FORMATION OF PHYTOSTEROLS OXIDES IN FRIED OIL PRODUCTS

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INTRODUCTION

The average American diet contains 700 to 1000 mg of sterols per day, of which approximately 30-50% are phytosterols (Grundy 1978). While autoxidation of cholesterol has been extensively studied, relatively little information is available on autoxidation of corresponding plant sterols (Smith 1981). The presence of cholesterol oxides in food products is of great concern due to their cytotoxicity, angiotoxicity, mutagenicity and carcinogenicity (Smith 1981). Cholesterol oxidation problems may be further complicated by the presence of phytosterols. Aringer et al. (1981) found sitosterol oxidation products to be similar in structure to those of cholesterol after biological oxidation.

This paper reports the presence of cholesterol and plant sterols oxides in selected snack type food products available in a supermarket in North America.

MATERIALS AND METHODS

Materials

A variety of fried food products were purchased in a local supermarket. Cholesterol and sterol standards were purchased from Sigma Chemicals and Steraloids Inc.. Sylon BTZ and pyridine were obtained from Supelco Canada Ltd. while Sep-Pac aminopropyl cartridge from Waters Canada Ltd.

Extraction and Isolation

Lipids were extracted from the individual food products using the Bligh and Dyer procedure (1959). Lipid extracts were evaporated to dryness, dissolved in chloroform and 10 mg of total lipids were loaded on aminopropyl cartridge. Neutral lipids, including sterols and sterol esters, were eluted with 8 ml of chloroform:2-propanol=2:1. The eluted lipids were dissolved in hexane, and applied to a second cartridge for further separation. Sterols esters were eluted with 8 ml of hexane and sterols with 12 ml of 5% v/v ethyl acetate in hexane.

Saponification

The eluates were evaporated to dryness and to residues 5 ml of 2N KOH in methanol and 1 ml of internal standard solution (containing 40 μg of 5α-cholestane per one ml of methanol) were

added. The mixtures were blanketed with nitrogen, and vigorously shaken until a monophasic system was obtained. Saponification was carried out overnight, in the dark at room temperature. The saponified mixture was diluted with 10 ml of glass distilled water and non-saponifiables extracted three times with 10 ml of diethyl ether. Combined extracts were washed twice with 5 ml of 0.5N KOH and three times with 5 ml of distilled water and evaporated to dryness. Water was removed by evaporation with 1 mL benzene and 2 mL 2-propanol.

Derivatization for Gas Chromatography

The dried ether extract residues were redissolved in 200µl pyridine and 100µl of Sylon BTZ added to derivatize the sterols. The reaction was conducted at room temperature for a minimum of 1 hr with double sonications for 2 min each.

Gas Chromatography and Mass Spectrometry

The derivatized sterols were separated on a fused silica capillary column DB-1 (30 m x 0.25 mm i.d.) with 0.25 µm phase film. Column temperature was programmed from 120 to 195 C at the rate 30 C/min, then to 235 C at 10 C/min and finally to 285 at 6 C/min. The lower temperature was held for 15 min. Approximately 0.5 - 1.0 µl of the sample mixture was injected with a split ratio 1:100 into the column.

A Hewlett-Packard 5190 GC equipped with 5970 Mass Selective Detector (MSD) was used. GC-MS analyses were performed on the same capillary column as described earlier and with splitless injection mode.

Recovery of Sterols

The recovery of sterols during saponification and extraction was studied. A mixture of standard oxides (30 µg each) with 40 µg of 5α-cholestane, as internal standard, were added to 1g of canola oil and the entire procedure carried out. Recoveries for each sterol also were determined to assess the effect of this procedure on the formation of oxides.

RESULTS AND DISCUSSION

Recovery and Precision of Methods

The recovery for the sterols was conducted separately from oxidized cholesterol products (OCP's), because saponification is often criticized for the formation of oxidized artifacts (Smith 1981). The reliability of the quantitative data using solid phase extraction (SPE) and cold saponification was checked by examining the recovery of sterols and OCP's (Table 1). Recoveries and standard deviation were calculated from six replications. No additional peaks were detected following

overnight saponification at room temperature. The peaks observed corresponded to the original sterol standards only. These results confirmed the absence of any oxidation and/or others artifacts formed during the procedure used in this study, and eliminated the need to add antioxidants to samples prior to cold saponification and sample handling.

Table 1. Recoveries of sterols and cholesterol oxides from oil samples.

Compound	Recovery (%)	Standard Deviation (%)
Cholesterol	98.6	1.1
ß-Sitosterol	97.2	1.3
Campestrol	100.1	2.6
Stigmasterol	99.8	4.1
7α-Hydroxycholesterol	97.9	2.3
7ß-Hydroxycholesterol	95.2	1.8
Cholesterol α-epoxide	97.8	3.2
7α-Ketocholesterol	98.1	2.8
25-Hydroxycholesterol	96.3	2.1
Cholestantriol	88.5	3.0

Similar observations on the absence of any OCP's formation during cold saponification were also reported by Park and Addis (1986). The actual recovery of sterols during sample preparation is shown in Table 1 and over 98% recovery was obtained for the majority of components analyzed. Using a similar procedure, Park and Addis (1986) accomplished a similar recovery with an internal standard present. The overall recovery of the standards analyzed of not less than 90% with a standard deviation below 5%, indicated sample preparation procedure described is accurate and reproducible (Park et al. 1986). The recovery of cholestantriol, even with the internal standards added at the beginning of the procedure, was only 88%. This necessitated the use of a correction factor to adjust for this lower recovery level.

Detection of Oxidation Products in Foodstuffs

Studies conducted to date have focused on the formation of cholesterol oxidized products in foods containing fat of animal origin. In this study the extent of oxidation in the snack foods is summarized in Tables 2-5. All results represent the mean of 6 replications. In Table 2. results of analyses of different sterols in snack food products are presented. With the exception of potato chips and ruffles, all other products were modified corn products, fried in soybean, canola and cottonseed oils or a mixture of some of these oils. Cheese Puffs, Cheese Twisties and Nacho Doritoes are all extruded corn products sprayed with cheese and a seasoning mixture. These products contained higher amounts of cholesterol as well as total sterols. Of the total amount of sterols analyzed, the three major ones for the cheese sprayed products were: ß-sitosterol, campestrol and cholesterol.

Table 2. Presence of sterols in snack food products in ppm.

Product	CH	BR	CM	STG	SIT
Cheese Puffs	232	ND	218	16	512
Cheese Twisties	186	7	226	20	652
Nacho Doritoes	264	ND	232	41	736
Salsa Rio Doritoes	36	18	272	TR	726
Nacho Grande	8	16	326	18	892
Natural Doritoes	14	27	307	8	926
Potato Chips	12	32	262	7	332
Ruffles	16	26	316	4	1026

CH = Cholesterol; BR = Brasicasterol; CM = Campestrol; STG = Stigmasterol; SIT = Sitosterol.

Identification of oxidized sterols was performed by comparison of mass spectra with a previously prepared library of compounds of interest using available standards as mentioned in methods above. For quantitation, two methods were applied: GC with internal standard and mass fragmentography. The latter method was used for quantitation utilising specific chosen ions with the highest abundance.

Table 3. Cholesterol oxidation products present in snack foods (ppm).

Product	7αH	7βH	αEP	βEP	7αK	25H	TROL
Cheese Puffs	8	5	11	4	3	ND	7
Cheese Twisties	ND	2	7	8	ND	7	4
Nacho Doritoes	TR	6	8	3	TR	12	5
Salsa Rio Doritoes	ND	9	5	5	2	ND	ND
Nacho Grande	ND	ND	2	ND	ND	4	ND
Natural Doritoes	1	3	7	2	4	ND	ND
Potato Chips	ND	ND	4	ND	ND	7	ND
Ruffles	ND	ND	TR	ND	2	TR	ND

7αH = 7α-Hydroxycholesterol; 7βH = 7β-Hydroxycholesterol; αEP and βEP = α and β- Cholesterol epoxides; 7αK = 7α-Ketocholesterol; 25H = 25-Hydroxycholesterol; TROL = 5α-Cholestane-5,6β-triol. ND = Non detected; TR = Traces.

The results presented in this study include only those components whose identification were confirmed with quantitative results within a 10% range between GC and mass fragmentography. The latter proved particularly useful at very low concentrations when full scanning spectra provided insufficient data for identification purposes. The MFG permitted the tentative conformation of components present in only trace amounts as shown in the Tables. Cholesterol oxides were found in all snack foods analyzed. The cheese-sprayed products contained the highest level of cholesterol oxides with α-epoxides being

predominant. This was followed by 7ß-hydroxycholesterol and ß-epoxide cholesterol with 7α-hydroxycholesterol and cholestantriol present at lower levels (Table 2). Sander et al. (1989) reported similar OCP's levels in fresh Cheddar and Blue cheese powders while Finocchiaro and co-workers (1983) found higher levels of oxides in grated cheese stored for 5-10 months in clear glass container as compared to cheese-sprayed products.

Table 4. ß-Sitosterol oxidation products identified in snack foods in ppm.

Product	αES	ßES	ST	7αHS	7ßHS	7KS
Cheese Puffs	2	ND	3	2	3	5
Cheese Twisties	ND	ND	2	5	7	3
Nacho Doritoes	ND	5	ND	TR	2	7
Salsa Rio Doritoes	6	2	1	5	3	5
Nacho Grande	TR	ND	ND	3	8	7
Natural Doritoes	7	4	5	3	6	2
Potato Chips	TR	4	3	5	7	2
Ruffles	1	3	7	TR	ND	8

αES and ßES = α and ß-Sitosterol epoxides; ST = Sitosteroltriol; 7α and 7ß = 7α and 7ßHydroxysitosterol; 7KS = 7α-Ketositosterol.

Data on the oxidation of plant sterols such as ß-sitosterol and campestrol, in snack food examined is presented in Table 4 and 5. Among these identified compounds, 7α-ketosterol and 7α-hydroxysitosterol were predominant followed by ß-campestrol epoxide and 7ß-hydroxysitosterol. The largest amount of oxides were found in Salsa Rio Doritoes followed by Cheese Puffs, Nacho Grande, Cheese Twisties and Nacho Doritoes in decreasing order of magnitude. Products in which cheese is sprayed on the outside layer provides a large surface area for oxidation.

Table 5. Presence of campestrol oxidation components in snack foods (ppm).

Product	αCE	ßCE	CT	7αCH	CK
Cheese Puffs	3	ND	TR	ND	ND
Cheese Twisties	1	1	3	2	TR
Nacho Doritoes	ND	ND	TR	ND	ND
Salsa Rio Doritoes	7	2	TR	1	3
Nacho Grande	2	1	ND	5	6
Natural Doritoes	1	ND	ND	1	8
Potato Chips	3	8	5	TR	ND
Ruffles	3	4	2	5	TR

αCE and ßCE= α and ß-Campestrol epoxides; CT=Campestrol-triol; 7αCH= 7α-Hydroxycampestrol; CK= 7α-Ketocampestrol.

This makes it easier for light and oxygen to penetrate into the lipids and accelerate sterol oxidation (Smith 1981; Finocchiaro

1983).The present study includes only those compounds confirmed by MS as there were a significant number of separated peaks that remained unidentified due to lack of standards and/or insufficient purity of spectrum to be positively interpreted and compound identified. This study confirms the presence of sterol oxides in selected snack food products available in North American supermarkets. The lack of information on the storage and production conditions of these products makes it difficult to explain the large differences between the foods examined. Abusive storage conditions in supermarkets as well as clear packages exposed to light, temperature and moisture could stimulate the formation of these components. The presence of phytosterols may be of importance in food products designed for infants and children where animal fats are replace by vegetable oils. Diet rich in plant sterols have been reported to induce 5 to 15 fold elevations of plasma phytosterols in children than that observed in adults (Mellies et al. 1976). The presence of plant sterol oxides in snack foods, reported for the first time in this study, necessitate further investigation in this area. In light of concern over cholesterol oxides, the health implications of plant sterol oxidation products should be evaluated.

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