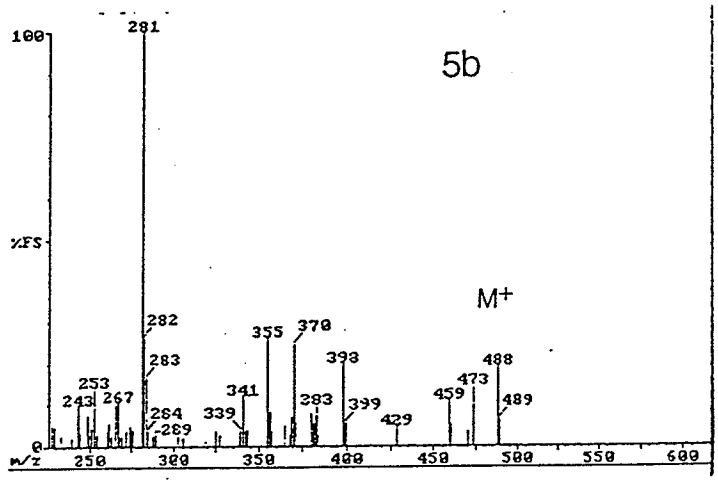


Fig. 4a: Mass spectrum of authentic sample of TMS-ether derivative of 5α, 6α-epoxysitosterol M^+ 502

Fig. 4b: Mass spectrum of authentic sample of TMS-ether derivative of 5α, 6α-epoxycampesterol M^+ 488

Fig. 4c: Mass spectrum of authentic sample of TMS-ether derivative of 5α, 6α-epoxystigmasterol M^+ 500





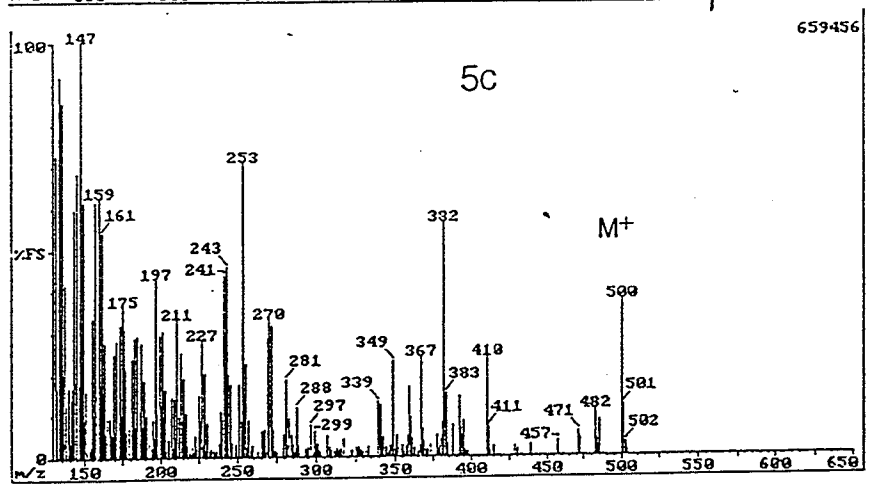


Fig. 5a: Mass spectrum of authentic sample of TMS-ether derivative of 5b, 6b-epoxysitosterol M^+ 502

Fig. 5b: Mass spectrum of authentic sample of TMS-ether derivative of 5β, 6β-epoxycampesterol M^+ 488

Fig.5c: Mass spectrum of authentic sample of TMS-ether derivative of 5β, 6β-epoxystigmasterol showing the molecular ion at 500

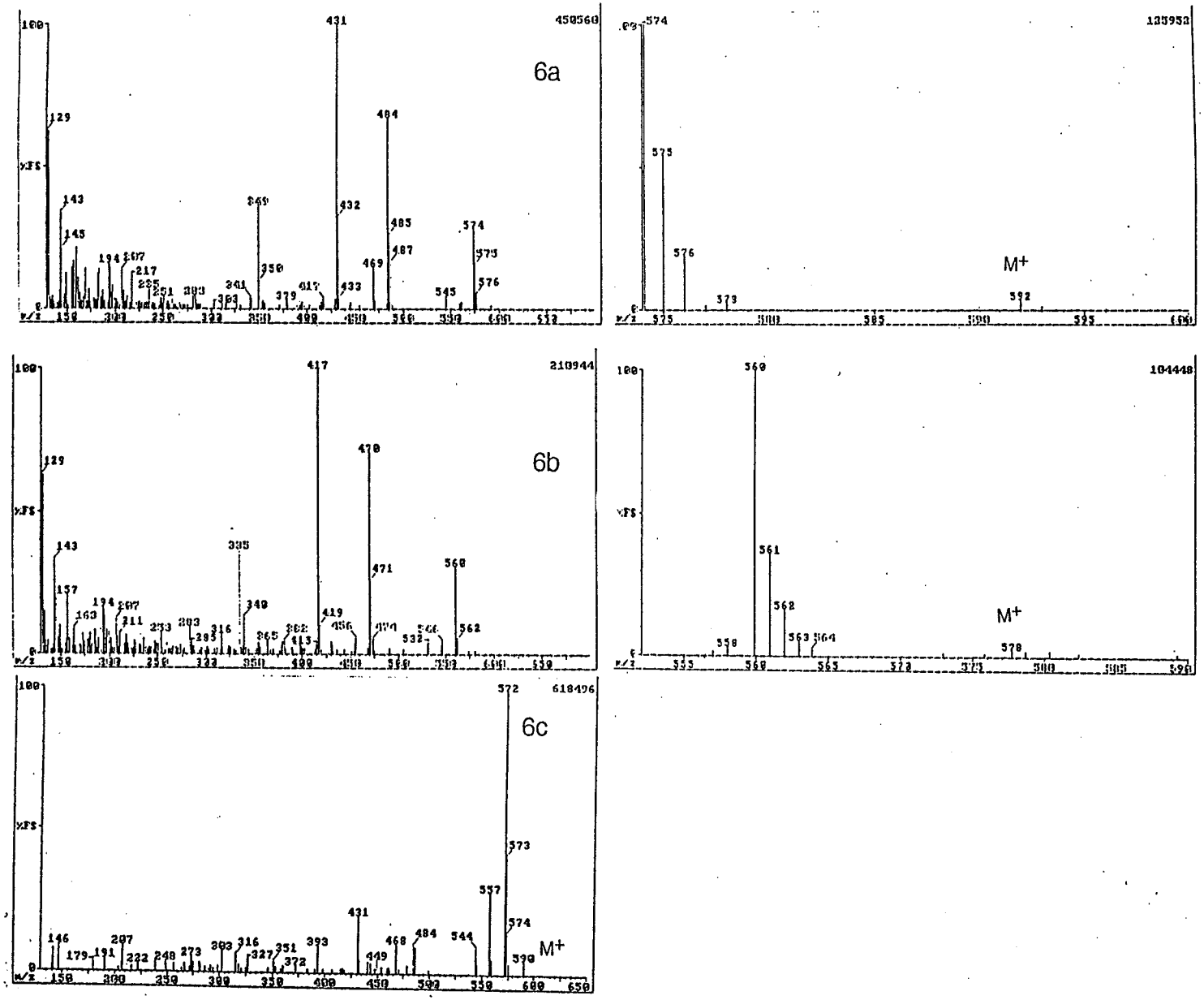


Fig.6a: Mass spectrum of authentic sample of di TMS-ether derivative of trihydroxysitosterol M^+ 592

Fig. 6b: Mass spectrum of authentic sample of di TMS-ether derivative of trihydroxycampesterol M^+ 578

Fig. 6c: Mass spectrum of authentic sample of di TMS-ether derivative of trihydroxystigmasterol M^+ 590