Lipid and Phytosterol Oxidation in Vegetable Oils and Fried Potato Products

Elham Tabee

Faculty of Natural Resources and Agricultural Sciences
Department of Food Science
Uppsala

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Abstract

This thesis provides new data on the levels of lipid and phytosterol oxidation products (POPs) in French fries and potato crisps consumed in Sweden. The levels of POPs in fried foods have attracted interest in recent years because of their potential harmful health effects. The analytical methods used here for the determination of POPs in commercial prefried French fries, French fries collected from fast food restaurants and commercial potato crisps included transesterification, SPE, GC and GC-MS. Higher amounts of total POPs were generally found in French fries prepared in restaurants (1.5-8.1 μg/g sample) than in prefried French fries (0.1-2.1 μg/g sample). In potato crisps, the total POPs content ranged from 0.5 to 6.8 μg/g sample. In general, no correlation could be established between the POPs content and the fatty acid profile, tocopherol, tocotrienol and sterol content in any of the fried potato products tested. However, these parameters provided information on the type of frying oil used in the preparation of the commercial fried potato products.

The possibility of minimising lipid oxidation and POPs formation in some regular and oleic acid-dominated vegetable oils was examined. The oxidative stability was evaluated in these vegetable oils with and without addition of α-tocopherol and the results showed that addition of α-tocopherol (500-20000 μg/g) increased Rancimat stability only in refined olive oil. After heating at 180 °C for up to 12 h, the levels of POPs generally increased over time in high oleic rapeseed oil, palm olein and refined olive oil. The addition of 0.2% α-tocopherol to refined olive oil decreased POPs formation significantly during heating.

Studies on the quality of French fries prepared at 180 °C in refined olive oil and palm olein in five batches at 1-hour intervals showed a higher content of POPs in French fries prepared in refined olive oil. However, all other frying quality parameters tested, such as total polar compounds, free fatty acids and *p*-anisidine value, were significantly lower in the French fries prepared in refined olive oil than in those prepared in palm olein.

Keywords: Deep frying, fatty acids, French fries, oxidative stability, phytosterol, phytosterol oxidation products, tocopherols, total polar compounds, vegetable oils

Author's address: Elham Tabee, Department of Food Science, Swedish University of Agricultural Sciences (SLU), P.O. Box 7051, SE-750 07, Uppsala, Sweden
E-mail: Elham.Tabee@lmv.slu.se

Contents

List of Publications

This thesis is based on the work contained in the following papers, which are referred to in the text by their Roman numerals:

I Elham Tabee, Sodeif Azadmard-Damirchi, Margaretha Jägerstad & Paresh C. Dutta (2008). Lipids and phytosterol oxidation in commercial French fries commonly consumed in Sweden. *Journal of Food Composition and Analysis 21*, 169-177.

II Elham Tabee, Margaretha Jägerstad & Paresh C. Dutta (2008). Lipids and phytosterol oxidation products in commercial potato crisps commonly consumed in Sweden. *European Food Research and Technology* 227, 745-755.

III Elham Tabee, Sodeif Azadmard-Damirchi, Margaretha Jägerstad & Paresh C. Dutta (2008). Effects of α-tocopherol on oxidative stability and phytosterol oxidation during heating in some regular and high-oleic vegetable oils. *Journal of the American Oil Chemists' Society 85*, 857-867.

IV Elham Tabee, Margaretha Jägerstad & Paresh C. Dutta (2008). Refined olive oil is superior to palm olein in preparing French fries (submitted).

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Elham Tabee's contribution to the papers:

I & II. Participated in the planning of experimental work together with the supervisors. Performed the laboratory work, evaluated results and wrote the papers. Took responsibility for submitting the papers and acted as corresponding author.

III & IV. Planned the work together with the supervisors. Performed the laboratory work, evaluated results, performed statistical analyses and wrote the papers. Took responsibility for submitting the papers and acted as corresponding author.

List of Abbreviations

CVD	Cardiovascular Disease
COPs	Cholesterol Oxidation Products
FAME	Fatty Acid Methyl Esters
FFA	Free Fatty Acids
FID	Flame Ionisation Detector
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
HPLC	High Performance Liquid Chromatography
IS	Internal Standard
LDL	Low-density Lipoprotein
OSI	Oil Stability Index
p-AV	*p*-Anisidine Value
PL	Phospholipid
POPs	Phytosterol Oxidation Products
P/S	Polyunsaturated Fatty Acid/ Saturated Fatty Acid Ratio
PS	Phytosterols
PV	Peroxide Value
SPE	Solid Phase Extraction
TAG	Triacylglycerols
TLC	Thin Layer Chromatography
TMS	Trimethylsilyl
TPC	Total Polar Compounds

Introduction

Frying is one of the fastest and oldest methods of cooking food and its use dates back to the ancient Egyptians, in around the sixth century BC. The Romans made extensive use of this cooking method and called it 'boiling in oil' (Banks, 1996).

In deep frying, the food is totally immersed in the hot fat/oil, which acts as a medium for heat transfer. Basically, frying is a dehydration process at high temperature (160-190 °C). The purpose of frying is the formation of a unique crust, colour, flavour and texture. Frying is useful in the cooking of all types of foods, such as meat, fish and vegetables. However, potato is probably the food most subjected to frying, since potatoes are used to produce French fries and potato crisps (Rossell, 2001a). French fries contain between 8 and 15% fat. In contrast, potato crisps contain rather high amounts of fats, up to 35% (Saguy & Dana, 2003).

During frying, oils are subjected to various deterioration processes such as hydrolysis, oxidation and thermal alteration, which generate a large number of compounds including free fatty acids, monoacylglycerols, diacylglycerols, volatile compounds, cyclic compounds, geometric isomers of unsaturated fatty acids, *etc.* Oxidised monomeric, dimeric and oligomeric triacylglycerols formed from the native triacylglycerols may have negative effects on human health (Dobarganes & Marquez-Ruiz, 2003; 2006).

The different vegetable oils used for frying are characterised by different fatty acid profiles (Erickson, 2006). Oils with a high content of saturated fatty acids are more stable in the frying process but because of the negative health attributions associated with these, the interest in using monounsaturated oils in frying has increased (McDonald & Eskin, 2006).

Minor components other than acyl-lipids, such as cholesterol (5-cholesten-3β-ol) and analogous sterols, can also undergo oxidation to numerous products, commonly known as cholesterol oxidation products (COPs). Phytosterols (plant sterols) are structurally similar to cholesterol and can also undergo oxidation, thereby producing a number of oxidation products similar to COPs, designated phytosterol oxidation products (POPs). COPs have received much attention due to their biological effects such as cytotoxicity, atherogenicity, sterol metabolism interference, mutagenicity, carcinogenicity and absorption of these compounds (Dutta *et al.,* 2006). At present, only limited information is available on the biological effects of POPs and their levels in foods and human plasma (Hovenkamp *et al.*, 2008). The relationship between the long-term consumption of lipid oxidation products and human health is not clear, but it is generally recognised that over-used and abused oils undoubtedly contain oxidised material, which if chronically consumed in large amounts could pose a human health risk (Dobarganes & Marquez-Ruiz, 2003).

A challenging aspect to study is the extent to which cooking might turn the phytosterols into possible harmful POPs, in order to minimise the adverse health effects of these products. The present work therefore focused mainly on qualitative and quantitative studies of lipids and POPs present and formed during deep frying of potatoes, *i.e.* French fries and potato crisps. The vegetable oils were studied in terms of lipid stability during heating as a way to limit the formation of lipid oxidation products in general and the occurrence of POPs in particular.

Frying oils

Types of frying media

Many different types of edible fats and oils are available for frying purposes. These include the animal fats (*i.e.* lard and tallow) and vegetable oils such as palm oil, rapeseed oil (low erucic acid), olive oil, soybean oil, cottonseed oil, corn oil, *etc.* (Rossell, 2001b). The choice of frying oil depends on many factors such as availability, price, flavour and stability. Resistance to oxidation during prolonged exposure to high temperature is one of the main properties that industrial frying oil should posses (Kochhar, 2001).

Palm oil is currently the oil most used for frying. It has high saturated fatty acid content and a good resistance to oxidation (Rossell, 2001b). Palm oil is used in many EU countries for frying French fries, potato crisps and other convenience foods (Kochhar, 2000). However, negative nutritional effects of this mostly saturated fat on serum total cholesterol, low density lipoprotein (LDL) and cardiovascular disease (CVD) are a health concern (McDonald & Eskin, 2006).

Vegetable oils can be modified to improve their stability in the frying process. This modification involves processes such as fractionation, hydrogenation and genetic modification of the fatty acid composition of plant seeds (Rossell, 2001b).

Oil fractionation has been used to reduce the saturated fatty acid content of palm oil (Rossell, 2001b). Palm olein has a lower content of saturated fatty acids and a higher content of monounsaturated fatty acid in comparison with palm oil (see Table 1). It is also widely used for manufacturing snack

foods in many EU countries and serves as good frying oil because of its high oxidative stability (Kochhar, 2001; Erickson, 2006).

Hydrogenation of oil is the chemical reaction of oil with hydrogen gas in the presence of catalysts to reduce the degree of unsaturation and thus improve stability against oxidation (Orthoefer & List, 2006). However, hydrogenation of oils or partial hydrogenation of edible oils not only increases the LDL content and the risk of atherogenic lipoprotein, but also decreases the level of HDL due to the production of *trans* fatty acids during the process (McDonald & Eskin, 2006).

Genetic modification of the fatty acid composition of oilseeds usually involves increasing the relative percentage of oleic acid and decreasing that of linoleic and linolenic acid in oilseeds (Warner & Knowlton, 1997). Many studies have examined oil stability in modified vegetable oils such as high oleic soybean, high oleic rapeseed oil and high oleic sunflower oil (Warner & Gupta, 2005; Matthaus, 2006; Normand *et al.*, 2006).

Olive oil can be another choice for frying purposes. Refined olive oil is obtained from virgin olive oils by refining methods that do not lead to any alteration in initial glyceride structure. Detailed definitions of other grades of olive oil have been produced by the International Olive Oil Council (IOOC, 2008). Due to the low level of saturated fatty acid, olive oil is regarded as more favourable from a nutritional point of view. In addition, the high content of monounsaturated fatty acids, low concentrations of linoleic (C18:2) and linolenic (C18:3) acids and content of antioxidants are considered valuable properties of olive oil (Quaglia & Bucarelli, 2001).

Composition of frying oils

Refined vegetable oils consist mainly of 98-99% triacyglycerols but also of plant sterols (phytosterol), tocopherols/tocotrienols and other minor compounds (Erickson, 2006).

Fatty acids

Vegetable oils differ in their composition of fatty acids. Fatty acids are saturated or unsaturated with one double bond (monounsaturated) or more than one double bond (polyunsaturated). The major unsaturated fatty acids are oleic acid (C18:1), linoleic acid (C18:2) and linolenic (C18:3) acid. The levels of five major fatty acids in some vegetable oils are shown in Table 1.

Table 1. Fatty acid composition (%) of some vegetable oils (adapted from Firestone & Reina, 1996)

Fatty acid	Palm oil	Palm olein	Olive oil	Rapeseed oil (Low erucic)	Sunflower oil(high oleic)	Corn oil
C16:0	40-46	38-43	7.5-20	3.3-6.0	2.7-4.2	10-17
C18:0	4-7	3.7-4.8	0.5-5.0	1.0-2.5	3-5	1.6-3.3
C18:1	36-41	40-44	55-83	52-67	80-87	25-42
C18:2	9-12	10-13	3.5-21	16-25	4-9	39-61
C18:3	0.1-0.4	0.1-0.6	0-1.5	6.0-14	-	0.7-1.3

Tocopherols and tocotrienols

Tocopherols and tocotrienols are derivatives of 2-methyl-6-chromanol with a side chain of three terpene units attached at C2. The terpenoid side chain is in saturated form for tocopherol and in unsaturated form for tocotrienols. Both tocopherols and tocotrienols are further divided into individual compounds that are designated by the Greek letter prefixes α, β, γ, δ depending on the number and position of methyl substitution on the chromanol ring (Gregory, 1996). Tocopherols are recognised as the principal natural antioxidants in vegetable oils (Nawar, 1996). Table 2 shows the levels of tocopherols and tocotrienols in some vegetable oils (Kamal-Eldin & Andersson, 1997).

Table 2. *Tocopherol and tocotrienol content (μg/g oil) of some vegetable oils (adapted from Kamal-Eldin & Andersson, 1997)*

Tocopherol/ Tocotrienol	Palm oil	Olive oil	Rapeseed oil	Sunflower oil	Corn oil
α-T	377	96	180	671	222
β-T	1	6	-	23	1
γ-T	4	12	340	4	570
δ-T	-	-	-	-	23
α-T3	52	-	-	-	54
β-T3	4	-	-	-	11
γ-T3	132	-	-	-	62

α-T, β-T, γ-T and δ-T (α, β, γ and δ-tocopherol); α-T3, β-T3 and γ-T3 (α, β and γ tocotrienol), respectively.

Phytosterols

Plant sterols or phytosterols (PS) are present in all plants and in food products of plant origin. Phytosterols regulate the fluidity and permeability of membranes and play an important role in adaptation of membranes to temperature (Piironen *et al.,* 2000). Cholesterol is the main sterol in animal cells but is only present in small amounts in plant cells (Heupel, 1989).

Phytosterols are made up of a tetracyclic cyclopenta (α) phenanthrene ring and flexible side-chain at C17. They are mainly 28-29 carbon atom steroid alcohols. Phytosterols have a similar backbone structure but differ in that they have an additional methyl or ethyl group in the side chain (Fig. 1). The main PS in vegetable oil are sitosterol, campesterol and stigmasterol. They have a hydroxyl group in the β-position at C3 and a double bond between C5-C6 (Δ^5). The hydroxyl group at C3 in free phytosterols can be esterified by a fatty acid to form steryl esters, or linked to a carbohydrate to form steryl glycosides or acylated steryl glycosides (Dutta *et al.*, 2006).

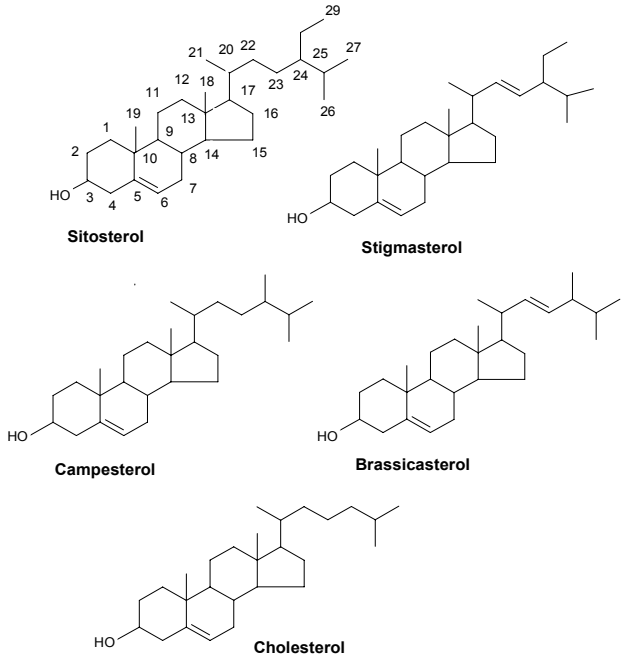

Figure 1. Chemical structure of some plant sterols (sitosterol, campesterol, stigmasterol and brassicasterol) and animal sterol (cholesterol).

In general, vegetable oils are the richest natural sources of phytosterols, followed by cereal grains and nuts. Among the vegetable oils, corn and rapeseed contain the highest amounts of phytosterols (Piironen *et al.,* 2000). The levels of the main phytosterols in some vegetable oils are presented in Table 3.

Table 3. *Phytosterol content (μg/g oil) in some edible refined oils (adapted from Piironen & Lampi, 2004)*

Phytosterol	Palm oil	Olive oil	Rapeseed oil	Sunflower oil	Corn oil
Campesterol	140-180	20-50	1640-3000	270-550	1230-1640
Stigmasterol	70-100	0-30	0-160	180-320	460-590
Sitosterol	350-410	1220-1300	3580-3950	1940-2570	4540-5430
Avenasterols	0-30	160-600	140-560	190-560	100-410
Brassicasterol	-	-	510-920	20	-

Fried potato products

Fresh potato contains very small amount of lipids ranging from 0.8 to 1.3 mg/g of fresh weight. The major fatty acids in potato are palmitic acid (C16:0, 16-19%), linoleic acid (C18:2, 52-60%), and linolenic acid (C18:2, 13-24%) (Galliard, 1973). In addition, small amounts of sterols have been reported in potato (~50 μg/g of fresh weight) (Piironen *et al.*, 2003). Since fresh potato contains very small amount of oils, the fat content of fried potato reflect that of the frying oil.

Potato crisps are thin slices of potato in either flat or wavy form, fried in fat/oil and salted or seasoned to taste (Bennett, 2001). They are extremely popular in the USA and other countries and the demand for potato crisps increases every year. Based on one report, the retail trade in the United States amounts to about $ 6 billion/year (Clark, 2003).

French fries are one of the most popular fried potato products in the world, attracting consumers, especially the younger generation, because of their characteristic flavour and texture. The initial step of pre-frying means that the potato is partially fried in the processing plant. Before consumption, prefried French fries are cooked by deep frying in oil or by heating in the oven/microwave. Thus, the heating process is partly carried out during industrial processing and partly in the kitchen (Keijbets, 2001).

Oil deterioration during frying

Chemical changes

During deep frying, fats and oils are exposed to elevated temperatures and a variety of reactions take place. In the presence of oxygen, food moisture and high temperature, the oil undergoes three main reactions: hydrolysis, oxidation and thermal alteration.

Hydrolysis is one of the major reactions occurring during deep frying due to the presence of moisture introduced with the food and the relatively high temperatures used. Hydrolysis of ester bonds in the lipids results in the formation of free fatty acids (FFA), mono- and di-acylglycerols and glycerols (Perkins, 2006).

Lipid oxidation is one of the important causes of food spoilage. Oxidation, which is accelerated at the high temperature used in deep frying, creates rancid flavours and reduces the organoleptic characteristics of fried food. Hydroperoxides are the major initial reaction products of lipid oxidation. However, they are not stable and decompose spontaneously to form other compounds such as aldehydes, ketones, alcohols, acids, hydrocarbons, *etc.* (Nawar, 1996).

In the high temperatures during the frying process, triacyglycerols undergo decomposition reactions such as isomerisation, cyclisation and polymerisation (Saguy & Dana, 2003; Sebedio & Juaneda, 2006). Dimeric and polymeric glycerides acids also can be formed by the thermal and oxidative combination of free radicals (Dobarganes & Marquez-Ruiz, 2006).

The ratio of polyunsaturated to saturated fatty acids (P/S) is considered to be a major factor affecting oil oxidation. The presence of a high content of polyunsaturated fatty acids increases the susceptibility of oil to oxidation (Quaglia & Bucarelli, 2001). Oil resistance to oxidation in the frying process depends mainly on the fatty acid composition and antioxidant content of the oil (Rossell, 2001b; Sanches-Silva *et al*., 2003; Nogala-Kalucka *et al.,* 2005; Przybylski & Eskin, 2006).

Measuring the quality of frying oil

A number of methods such as peroxide value (PV), *p*-anisidine value (*p*-AV), viscosity, colour, free fatty acids (FFA), sensory analysis, smoke point, foaming and total polar compounds (TPC) have been used to test the quality of frying oils and fried products (Stier, 2001).

Peroxide value (PV)

Peroxide value (PV) is one of the methods for determination of hydroperoxides as the initial lipid oxidation products. The PV is expressed as milliequivalents oxygen per kg of fat/oil (Nawar, 1996). Since hydroperoxides do not accumulate due to their instability at frying temperature, PV determination is not suitable for assessment of used frying oils (Matthaus, 2006; Suleiman *et al.,* 2006).

p-Anisidine value (p-AV)

p-Anisidine value (*p*-AV) is a method for measuring secondary decomposition products such as aldehydes (Mariod *et al.,* 2006). Aldehydes are the carbonyl compounds formed by decomposition of hydroperoxides and can be used as markers to determine degradation of peroxidised materials produced by the heating process (Stier, 2001).

Free fatty acids (FFA)

Free fatty acids (FFA) are a measure of the amount of fatty acids hydrolysed on the triacylglycerol backbone. They are used as a chemical marker for monitoring the quality of frying operations (Stier, 2001). This parameter is often used for assessment of the suitability of frying oils for human consumption and a value of 2% is defined as the limit for oil rejection (Matthaus, 2006).

Total polar compounds (TPC)

Total polar compounds (TPC) content is the most important measure for evaluating the deterioration of oils and fats during deep frying. During frying, as oil breaks down, many polar compounds such as peroxides, acids, monoacylglycerol, diacylglycerol and cyclic compounds, as well as oxidised monomeric, dimeric and oligomeric triacylglycerol are formed (Dobarganes & Marquez-Ruiz, 2006). The maximum permitted TPC level in frying oil in several European countries is 24-27% (Mariod *et al.,* 2006).

Prevention of frying oil deterioration

The oxidation reaction can be inhibited by antioxidants that are naturally present in the oils or added in order to increase the stability. Tocopherols and tocotrienols are natural antioxidants present in vegetable oils. Some synthetic antioxidants are also available for increasing oil stability (Nawar, 1996). Antioxidants delay the oxidation reaction and inhibit the formation of free radicals by donating their phenolic hydrogen atoms to the free radicals (Kochhar, 2000).

Investigations on the addition of tocopherols or tocotrienols to increase oil stability at high temperatures have shown that the antioxidant effect of these compounds depends mainly on the fatty acid composition and the type and content of tocopherols in the oil (Wagner & Elmadfa, 2000; Wagner *et al.,* 2001). The addition of tocopherols at high concentrations can act as a pro-oxidant and decrease the stability of fats or oils (Nogala-Kalucka *et al.,* 2005).

Phytosterol oxidation

By virtue of the double bond at the 5-position, phytosterols are prone to oxidation, which generates phytosterol oxidation products (POPs). Although studies on the oxidation of phytosterols were started in 1940s, some POPs in plants and vegetable oils were not identified until the mid-1960s (Dutta & Savage, 2002). The oxidation of cholesterol has been studied extensively. Due to the similar structure of phytosterol and cholesterol, most of the information on sterol oxidation mechanisms refers to cholesterol oxidation. Oxidation of phytosterols is facilitated by many factors, for instance temperature, light, oxygen, metal ions and free radical initiators (Lercker & Rodriguez-Estrada, 2002; Dutta *et al.,* 2006).

Autoxidation of phytosterols

The most common oxidation mechanism of sterols is autoxidation, which involves abstraction of the reactive allylic hydrogen atom at C7 to produce a free radical or addition of the peroxyl radical at either end of the double bond at C5-C6. The radical molecule formed by hydrogen abstraction reacts with the triplet oxygen ($^3O_2$) to form 7-peroxyl radical. The 7-peroxyl radicals are stabilised in turn by hydrogen abstraction to produce more stable products such as 7-hydroperoxides. The 7-hydroperoxides undergo degradation and form the major oxidation products 7α-hydroxysterols and 7β-hydroxysterols, as well as 7-ketosterols (Fig. 2). Hydration of epoxides in an acidic environment generates triols (Kamal-Eldin & Lampi, 2008).

The oxidation of phytosterols during the heating process has been studied and formation of 7α-hydroxy, 7β-hydroxy, 7-keto, 5α,6α-epoxides, 5β,6β-epoxides and triols from sitosterol, campesterol and stigmasterol has been

reported (Dutta, 1997; Dutta & Appelqvist, 1997; Zhang *et al.,* 2005; Johnsson & Dutta, 2006).

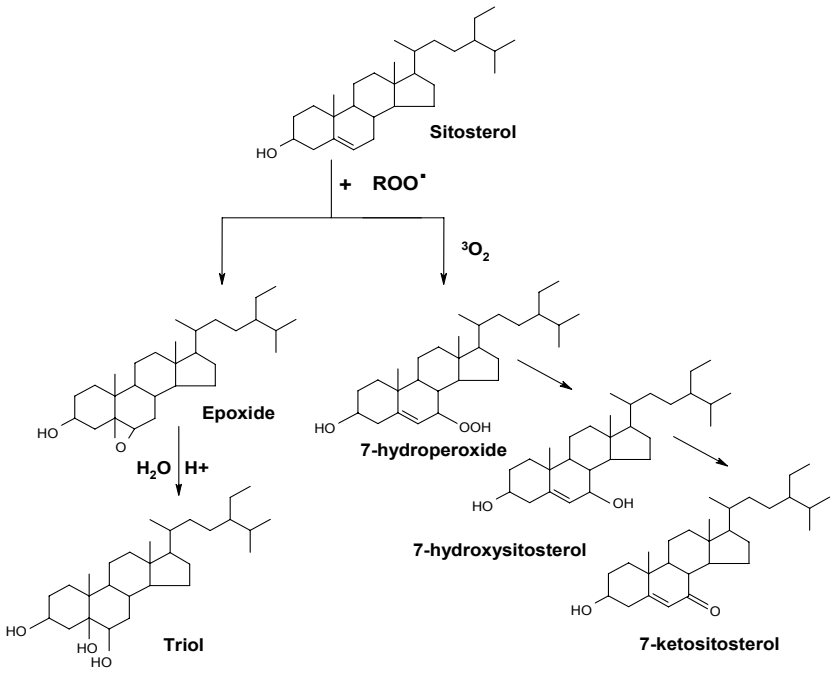

Figure 2. Common pathways of phytosterol oxidation (adapted from Kamal-Eldin & Lampi, 2008).

Occurrence of POPs in foods

Content of POPs in vegetable oils

Studies on the occurrence of POPs in heated vegetable oils are limited. However POPs content appears to be dependent on many factors such as fatty acid composition, sterol content, frying conditions, *etc.* (Dutta *et al.,* 2006). Formation of POPs in rapeseed oil heated at 180 °C for up to 24 h was investigated by Lampi *et al.* (2002). No detectable amounts of POPs were reported prior to heating in this study. However, the amounts of total oxidation products increased steadily during the experiment. After 24 h of heating, the total amount of POPs was more than 1000 μg/g oil.

The concentration of sitosterol oxides before and after heating has been investigated for sunflower and olive oil at 150 °C and 200 °C for 60 min (Zhang *et al.,* 2005). The content of sitosterol oxides in olive oil increased from not detectable to 37 and 365 μg/g oil during 60 min, at 150 °C and 200 °C, respectively. The corresponding figures for sunflower oil were 51 to 241 and 815 μg/g oil at 150 °C and 200 °C, respectively. The levels of various POPs have also been reported in three vegetable oils heated at 180 °C for 0-2 h (Johnsson & Dutta, 2006). The level of total POPs increased in olive and maize oil from 7.7 to 17.6 μg/g oil and 4.3 to 12.2 μg/g oil, respectively. However, during heating of peanut oil, the levels of total POPs remained almost unchanged (7.1 to 6.8 μg/g oil).

Content of POPs in deep fried potato

Few studies have examined the level of POPs in fried potato (Dutta *et al.,* 2006). One study reported the content of a few POPs in potato crisps fried in cotton seed oil and packed in foil during storage (Lee *et al.,* 1985). It was demonstrated that the oxidation products were the result of elevated temperature. Samples stored at 23 °C for 150 days did not produce detectable amounts of POPs. In contrast, potato crisps stored at 40 °C for 95 days contained sitosterol 5β,6β-epoxide, 7α-hydroxysitosterol and 7β-hydroxysitosterol at levels of 6, 13 and 9 μg/g lipids, respectively.

The level of POPs in potato crisps fried in palm oil, sunflower oil and high oleic sunflower oil under industrial conditions and vacuum packed was studied during storage of up to 25 weeks at room temperature by Dutta & Appelqvist (1997). During this period of storage, the levels of total POPs in oil extracted from potato crisps fried in palm oil and high oleic sunflower oil increased from 5.0 to 8.6 and from 35.1 to 58.5 μg/g oil, respectively. However, there was virtually no change during storage in POPs content in potato crisps fried in conventional sunflower oil (45.8 to 47.1 μg/g oil).

Other studies examined the POPs content in French fries fried in blends of palm oil/rapeseed oil, sunflower oil and high oleic sunflower oil at 250°C and 200 °C for 15 min (Dutta & Appelqvist, 1996; Dutta, 1997). A lower content of total POPs was found in French fries prepared in the oils heated at a lower temperature (200 °C) (Table 4).

Table 4. *Level of total POPs (μg/g oil) in oil extracted from French fries prepared in different vegetable oils at 200 °C and 250 °C for 15 min (Dutta & Appelqvist, 1996; Dutta, 1997)*

Frying oil	200 °C	250 °C
Palm oil/Rapeseed oil	32	191
Sunflower oil	37	39
High oleic sunflower oil	54	69

Health implications of POPs

Oxidation products of cholesterol are of considerable interest because of their possible negative effect on human health. Cholesterol oxidation products (COPs) are reported to be mutagenic, cytotoxic and carcinogenic (Osada, 2002). The effects of COPs on atherosclerosis and coronary heart disease have been studied extensively (Garcia-Cruset *et al.,* 2002). In one study by Ryan *et al.* (2005), similar toxic effects of POPs and COPs were found in human cell lines, although higher concentrations of POPs were required for comparable levels of toxicity. Meanwhile, in a recent *in vivo* study, no evidence of genotoxic potential of POPs was found in mice blood after injecting different doses of epoxide and triols (Abramsson-Zetterberg *et al.*, 2007). During the past decade, absorption and subsequent toxicological effects of POPs have been examined in *in vivo* and *in vitro* studies, but the data on biological effects are still rather unclear (Hovenkamp *et al.*, 2008).

Analysis of POPs

The basic principles of POPs analysis are similar to those of COPs analysis. Determination of POPs comprises several steps, *e.g.* extraction of lipids, purification, identification and quantification (Dutta *et al.,* 2006).

Extraction of lipids

Different solvent systems are used for extraction of lipids. The combination of a mixture of chloroform/methanol with water has been used for lipid extraction for many years (Folch *et al.,* 1956; Lee *et al.,* 1985). A two-phase system forms after equal volumes of water and chloroform are added to the system. The extracted lipid in chloroform is collected in the bottom phase, while the upper phase contains the contaminants (Dutta *et al.*, 2006). A mixture of hexane and isopropanol (HIP) is another solvent system used for extraction of lipids (Hara & Radin, 1978). Application of the HIP (3:2, v:v) solvent system is interesting because of its lower toxicity (Dutta *et al.,* 2006). HIP solvent has been used for lipid extraction from French fries, potato

crisps and margarine (Dutta & Appelqvist, 1996; Dutta & Appelqvist, 1997; Johnsson & Dutta, 2006).

Purification and enrichment of POPs

It must be borne in mind that POPs are only minor components in lipid extracts and therefore purification is essential before quantification in order to improve the efficiency of analysis (Guardiola *et al.*, 2004). The initial step in the purification is generally either cold saponification conducted at room temperature or transesterification of the bulk lipids (Dutta *et al.,* 2006).

Cold saponification is an alkaline hydrolysis that has been used in many studies (Dutta & Appelqvist, 1997; Lampi *et al.,* 2002; Soupas *et al.*, 2004; Zhang *et al.,* 2005). Using cold saponification takes a long time, usually overnight or about 18 h. The basic principle of saponification is to hydrolyse ester bonds of triacylglycerols (TAG), phospholipids (PL) and other sterol esters in an alkaline medium, such as KOH in methanol. The non-saponifiable layer contains sterols and their oxidation products (Dutta *et al.*, 2006).

Transesterification is an alternative method to cold saponification. One of the advantages of this method is that is less time-consuming than cold saponification. During transesterification, fatty acids attached to sterols, sterol oxides and TAG are converted to fatty acid methyl esters (FAME) (Schmarr *et al.*, 1996). After transesterification, the lipid primarily consists of FAME, free sterols and its oxidation products, and some other minor components (Guardiola *et al.,* 2004).

Complete removal of un-oxidised phytosterols is important for subsequent GC analysis due to overlap with POPs in GC chromatograms (Dutta, 2002). After saponification or transesterification, POPs are further separated from other components using column chromatography. The solid phase extraction (SPE) method is a simple, fast and effective technique (Dutta *et al.,* 2006). The common oxidation products of sterols (cholesterol and phytosterols) are more polar than their un-oxidised counterparts, and therefore the SPE procedure has to be optimised to elute apolar components up to the polarity of free sterols (Schmarr *et al.,* 1996). Silica (Si) and aminopropyl (NH_2) cartridges are the most commonly used SPE columns (Guardiola *et al.,* 2004). Many different SPE methods have been reported in the literature for separation of POPs from the rest of the lipid matrix. Table 5 lists different SPE methods used to purify POPs.

Table 5. *The SPE methods currently used to enrich POPs prior to determination by GC*

Sample	Stationary phase	Solvent system	Reference
Interesterified olive oil and palm stearin	Silica (1g)	Hexane:diethyl ether (9:1) Hexane:diethyl ether (1:1) Acetone[a]	Azadmard-Damirchi & Dutta, 2008
Olive oil, peanut oil and maize oil	NH_2 (500mg)[b]	Hexane Hexane:MTBE(2:1) Acetone[a]	Johnsson & Dutta, 2006
Sunflower oil, olive oil and butter	Silica (500mg)	Cyclohexane:diethyl ether (9:1) Cyclohexane:diethyl ether (1:1) Acetone[a]	Zhang *et al.,* 2005
Crude vegetable oils	Silica (1g)	Hexane:diethyl ether (1:1) Diethylether:methnol (1:1)[a]	Bortolomeazzi *et al.,* 2003
Rapeseed oil	Silica (500mg)	Hexane:diethyl ether (9:1) Hexane:diethyl ether (1:1) Acetone[a]	Lampi *et al.,* 2002
Potato crisps prepared in vegetable oils	Silica (500mg)[b]	Hexane:diethyl ether (75:25) Hexane:diethyl ether (60:40) Acetone[a]	Dutta & Appelqvist, 1997

[a]elution solvent, containing POPs

[b]two-fold SPE procedure carried out

Determination of POPs

Determination of POPs is performed mostly by capillary GC after derivatisation to trimethylsilyl (TMS) ethers. Oxides are detected with a flame ionisation detector (FID) because of its high specificity and the ability to detect components at low levels (Dutta *et al.,* 2006). For the separation of POPs in GC, different capillary GC columns (nonpolar, mid-polar or a combination of both) have been used (Lampi *et al.,* 2002; Soupas *et al.,* 2004; Johnsson & Dutta, 2005).

HPLC and HPLC-MS have been used for the analysis of POPs in a few studies (Säynäjoki *et al.,* 2003; Kemmo *et al.,* 2008). However, this technique is not sufficiently effective to separate large numbers of POPs (Dutta *et al.*, 2006).

Capillary GC with MS as a detector has provided a powerful separation and identification technique for POPs. Identification in chromatographic methods is generally performed by comparison of retention data for the compounds analysed with data for authentic standards if available. It is common to include mass spectrometry (MS) (electron impact) in identification. Molecular fragmentation data and mass spectra of trimethylsilyl (TMS) ether derivatives from phytosterols and some phytosterol oxides have been reported (Aringer & Nordstrom, 1981; Dutta, 2002; Johnsson & Dutta, 2003; Johnsson *et al.,* 2003; Lambelet *et al.,* 2003).

Quantification of POPs is performed using an internal standard (IS) such as 5α-cholestane and cholesterol oxidation products. Since 5α-cholestane is less polar than POPs, it must be added after the purification steps (Guardiola *et al.,* 2004).

Objectives

The overall aim of this thesis was to collect data on the levels of phytosterol oxidation products (POPs) in commercial deep fried potato products commonly consumed in Sweden and on the current status of oils used for industrial frying. We also hypothesised that it might be possible to select frying oils containing less saturated fatty acids with improved oxidative stability in order to reduce POPs in deep fried potato products.

The main objectives of the present work were to:

- Study some lipid parameters and the content of POPs in prefried French fries, French fries prepared in fast food restaurants and commercial potato crisps consumed in Sweden **(Papers I & II).**

- Compare and rank five different vegetable oils by characterising their lipid profile (P/S ratio) and content of tocopherols in relation to their oxidative stability during heating, with and without added α-tocopherol (**Paper III).**

- Study some oxidation parameters such as peroxide value (PV), *p*-anisidine value (*p*-AV) and POPs in monounsaturated-dominated vegetable oils during laboratory heating at 180 °C for 0-12 h **(Paper III).**

- Assess some frying oil quality parameters (total polar compounds, free fatty acids, *p*-anisidine value and POPs) in oil extracted from French fries prepared in two monounsaturated vegetable oils selected on the basis of the results from Paper III (**Paper IV**).

Materials and methods

This section summarises the materials and methods used in the four studies of the thesis. More detailed descriptions are given in **Papers I-IV**.

Samples

Five packets of prefried frozen French fries with different fat contents (2.5-8% fat labelled value), produced by four different companies, were collected from local food outlets in Uppsala, Sweden. All samples were stored at -20 °C until analysis. Prior to extraction, they were heated at 225-250 °C for 20-30 min (according to the instructions on the packaging) in a laboratory oven. Four portions of French fries were collected from different fast food restaurants in Uppsala and Stockholm, Sweden. The samples obtained from each restaurant were stored at -20 °C until analysis **(Paper I)**.

Eleven packets of different high fat (HF) potato crisps (27-38% fat) and five packets of different low fat (LF) potato crisps (15-25% fat) were collected randomly from local food retailers in Uppsala. All the samples were stored at -20 °C before analysis **(Paper II)**.

Vegetable oils (high oleic rapeseed oil, palm olein, refined (deodorised) olive oil, low erucic acid rapeseed oil and sunflower oil) were obtained from AarhusKarlshamns Sweden AB **(Papers III & IV).**

Raw potatoes (cv. Melody, Skänninge, Sweden) were purchased from a local supermarket (ICA) in Uppsala, Sweden, and stored at 5 °C until frying operations **(Paper IV)**.

Methods

Rancimat testing for oil stability index (OSI)

The oxidative stability of oil samples was determined by Rancimat 679 (Metrohm KEBO Lab AB, Stockholm, Sweden) **(Paper III)**. In brief, 2.5 g of the vegetable oils were weighed into the reaction vessel and heated to 110 °C with an air flow of 10 L/h. Volatile products released during the oxidation process were collected in a flask containing distilled water. The oxidation process was recorded automatically by measuring the change in conductivity of the distilled water due to the formation of volatile compounds and the oil stability index (OSI) is expressed in hours (h). Various levels of α-tocopherol (500-20000 μg/g oil) were added to the oil containing indigenous α-tocopherol in darkness and their OSI value was measured.

Thermal experiment

Vegetable oils were heated at 180 ± 5 °C in a glycerin bath for a total time of 12 h **(Paper III)**. The oils were heated at 180 ± 5 °C for 6 h on day 1 and oil samples were collected at 3 and 6 h, cooled and stored at -20 °C for further analysis. The remaining oils were covered, allowed to cool and left overnight for further heating. On the following day, the remaining oils were heated again at the same temperature and the samples were collected after 9 and 12 hours of total heating.

Deep frying of French fries

Deep frying was performed in a deep fryer in our laboratory **(Paper IV)**. Oil (2.1 L) was introduced into the fryer and heated to 180 ± 5 °C for 10 min. The oil was held at this temperature for 1 h. The raw potatoes were peeled and sliced, rinsed several times in cold water and dried before frying. About 320 g of potato slices were introduced into hot oil and fried for 6 min. A 1:6 (w:w) ratio of food to oil was used, as recommended by Normand *et al.* (2006). Five consecutive batches were fried and prior to each batch, the oil was kept at 180 ± 5 °C for 1 h (Matthaus, 2006). This resulted in oil heating times of approx. 1-5 hours. French fries were stored in aluminium foil at -20 °C for subsequent analyses.

Lipid extraction and characterisation

The extraction of lipids from potato products was performed according to Dutta (1997) (**Papers I, II & IV).** The oil samples were extracted from fried potato for determination of total polar compounds according to Masson *et al.* (2002) (**Paper IV**). Analysis of the fatty acid composition as fatty acid methyl esters (FAME) and total sterols was carried out according to Savage *et al.* (1997) (**Papers I-IV**). Analysis of tocopherols by HPLC was performed according to the method described by Savage *et al.* (1999) (**Papers I, II & III**).

Peroxide value, p-anisidine value and free fatty acids

The method used for evaluating the peroxide value (PV) in this study refers to IDF Standard 74A (International Dairy Federation, 1991) **(Paper III).** The *p*-anisidine value *(p*-AV) was determined following the standard method (Paqout, 1987) **(Papers III & IV)**. The free fatty acids (FFA) content was measured using the AOCS official method (AOCS, 1994) **(Paper IV)**.

Total polar compounds

Total polar compounds (TPC) content was determined gravimetrically according to a mini column method described previously with slight modification (Dobarganes *et al.,* 2000) **(Paper IV)**. In brief, about 0.3 g of extracted oil was weighed into a 10 mL volumetric flask and dissolved in a mixture of petroleum ether and diethyl ether (90:10; v:v). Five mL of this solution were put into a silica gel column (5 g silica, 20 mL) previously conditioned with a 25 mL mixture of petroleum ether and diethyl ether (90:10; v:v). The non-polar compounds were eluted with 90 mL of a mixture of petroleum ether and diethyl ether (90:10; v:v). The polar compounds were eluted with 80 mL diethyl ether into a weighed flask. The solvent was removed by rotary evaporation and afterwards the flask was flushed under a stream of nitrogen for complete dryness.

Determination of POPs

The transesterification procedure used in this study was slightly modified from a method previously described by Schmarr *et al.* (1996) (**Papers I-IV**).

In **Papers I & II**, the SPE enrichment was performed according to Schmarr *et al.* (1996) with slight modification. In brief, the transesterified sample was dissolved in 250 μL chloroform and applied onto a cartridge

(NH_2, 0.5 g, 6 mL) previously conditioned with 5 mL hexane. The sample tube was washed with 5 mL hexane and transferred onto the cartridge. The column was washed with 5 mL hexane, 15 mL hexane:*tert*-butyl methyl ether (5:1, v:v) and 20 mL hexane:*tert*-butyl methyl ether (3:1, v:v) to separate the apolar components and un-oxidised phytosterols. The POPs were eluted with 3 mL acetone. The internal standard 5α-cholestane was added and the solvent was evaporated to dryness using nitrogen.

In **Papers III & IV,** the separation of POPs was carried out according to Azadmard-Damirchi & Dutta (2008). The transesterified sample was dissolved in 1 mL of hexane:diethyl ether (9:1, v:v) and applied onto an SPE cartridge (silica, 1 g, 6 mL) previously conditioned with 5 mL hexane. The non-POPs compounds were removed with 15 and 10 mL of hexane/diethyl ether (9:1, v:v) and (1:1, v:v), respectively. The POPs were eluted with 10 mL acetone. The internal standard 5α-cholestane was added and the solvent was evaporated to dryness by using nitrogen **(Papers III & IV).** Prior to GC and GC-MS analysis, the mixture was derivatised to trimethylsilyl (TMS) ethers and analysed by GC and GC-MS **(Papers I-IV).**

In **Papers I & II**, a combination of two fused silica capillary columns, DB-5MS and DB-17MS (J&W Scientific, Folsom, CA, USA) with the same dimensions (length 10 m, i.d. 0.18 mm and film thickness 0.18 μm) connected to the GC Varian Star 3400 CX instrument (Varian, Palo Alto, CA, USA) coupled with FID, was used. In **Papers III & IV**, TMS-ether derivatives of POPs were analysed using another combination of DB-5MS (length 15 m, i.d. 0.18 mm and film thickness 0.18 μm) and a DB-35MS (length 10 m, i.d. 0.2 mm, and film thickness 0.33 μm) were connected to GC model 6890 N (Agilent Technologies, Wilmington, DE, USA). In all GC analyses, helium was used as the carrier gas and nitrogen as the make-up gas.

To identify the POPs in **Papers I & II**, GC-MS analyses were performed on a GC 8000 Top Series gas chromatograph (CE Instruments, Milan, Italy) coupled to a Voyager mass spectrometer with MassLab data system version 1.4 V (Finnigan, Manchester, UK). In **Papers III & IV** the same gas chromatograph was coupled to a Voyager mass spectrometer operated with Xcalibur v.1.2 (ThermoQuest, Manchester, UK). The full scan mass spectra were recorded at electron energy of 70 eV and ion source temperature of 200 °C. More details regarding the GC and GC-MS analyses are presented in **Papers I-IV**. An overall summary of the samples and analyses is presented in Table 6.

Table 6. *Summary of tasks conducted in* ***Papers I-IV***

Paper I **Sample: French fries**	**Paper II** **Sample: Potato crisps**	**Paper III** **Sample: Vegetable oils**	**Paper IV** **Sample: French fries**
1) Extraction of lipids	1) Extraction of lipids	1) Measurement of oil stability	1) Preparation of French fries
2) Analysis of FAME	2) Analysis of FAME	2) Analysis of FAME	2) Extraction of lipids
3) Analysis of tocopherols and tocotrienols	3) Analysis of tocopherols and tocotrienols	3) Analysis of tocopherols and tocotrienols	3) Analysis of FAME
4) Analysis of sterols	4) Analysis of sterols	4) Analysis of sterols	4) Analysis of sterols
5) Optimisation of SPE method	5) Analysis of POPs	5) Enrichment with α-tocopherol and measurement of oil stability	5) Analysis of total polar compounds
6) Recovery test for POPs analysis		6) Thermal experiment	6) Analysis of free fatty acids
7) Analysis of POPs		7) Analysis of PV	7) Analysis of *p*-AV
		8) Analysis of *p*-AV	8) Analysis of POPs
		9) Analysis of POPs	

Results and discussion

Methodological considerations for POPs in fried potato products (Papers I & II)

The POPs content in the commercial fried potato samples was determined by transesterification, enrichment with SPE and quantification by GC. Identification of the individual POPs was performed by GC-MS following recent methods for POPs analysis developed in our laboratory (Johnsson & Dutta, 2006; Azadmard-Damirchi & Dutta, 2008). However, further optimisation of purification and enrichment steps was performed prior to analysis by GC and GC-MS. The separation of POPs from un-oxidised sterols is the most critical step in POPs analysis. The first step in this procedure is transesterification. Using the methods described by Schmarr *et al.* (1996) and Johnsson & Dutta (2006), further optimisation was performed and the completeness of the reaction was visually checked by TLC. The optimisation resulted in certain modification, *e.g.* slightly increased volumes of some of the reagents. We also investigated whether a single SPE step was enough for the purification of POPs in an SPE cartridge (NH_2, 0.5 g, 6 mL) and how the SPE step could be optimised. Various proportions of hexane:tert-butyl methyl ether were checked and the results were evaluated visually by TLC. It was found that the most efficient combinations for elution of FAME and other unwanted compounds were hexane:tert-butyl methyl ether 15 mL (5:1, v:v) and 20 mL (3:1, v:v), respectively. The elution of POPs was investigated with different amounts of acetone and it was found that 3 mL was suitable in order to quantitatively elute the POPs using a flow rate of approximately 0.5-0.8 mL/min. TLC analysis of the acetone fraction confirmed that the SPE method used was efficient in separating non-desirable compounds from POPs (Fig. 3).

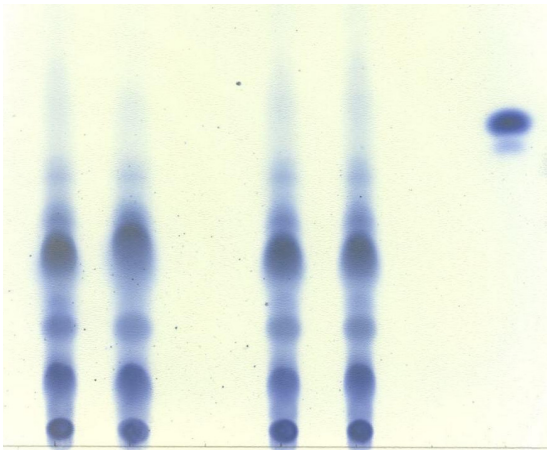

Figure 3. Purity of POPs separated by the SPE method on analytical TLC. Developing solvent: diethyl ether:cyclohexane (90:10; v:v). S1, S2, S3 and S4 are the oil samples extracted from different French fries.

In order to evaluate the efficiency of the method and to check the possible loss of POPs during enrichment, a recovery test was performed by spiking a lipid extract from one of our samples with standard COPs samples. Since standard samples of POPs are not commercially available and since cholesterol is structurally similar to phytosterols, we used a few standard samples of COPs to check recovery, in accordance with previously published work by Lampi *et al.* (2002). The recovery for 7α–hydroxycholesterol, 7β–hydroxycholesterol and 7-ketocholesterol was 93%, 89% and 96%, respectively. The coefficient of variation (CV%; n=5) for each of the three standard COPs was less than 14%, which is similar to other studies (Ubhayasekera *et al.,* 2004; Johnsson & Dutta, 2006).

Levels of POPs in French fries and potato crisps (Papers I & II)

Lipid extracts from commercial prefried French fries oven-heated according to the instructions given on the packaging (n=5), French fries collected from different restaurants (n=4), and different commercial varieties of potato crisps (n=16) obtained from the Swedish food market were analysed for individual and total POPs.

The POPs detected for French fries samples were 7α-hydroxycampesterol, 7β-hydroxycampesterol, 7α-hydroxystigmasterol, 7β-hydroxystigmasterol, 7α-hydroxysitosterol, 7β-hydroxysitosterol, sitosterol 5α,6α-epoxide, campestanetriol, stigmastentriol, sitostaneteriol, 7-ketocampesterol, 7-ketostigmasterol and 7-ketositosterol. The total POPs content in prefried samples ranged from 0.1 to 2.1 μg/g. The corresponding figures for restaurant French fries ranged from 1.5 to 8.1 μg/g (Table 7). Thus, the amounts of POPs in prefried commercial French fries were generally lower than in samples collected from fast food restaurants, in spite of the two cooking steps for prefried French fries. Taken together, these figures for POPs content are lower than those reported earlier for French fries prepared in different vegetable oils (Dutta & Appelqvist, 1996; Dutta, 1997). Whether this difference in POPs content compared with the present study can be explained by improved analytical methodology or differences in the cooking conditions and frying oils selected needs to be further studied.

The individual POPs detected for potato crisps were generally similar to those in French fries. The total content of POPs in commercial potato crisps ranged from 0.5 to 6.8 μg/g (Table 8). The content of POPs in this study was in line with figures reported previously for potato crisps fried in palm oil (Dutta & Appelqvist, 1997).

Lipid parameters in French fries and potato crisps (**Papers I & II**)

Total fat and fatty acid composition varied substantially between the potato samples and might have had an impact on POPs formation. For instance, total fat in prefried French fries was less than half that in French fries collected from restaurants, 2.5-8% compared with 14-18%. The most abundant fatty acids in the lipid extracted from prefried French fries were palmitic acid (C16:0, 7-42%), oleic acid (C18:1, 23-41%) and linoleic acid (C18:2, 10-61%). The fatty acid profiles indicated that the vegetable oils used in the prefried samples differed (Firestone & Reina, 1996). The prefried French fries samples S2, S3 and S5 contained tocotrienols and high levels of saturated fatty acids, which indicated the presence of palm oil. Prefried samples S1 and S4 were comparable in fatty acid composition and tocopherol and tocotrienol content, and it seems that sunflower oil might have been used for the preparation of these samples. In contrast, the fatty acid profiles of French fries collected from restaurants were not comparable

with any known edible vegetable oils and fats, and therefore these samples were most probably prepared in a mixture of fats and oils. Total lipid content in the potato crisps varied from 15 to 38%. The main fatty acids were palmitic acid (C16:0, ~23-43%), oleic acid (C18:1, ~38-60%) and linoleic acid (C18:2, ~10-23 %). Based on the fatty acid profiles obtained in our laboratory and other literature (Firestone & Reina, 1996), we suggested that the vegetable oil used for the most of the crisp samples (thirteen of sixteen) could be palm oil. The three remaining samples showed other fatty acid profiles and we concluded that blends of oils might have been used in preparation of these samples.

Among the prefried French fries, only α-tocopherol could be quantified. The lack of γ-tocopherol in these samples may be because palm oil and sunflower oil contain low levels of this tocopherol (Kamal-Eldin & Andersson, 1997). In the prefried French fries, the content of total tocotrienols (α-tocotrienol, γ-tocotrienol and δ-tocotrienol) ranged from not detectable to 5 μg/g (Table 7). In French fries samples collected from restaurants, the total content of tocopherols (α-tocopherol, γ- tocopherol) ranged from 1 to 63 μg/g sample and the total tocotrienols (α-tocotrienol, γ-tocotrienol and δ-tocotrienol) ranged from not detectable to 12 μg/g (Table 7). For the potato crisps, α-tocopherol was generally the dominant tocopherol and the total content of tocopherols (α-tocopherol and γ-tocopherol) ranged from not detectable to 102 μg/g sample. In addition, the content of total tocotrienols (α-tocotrienol, γ-tocotrienol and δ-tocotrienol) ranged from 5 to 103 μg/g sample (Table 8).

The content of total sterols in the prefried French fries ranged from 58 to 195 μg/g sample. The corresponding range for the French fries collected from different restaurants was 262-1118 μg/g. For the potato crisps, the content of total sterol ranged from 102 to 931 μg/g sample. The dominant sterol was sitosterol, followed by campesterol, stigmasterol, Δ^5-avenasterol and cholesterol. An overall summary of results from **Papers I & II** is presented in Tables 7 and 8.

.

Table 7. *Overview of the lipid parameters and cooking conditions in prefried French fries and in French fries collected from different restaurants* ***(Paper I)***

Sample	Lipids (g/100g)	Saturated fatty acids (g/100 g)	Monounsaturated fatty acids (g/100 g)	Polyunsaturated fatty acids (g/100 g)	Total tocopherols (µg/g)	Total tocotrienols (µg/g)	Total sterols (µg/g)	Total POPs (µg/g)	Cooking conditions
Prefried samples									
S1	8.0[a]	0.9	2.0	4.9	18	-	195	1.2	250 °C/20 min
S2	7.5[a]	3.0	2.8	1.6	5	5	58	2.1	225 °C/20 min
S3	2.5[a]	1.2	1.0	0.3	2	3	14	0.1	225 °C/30 min
S4	2.5[a]	0.3	0.6	1.5	9	-	61	0.3	225 °C/20 min
S5	3.5[a]	1.7	1.4	0.4	1	2	17	0.2	225 °C/30 min
Restaurant samples									
R1	13.6[b]	4.3	6.4	1.0	1	4	422	8.1	Unknown
R2	17.8[b]	3.2	8.6	0.9	63	-	1118	2.1	Unknown
R3	15.1[b]	2.0	8.7	2.7	38	3	568	1.5	Unknown
R4	14.1[b]	3.3	8.0	2.4	29	12	262	1.7	Unknown

S1, S2, S3, S4 and S5 are prefried commercial French fries; R1, R2, R3 and R4 are French fries samples collected from four different local restaurants; [a]label value; [b]analysed value.

Multiple parameters such as lipid content, fatty acid profile, type and content of tocopherols and content of sterols might have affected the formation of POPs in these samples, as previously suggested by other studies (Dutta & Appelqvist, 1996; Rudzinska *et al.,* 2004; Soupas *et al.,* 2007). In general, no correlation ($P<0.05$) could be established between content of POPs and fatty acid composition, total tocopherol, total tocotrienol, and total sterol content among the fried potato products, either in μg/g lipid or in mg/100g.

The wide range of parameters affecting POPs formation in these screening studies encouraged us to evaluate some vegetable frying oils with better health properties in terms of the possibility to minimise lipid oxidation, with subsequent reduction of POPs, during heating at high temperature.

Table 8. *Overview of lipid parameters in potato crisps* ***(Paper II)***

Sample	Lipids (g/100g)	Saturated fatty acids (g/100g)	Monounsaturated fatty acids (g/100g)	Polyunsaturated fatty acids (g/100g)	Total tocopherols (µg/g)	Total tocotrienols (µg/g)	Total sterols (µg/g)	Total POPs (µg/g)
ICA	33	15.6	13.4	3.4	24	51	255	1.7
Fragancia	32	14.0	13.8	3.6	30	68	163	6.8
WILLYS	35	16.2	14.5	3.6	35	103	234	1.2
Lant	27	11.4	11.0	4.1	7	15	238	1.8
STAR	35	16.7	14.1	3.5	28	60	190	0.5
Estrella 1	32	14.3	13.7	3.4	-	10	238	2.6
Estrella 2	20	8.7	8.7	2.2	12	29	139	0.6
Estrella 3	20	8.8	8.7	2.1	8	25	166	1.9
Estrella 4	20	8.7	8.9	2.1	17	36	174	0.8
First Price	34	16.4	13.5	3.5	13	53	307	1.5
OLW 1	34	15.3	14.6	3.7	19	51	240	1.5
OLW 2	15	6.6	6.7	1.5	-	5	102	1.6
ELDORADO	35	17.3	13.6	3.4	36	95	209	1.1
Pringles 1	38	13.0	14.4	8.7	102	84	931	0.9
Pringles 2	25	9.0	9.8	5.7	44	37	419	1.2
Lay´s	35	9.9	20.9	3.4	91	57	514	1.5

Evaluation of vegetable oil stability during heating (**Paper III**)

A particular interest in this study was to identify vegetable oils with a combination of monounsaturated fatty acids and high oxidative stability. We also evaluated the effect of varying α-tocopherol concentration in order to increase the oxidative stability. In addition, some oxidation criteria such as peroxide value (PV), *p*-anisidine value (P-AV) and formation of POPs were determined in selected monounsaturated vegetable oil during heating.

Chemical characterisation

Lipid composition of five regular and high oleic vegetable oils (high oleic rapeseed oil, low erucic acid rapeseed oil, palm olein, refined olive oil and sunflower oil) was monitored. Saturated fatty acids were usually below 10% except for palm olein (44%) and refined olive oil (14%). Oleic acid was the major fatty acid (C18:1, 40-70%) followed by linoleic acid (C18:2, 9-19%) for four of these oils. Sunflower oil was the exception, containing 25.5% oleic acid and 61.5% linoleic acid. The P/S ratio followed the order sunflower oil (5.8) > low erucic acid rapeseed oil (4.0) > high oleic rapeseed oil (1.9) > refined olive oil (0.7) > palm olein (0.3).

The total content of sterols varied 10-fold; 730-7500 μg/g oil. The lowest content was found in palm olein, while the highest amount was found in low erucic acid rapeseed oil. Contents of total tocopherols (α, β, γ and δ) ranged from 155 μg/g in palm olein to 632 μg/g in high oleic rapeseed oil. In addition, palm olein had a content of 384 μg/g of total tocotrienols (α, γ and δ). The highest and lowest amounts of total tocopherols and tocotrienols were found in high oleic rapeseed oil and refined olive oil, respectively.

Oxidative stability with and without added α-tocopherol

The oxidative stability of the five oil samples was determined by heating using Rancimat. The oil stability index (OSI) values varied approximately five-fold; 5.1-27.6 h. The highest OSI value was found in palm olein, while sunflower oil had the lowest value. It has been demonstrated that a higher P/S ratio generally results in lower oxidative stability (Wagner & Elmadfa, 2000).

On the other hand, other authors have suggested that minor compounds such as tocopherol may play an important role in oxidative stability (Przybylski & Eskin, 2006). The total indigenous content of tocopherols and

tocotrienols ranged from ~500 to 600 μg/g for these oils except for refined olive oil, which only contained ~160 μg/g. In this study the effect of addition of various levels of α-tocopherol (500-20000 μg/g oil) on the OSI value were investigated. Interestingly, refined olive oil was the only sample in which the OSI value was increased by addition of α-tocopherol (Table 9). Therefore, addition of α-tocopherol seems to have had a pro-oxidant effect on oxidative stability in high oleic rapeseed oil, palm olein, low erucic rapeseed oil and sunflower oil after doubling of the indigenous concentration. The reason for the antioxidative effect of addition of α-tocopherol to refined olive oil was probably its much lower content of native tocopherols and tocotrienols compared with the other oils. However further study is needed to clarify this.

Table 9. *Effect of adding α-tocopherol to different oils on their oxidative stability expressed as OSI (h)*

Addition of α-tocopherol (μg/g oil)	**HORO**	**RO**	**PO**	**ROO**	**SO**
0	18.8	8.5	27.6	17.5	5.1
500	17.0	8.2	25.9	17.6	5.5
1000	15.9	7.6	25.1	17.9	5.8
2000	14.9	7.1	24.8	18.5	5.9
6000	-	-	-	21.0	-
10000	-	-	-	22.3	6.4
15000	-	-	-	22.5	6.3
17500	-	-	-	-	4.3
20000	-	-	-	23.1	3.8

HORO, high oleic rapeseed oil; RO, low erucic acid rapeseed oil; PO, palm olein; ROO, refined olive oil; SO, sunflower oil.

Effect of α-tocopherol on peroxide value and p-anisidine value in selected oils

Based on the results obtained regarding oxidative stability (OSI) for the five different vegetable oils tested, three of these were selected for further heating experiments: high oleic rapeseed oil, palm olein and refined olive oil, with and without addition of 2000 μg/g oil (0.2%) α-tocopherol. Hence low erucic acid rapeseed oil and sunflower oil were dropped from the study.

Formation of hydroperoxides was measured as PV value at 0, 3 and 6 hours in heating at 180 ± 5 °C (Fig. 4). The PV values for all the fresh oils ranged between 1 and 2 meq oxygen/kg oil. During heating, the PV values gradually increased in all samples except for palm olein. Refined olive oil with additional 0.2% α-tocopherol showed a higher content of PV than refined olive oil without addition of α-tocopherol. It seems that addition of 0.2% α-tocopherol stabilised hydroperoxides and prevented their decomposition (Mäkinen *et al.,* 2001)

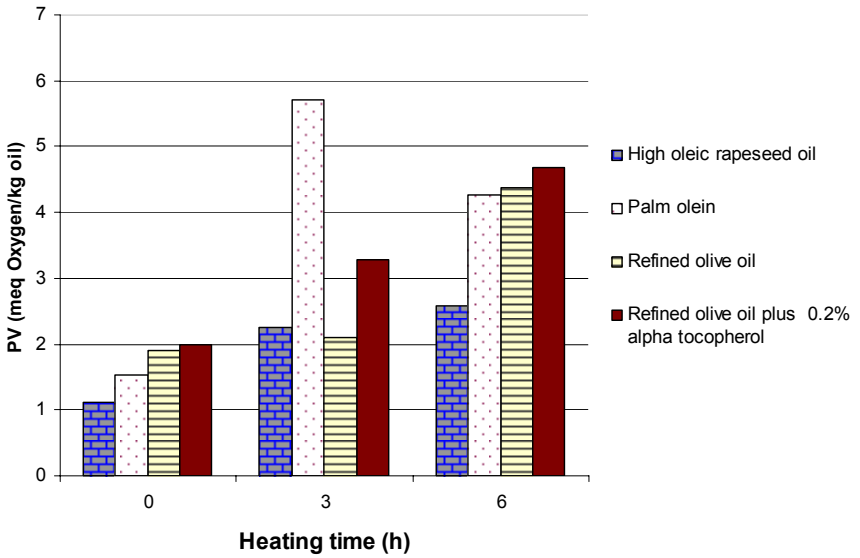

Figure 4. PV values in high oleic rapeseed oil, palm olein, refined olive oil and refined olive oil plus 0.2% α-tocopherol during heating at 180 ± 5 °C for 0-6 h.

During heating at 180 ± 5 °C for up to 12 h, the *p*-AV increased in all samples (Fig. 5). Palm olein and refined olive oil contained lower amounts of *p*-AV compared with rapeseed oil. The results demonstrated that after 6 h of heating, the increase in *p*-AV was rather similar for all the samples except olive oil containing 0.2% α-tocopherol, which had slightly higher levels.

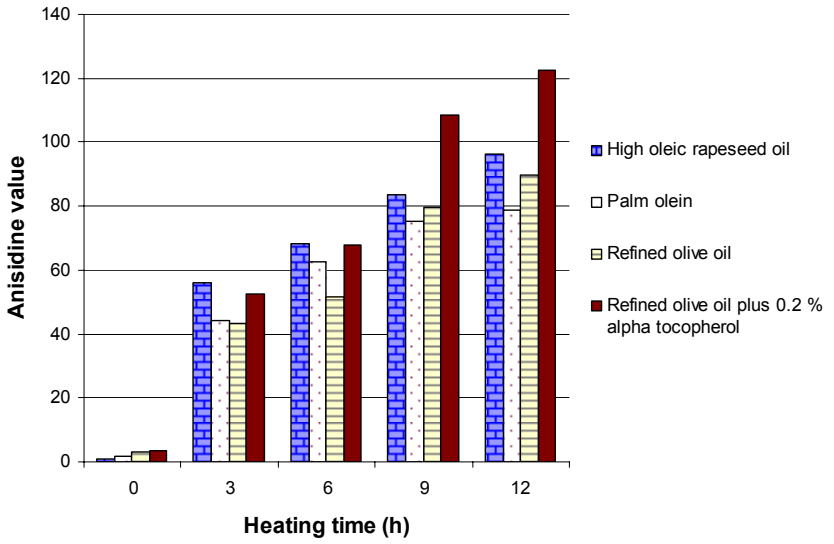

Figure 5. *p*-AV in high oleic rapeseed oil, palm olein, refined olive oil and refined olive oil plus 0.2% α-tocopherol during heating at 180 ± 5 °C for 0-12 h.

Similar increases in primary and secondary oxidation products have been observed for addition of α-tocopherol at levels between 0.01 and 0.1% to a mixture of partially hydrogenated rapeseed oil and palm oil during heating at 160 °C for 0-2 h (Nogala-Kalucka *et al.,* 2005).

Formation of POPs in selected oils

The major POPs originated primarily from sitosterol, followed by campesterol and stigmasterol. The highest level of total POPs was found in high oleic rapeseed oil, ranging from 66.3 to 92.3 μg/g oil. The corresponding figures for palm olein and refined olive oil were 4.7-15.9 μg/g oil and 8.7-24.9 μg/g oil, respectively.

The total POPs content generally increased during the 12 h of heating except for high oleic rapeseed oil after 3 h at 180 ± 5 °C (Table 10). The

decreasing amount of total POPs in rapeseed oil after 3 h of heating might be a consequence of simultaneous reactions in the oil, such as isomerisation and decomposition of sterol structure (Osada *et al.,* 1993; Johnsson & Dutta, 2006). The results showed that addition of α-tocopherol can inhibit the formation of POPs in refined olive oil after 6, 9 and 12 h of heating by approximately 30-40% (Table 10). It has been shown that α-tocopherol is a strong inhibitor of stigmasterol oxidation in purified TAG from sunflower oil during incubation at 60 °C for 0-9 days (Rudzinska *et al.,* 2004).

Table 10. *Content of POPs (μg/g oils) in high oleic rapeseed oil, palm olein, refined olive oil and refined olive oil plus 0.2% α-tocopherol during heating at 180 ± 5 °C for 0-12 h*

Vegetable oil	**0h**	**3h**	**6h**	**9h**	**12h**
HORO	81.1	66.3	87.9	91.7	92.3
PO	4.7	6.8	8.1	9.7	15.9
ROO	8.7	14.9	21.3	22.0	24.9
ROO plus 0.2% α-tocopherol	8.2	11.6	13.2	13.8	17.7

HORO, high oleic rapeseed oil; PO, palm olein; ROO, refined olive oil.
Each value mean of triplicate analyses.

Among the POPs identified, 6-hydroxysitostanol was dominant. Similar results have been obtained in another study on a blend of interesterified refined olive oil and palm stearin (50:50; w:w) at different temperatures (Azadmard-Damirchi & Dutta, 2008). In heated high oleic rapeseed oil, 6-hydroxycampestanol and 7-ketobrassicasterol were also found in high amounts. A feature in common for these three major POPs was that they decreased to various extents during heating. However, the total POPs were highest after 12 h of heating because other POPs were formed continuously during heating.

These results are the most extensive evaluation to date of the formation of individual POPs in high-oleic vegetable oils. The data clearly indicate that enrichment of refined olive oil with α-tocopherol can inhibit formation of total POPs, resulting in levels comparable with palm olein during heating. In addition, this study included other parameters such as composition of fatty acids, sterol, tocopherols, tocotrienols, OSI, PV and *p*-AV values. These results formed the base for the next paper **(Paper IV)** in which palm olein and refined olive oil were evaluated as frying oils for preparation of French fries.

Evaluation of refined olive oil and palm olein for deep frying of French fries (Paper IV)

Based on previous work **(Paper III),** it was decided to evaluate and compare the stability of two high oleic vegetable oils (palm olein and refined olive oil) using deep frying of potato as household cooking. The fatty acid composition and some oxidation criteria, *e.g.* free fatty acid (FFA), *p*-AV, total polar compounds (TPC), and level of POPs, were determined in oil extracted from French fries subjected to five consecutive batches prepared at approximately 1-hour intervals.

The effect on fatty acid composition was evaluated in terms of polyunsaturated/saturated ratio (P/S). The P/S ratio changed from 0.75 in fresh olive oil to 0.72 in oil extracted from French fries prepared in this oil at different frying times up to 5 h (Table 11). The corresponding figures for palm olein were 0.25 in fresh oil and 0.23 in oil extracted from fried potato from the fifth batch. It was suggested that heat treatment of oil decreased fatty acids with two or three double bonds (Orthoefer & Cooper, 1996).

Free fatty acids (FFA) show the amount of fatty acids hydrolysed from the triglyceride (TAG).The level of FFA increased about 2-fold from the initial value of 0.06% in fresh refined olive oil to 0.11% in oil extracted from French fries prepared in this oil heated for about 5 h. The corresponding figures for palm olein were 0.04% and 0.13%, respectively, which is still far below the acceptable limit of 2% (Matthaus, 2006) (Table 11). An elevated percentage of FFA can be expected in the frying process due to an increased rate of hydrolysis, since water is introduced into the frying system by the raw potato (Suleiman *et al.*, 2006).

During five consecutive batches fried at 1-hour intervals, the increase in *p*-AV was more than 20-fold for palm olein, from 2.5 to 53, whereas the corresponding increase was only 9-fold for refined olive oil, from ~4 to 33, (Table 11). After only 1 h of heating, the *p*-AV exceeded the recommended limit value of 10 for good quality (Che Man & Wan Hussin, 1998).

The level of polar compounds is a good indicator of the quality of used frying oils and shows the total amount of newly formed compounds having higher polarity than that of triacyglycerols (Dobarganes & Marquez-Ruiz, 2006). Therefore, polar materials provide a reliable measure of the degree of fat alteration during deep frying. Several European countries have established limits for polar compounds of between 24 and 27% in the worst

case (Mariod *et al.,* 2006). The level of TPC increased with frying time and the highest value of TPC (13.81%) was found in oil extracted from French fries prepared in palm olein after five batches of frying (Table 11). The level of TPC in all samples in our study was acceptable with respect to the EU limit value. It has been observed that TPC in oils increases during frying and that the content is correlated with frying time (Suleiman *et al.*, 2006).

The level of total POPs was determined for fresh oils and for oils extracted from potato products prepared in five different batches over 5 h. The total POPs content doubled for both oils after frying five batches of French fries. The total POPs content in lipids extracted from French fries prepared in refined olive oil was generally twice as high as that in lipid extracts from French fries cooked in palm olein (Table 11).

Among the five parameters for lipid oxidation investigated in **Paper IV**, formation of TPC, (*p*-AV) and formation of POPs changed gradually. The P/S ratio was less usable and changes in free fatty acid concentrations took place mainly during the first hour of heating, before levelling out.

An overview of the results obtained showed that in contrast to POPs content, lower levels of other lipid oxidation parameters were found in oil extracted from fried potato prepared in refined olive oil compared with palm olein during frying up to 5 h (Table 11).

Table 11. *Summary of P/S, TPC (%), FFA (%), p-AV and content of POPs (μg/g oil) in fresh oil and in oil extracted from 5 different batches of French fries during frying* at 180 ± 5 °C *up to 5 h* ***(Paper IV)***

Parameter	Refined olive oil						Palm olein					
	Fresh	**1**[b]	**2**[b]	**3**[b]	**4**[b]	**5**[b]	**Fresh**	**1**[b]	**2**[b]	**3**[b]	**4**[b]	**5**[b]
P/S	0.75	0.75	0.74	0.74	0.73	0.72	0.25	0.24	0.24	0.24	0.23	0.23
TPC%	4.6	5.5	6.0	6.3	6.7	7.3	9.8	10.7	11.4	12.4	13.0	13.8
FFA%	0.06	0.09	0.09	0.09	0.10	0.11	0.04	0.10	0.11	0.12	0.12	0.13
p-AV	3.7	11.8	18.1	23.4	28.5	32.8	2.5	17.9	30.7	39.4	48.6	53.4
POPs (μg/g oil)	5.1	6.6	6.9	7.3	8.4	9.6	1.9	3.0	3.6	3.8	4.3	5.3

[b]number of frying operations

Concluding remarks

The types of frying oils used in industrial production of fried food are undergoing continuous evolution. Among the secondary lipid oxidation products, formation of phytosterol oxidation products (POPs) has gained interest during recent years because of their possible health effects. Current knowledge on the occurrence of POPs in foods is scarce and mainly relates to some fresh and heated vegetable oils and sterol-enriched spreads. About 20 commercial products of French fries and potato crisps were analysed for individual and total POPs using recent methodological developments including transesterification, SPE-enrichment, GC and GC-MS. Prefried French fries (oven heated) were lowest in total POPs (10-210 μg/100g), followed by potato crisps (50-680 μg/100g), while French fries samples collected from restaurants contained the highest level (150-810 μg/100g).

These wide ranges in total POPs were reflected in variations in content of factors known to be important for sterol oxidation, *i.e.* total lipids and fatty acid profile, phytosterols and antioxidants. In addition, processing and frying conditions cause different thermal intensities during preparation of deep fried potato products, which is another major factor behind POPs formation.

Since the POPs in deep fried potato products originate mainly from the frying oils, better knowledge about the composition and stability of vegetable oils during frying could be a way to explain the wide variations found for POPs in deep fried potato products. Such knowledge could be useful for selecting the most healthy and stable vegetable frying oils. Therefore, we ranked five different regular and monounsaturated vegetable oils (palm olein, refined olive oil, high oleic rapeseed oil, low erucic acid rapeseed oil and sunflower oil) for their oxidative stability. Their nutritional profiles in terms of some major lipids were characterised, *e.g.* fatty acid

composition and P/S ratio, content of phytosterols, tocopherols and tocotrienols. The oil stability index (OSI) of these five oils was assessed in heating experiments using Rancimat. The results showed oxidative stability in the order of palm olein > high oleic rapeseed oil > refined olive oil > low erucic acid rapeseed oil > sunflower oil. Enrichment with α-tocopherol in the range 0.05-2% revealed mainly pro-oxidative effects on oxidative stability in the oils except for refined olive oil. In another part of study, the monounsaturated vegetable oils (high oleic rapeseed oil, palm olein, refined olive oil and refined olive oil with addition 0.2% α-tocopherol) were assessed for other oxidation parameters such as peroxide value, *p*-anisidine value and POPs content after heating at 180 ± 5 °C for 0-12 h. All of these factors generally increased during heating, and addition of 0.2% α-tocopherol decreased the formation of total POPs in refined olive oil. Since sunflower oil was the least stable frying oil and since the rapeseed oils with their higher sterol content resulted in high yields of POPs, palm olein and refined olive oil were selected as the two most promising candidates for preparation of French fries at laboratory scale similar to household conditions.

The chemical characteristics of the oil residues extracted from fried French fries were assayed for five consecutive batches fried for 6 min (180 °C) at 1-hour intervals. Refined olive oil as frying oil produced lower figures of total polar compounds, free fatty acids and *p*-anisidine values than palm olein. In addition, refined olive oil had a better fatty acid profile, with higher P/S ratio and sterol content, which however resulted in double the amount of POPs in French fries prepared in refined olive oil compared with palm olein. This difference in POPs formation between the two frying oils could be minimised by enrichment of refined olive oil with α-tocopherol.

The fact that refined olive oil was superior in most of the characteristics to palm olein in preparing French fries was unexpected. Refined olive oil has not been assessed as a candidate for deep frying as much as palm oil/palm olein. Thus the extensive evaluation of lipid stability presented here provides new and important information on this common healthy vegetable oil.

Future prospects

Fried foods are popular among a wide range of consumers, especially the younger generation all over the world. It is imperative to use frying oils with less harmful effects in long-term consumption of fried foods.

Frying oils dominated by monounsaturated fatty acids are a good compromise compared with saturated and polyunsaturated fatty acid-dominated frying oils. Refined olive oil is an interesting candidate for its potential in industrial food preparation. In the future:

- Refined olive oil can be utilised in industrial preparation of potato and other food products. However, sensory and storage studies are needed. Additional technological parameters can be tested to minimise formation of POPs.

- Studies are needed to accumulate additional data on the levels of POPs in various foods. Such data can be valuable for future investigations on the biological effects of POPs in humans.

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Swedish University of Agricultural Sciences
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