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Oxidation of Cholesterol and β-Sitosterol and Prevention by Natural Antioxidants

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Consumption of cholesterol oxidation products (COPs) is a growing health concern, but little is known about the intake of β-sitosterol oxidation products (SOPs). The present study was performed (i) to compare the oxidative stability of cholesterol with that of β-sitosterol; (ii) to investigate the oxidative pattern of cholesterol and β-sitosterol in lard, corn oil, and olive oil; and (iii) to examine the effectiveness of green tea catechins (GTC), α-tocopherol, and quercetin in prevention of cholesterol and β-sitosterol oxidation compared with butylated hydroxytoluene (BHT). Results showed both cholesterol and β-sitosterol were thermally unstable with 75% of cholesterol and β-sitosterol being oxidized at 180 °C for 2 h. The oxidation behavior of β-sitosterol was similar to that of cholesterol in terms of oxidative rate and oxidation products. The major COPs produced were 7-ketocholesterol, 7α-hydroxycholesterol, 7β-hydroxycholesterol 5,6α-epoxycholesterol, and 5,6β-epoxycholesterol, whereas the major SOPs were 7-ketositosterol, 7α-hydroxysitosterol, 7β-hydroxysitosterol, 5,6α-epoxysitosterol, and 5,6β-epoxysitosterol. Under the same experimental conditions, both cholesterol and β-sitosterol were oxidized more slowly in corn oil, lard, and olive oil, attributable to the unsaponified antioxidants present in these fat and oils. GTC, α-tocopherol, and quercetin were more effective than BHT in preventing the oxidation of cholesterol and β-sitosterol.

KEYWORDS: Cholesterol; catechins; COPs; quercetin; β-sitosterol; oxidation; SOPs; α-tocopherol

INTRODUCTION

Human diets contain both cholesterol and phytosterols. The former is mainly present in foods of animal origin, whereas the latter are mainly found in foods of plant origin. Cholesterol is susceptible to oxidation to form a series of cholesterol oxidation products (COPs) under various food-processing conditions. COPs in foods can reach up to 10% total cholesterol (*1*), particularly in Western countries where total fat intake is high and fried foods are popular (*2*). Major COPs identified in foods are 7α-hydroxycholesterol, 7β-hydroxycholesterol, 7α-hydroxycholesterol, 5α-hydroxycholesterol, 7-ketocholesterol, and epoxycholesterol (*3*,*4*). COPs have been extensively studied for their cytotoxicity, mutagenesis, carcinogenesis, acceleration of fatty streak lesion formation, and promotion of atherosclerosis (*5*−*10*).

Phytosterols are a group of plant sterols including β-sitosterol, stigmasterol, campesterol, and brassicasterol with β-sitosterol being a major isomer. Phytosterols have been sold as a functional cholesterol-lowering nutraceutical in Europe, the United States, and Australia. One of the major applications is the addition of phytosterols into spreads and vegetable oils. It is estimated that phytosterol intake in humans can reach 300 mg per day. Like cholesterol, β-sitosterol can also undergo oxidation and produce a series of β-sitosterol oxidation products (SOPs), namely, 7β-hydroxysitosterol, 7α-hydroxysitosterol, 5α-hydroxysitosterol, 7-ketositosterol, and epoxysitosterol. Although the adverse effect of COPs has been well documented, information on the health effect of SOPs is scarce (*11*).

It is essential to protect cholesterol and β-sitosterol from autoxidation and to minimize the production of COPs and SOPs before they are consumed. The present study was therefore performed (i) to compare the relative oxidative stability of cholesterol with that of β-sitosterol; (ii) to investigate the oxidative pattern of cholesterol and β-sitosterol in lard, corn oil, and olive oil; and (iii) to ascertain if synthetic antioxidant butylated hydroxytoluene (BHT) and natural antioxidants, namely, green tea catechins (GTC), α-tocopherol, and quercetin, were able to inhibit the oxidation of cholesterol and β-sitosterol.

MATERIALS AND METHODS

Materials and Reagents. α-Tocopherol, BHT, cholesterol, β-sitosterol, 5α-cholestane, 7α-hydroxycholesterol, 7β-hydroxycholesterol, 5,6β-epoxycholesterol, 5,6α-epoxycholesterol, 7-ketocholesterol, quercetin, and trimethylsilyl (TMS) reagent were purchased from Sigma (St. Louis, MO). Lard, corn oil, and olive oil were purchased from a local supermarket. All solvents were of analytical grade.

Preparation of GTC. GTC was isolated from jasmine green tea according to our previous study (*12*). Dry tea leaves (10 g) were soaked in 150 mL of hot distilled water (80 °C). The infusion was cooled at room temperature, filtered, and extracted with an equal volume of chloroform to

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remove caffeine and pigment. GTC was then extracted into ethyl acetate and freeze-dried overnight. Each catechin was quantified using a HP-1100 HPLC equipped with a ternary pump. The yield in this study reached 7.5 g of GTC/100 g of jasmine green tea leaves with a purity of 95%, in which (−)-epigallocatechin gallate (EGCG), (−)-epigallocatechin (EGC), (−)-epicatechin gallate (ECG), and (−)-epicatechin (EC) accounted for 62.3, 19.2, 8.3, and 4.6%, respectively.

Oxidation of Pure Cholesterol and β-Sitosterol. Cholesterol and β-sitosterol (0.2 mg each) in 200 μL of ethyl acetate were added into a test tube, and the ethyl acetate was removed under a gentle stream of nitrogen gas. Each test tube was then heated at 180 °C for 30, 60, 90, and 120 min. An additional set of the samples was similarly prepared and oxidized at 120, 150, and 180 °C for 60 min. After oxidation, all of the samples were allowed to cool at room temperature. Cholesterol, β-sitosterol, and their oxides were then converted to their TMS derivatives and subjected to GC analyses.

Oxidation of Cholesterol and β-Sitosterol in Lard, Corn Oil, and Olive Oil. Oxidations of cholesterol and β-sitosterol in lard, corn oil, and olive oil were conducted under similar conditions. In brief, lard, corn oil, and olive oil were dissolved in hexane at a concentration of 10%. After cholesterol or β-sitosterol was added into the lard, corn oil, and olive oil and thoroughly mixed, the hexane was evaporated under a gentle stream of nitrogen. Each test tube contained 10 mg of oil and 0.2 mg of cholesterol or β-sitosterol and heated at 120, 150, and 180 °C for 60 min. The samples were subjected to cold saponification overnight at the room temperature. The unsaponifiable materials were extracted with hexane, and purification of oxides was carried out on a solid-phase extraction (SPE) cartridge (LC-Si, 3 mL/500 mg; Supelco, Bellefonte, PA). In brief, the SPE cartridge was first conditioned with 4 mL of hexane. The unsaponifiable matter was dissolved in 1 mL of a solution of hexane/diethyl ether (9:1, v/v) and was then loaded onto the cartridge. Low-polarity lipids and nonoxidized sterols were eluted with 4 mL of hexane/diethyl ether (9:1, v/v) followed by 4 mL of hexane/diethyl ether (6:4, v/v). Finally, the sterol oxides were eluted with 4 mL of acetone and collected into a tube containing 40 μg of 5α-cholestane, which was used an internal standard to quantify the COPs or SOPs. The samples were subjected to TMS derivatization and GC analysis. Changes in COPs and SOPs were expressed as percentages of the initial amount of cholesterol and β-sitosterol.

Effect of Unsaponified Substance from Lard, Corn Oil, and Olive Oil on Oxidation of Cholesterol and β-Sitosterol. Corn oil, lard, and olive oil were subjected to cold saponification overnight at room temperature, and their corresponding unsaponified substances were collected. Similarly, cholesterol or β-sitosterol (0.2 mg) was oxidized with or without the presence of unsaponfied substances in amounts equivalent to those from 10 mg of corn oil, lard, and olive oil, respectively, at 180 °C for 60 min.

Effect of BHT and Natural Antioxidants on Stability of Cholesterol and β-Sitosterol. To investigate which antioxidant was more effective in preventing oxidation of cholesterol and β-sitosterol, 200 ppm BHT, GTC, α-tocopherol, or quercetin was dissolved in ethyl acetate and added into the reaction mixture containing cholesterol or β-sitosterol with or without lard, olive oil, and corn oil. The ethyl acetate was then evaporated under a gentle stream of nitrogen. The rationale for choosing this concentration is that the maximum 200 ppm of antioxidant is generally permitted in fats and oils in most countries. All of the experiments were conducted in triplicate, and the mixture was sampled at 30, 60, 90, and 120 min at 180 °C. All of the samples were subjected to TMS derivatization and GC analyses as described above.

Analyses of COPs and SOPs. Cholesterol, β-sitosterol, COPs, and SOPs were quantified in a Shimadzu GC-2010 gas−liquid chromatograph equipped with a flame ionization detector and an automated injector (Tokyo, Japan). Briefly, 40 μg of 5α-cholestane in ethyl acetate as an internal standard was added into each sample. After the removal of ethyl acetate under a gentle stream of nitrogen, 100 μL of TMS reagent was added to derivatize COPs and SOPs at 60 °C for 60 min. The TMS-ether derivatives were dissolved into 400 μL of hexane with 1 μL being injected onto a fused silica capillary column (SAC-5, 30 m × 0.25 mm, i.d.; Supelco). Helium was used as a carrier gas at a constant flow of 1.0 mL/min. Column temperature was programmed from 90 to 270 °C at a rate of 30 °C /min and then to 300 °C at 1.5 °C/min, at which it was held for 12 min. Both injector and detector temperatures were set at 300 °C. Individual COPs and SOPs were quantified according to the amount of internal standard added.

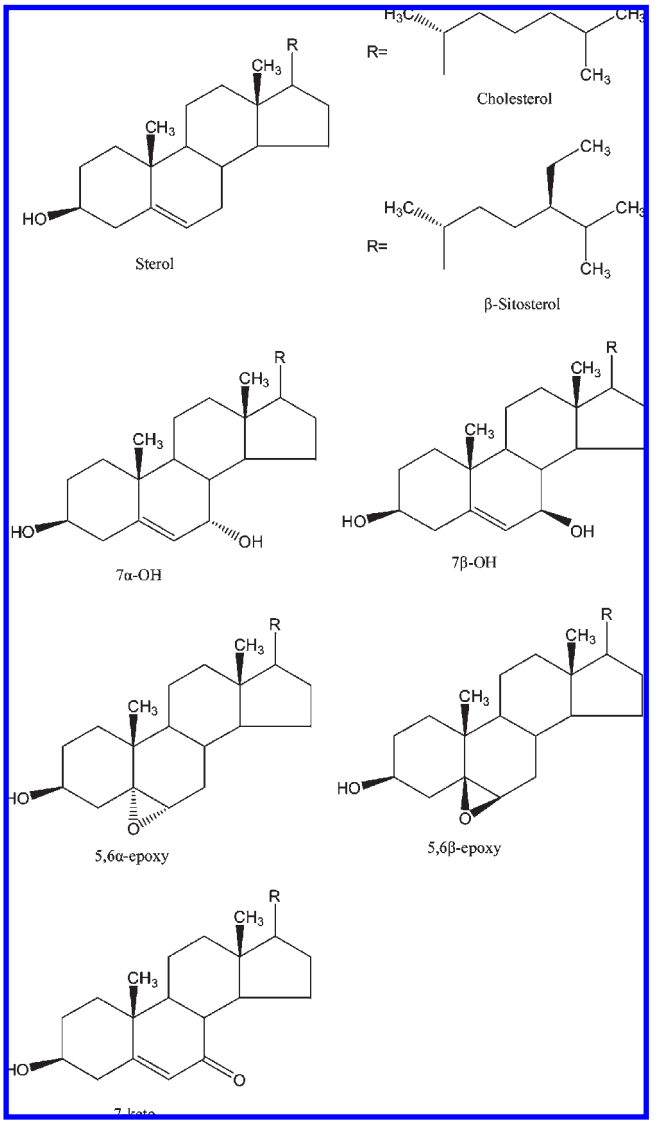

Figure 1. Chemical structures of the most common cholesterol oxidation products (COPs) and β-sitosterol oxidation products (SOPs).

GC-MS Identification of COPs and SOPs. Identification of cholesterol/β-sitosterol and their oxides was carried out on a 6890 series gas chromatograph coupled to an Agilent 5973 mass spectrometer (Agilent Technologies Inc., Palo Alto, CA) with a full scan mode (*m*/*z* 45−600). Similarly, the TMS derivatives of COPs and SOPs were separated on a SAC-5 column. Helium was used as a carrier gas at a constant flow of 1.0 mL/min. The column temperature was programmed from 90 to 270 °C at a rate of 30 °C /min and then to 300 °C at 1.5 °C/min, at which it was held for 12 min. The MS interface temperature was 280 °C, and the ion source was 230 °C. Electron ionization energy was set at 70 eV. Individual COPs and SOPs were identified according to their retention times of authentic standards and specific characteristics of mass spectra ions as previously published (*13*−*15*).

RESULTS

Comparison of COPs and SOPs. The GC profiles of COPs and SOPs demonstrated that cholesterol and β-sitosterol were similarly oxidized (**Figures 1** and **2**). Although the retention time of individual SOPs was longer compared with their corresponding COPs, the peak abundance and eluting order in the former were the same as those in the latter. When cholesterol was

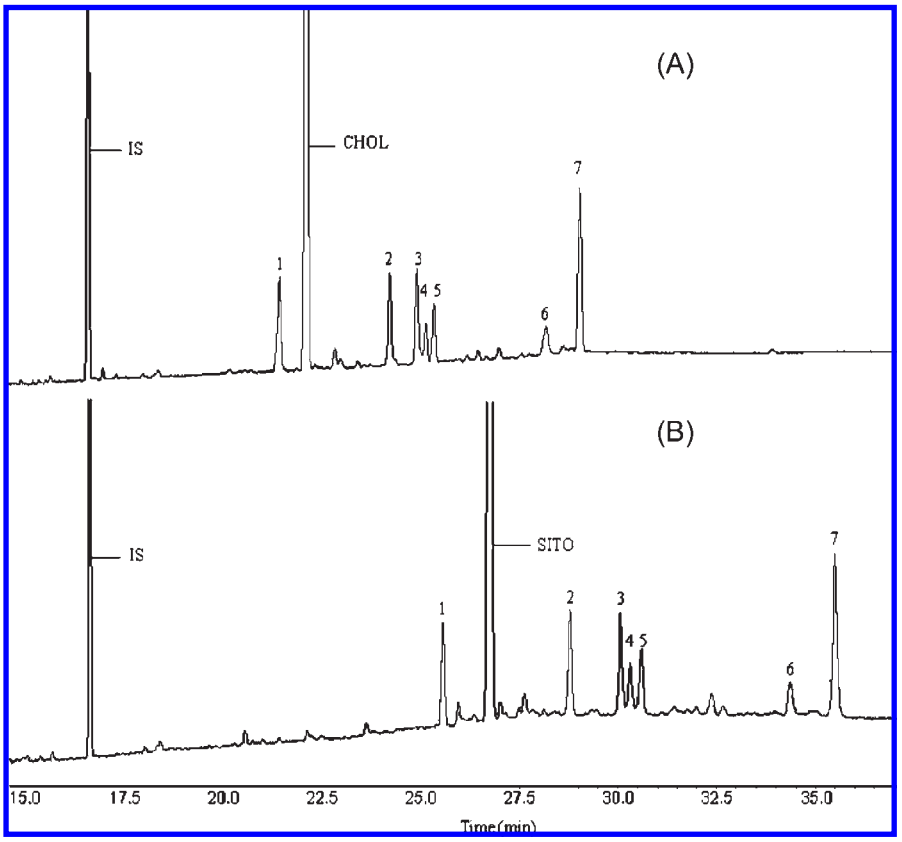

Figure 2. Typical GC profiles of cholesterol oxidation products (COPs) and β-sitosterol oxidation products (SOPs). Peak identification in (**A**): IS, internal standard (5α-chlestane); CHOL, cholesterol; 1, 7α-hydroxycholesterol; 2, 7β-hydroxycholesterol; 3, unknown; 4, 5,6β-epoxycholesterol; 5, 5,6α-epoxycholesterol; 6, unknown; 7, 7-ketocholesterol. Peak identification in (**B**): IS, internal standard (5α-chlestane); SITO, β-sitosterol; 1, 7α-hydroxysitosterol; 2, 7β-hydroxysitosterol; 3, unknown; 4, 5,6β-epoxysitosterol; 5, 5,6α-epoxysitosterol; 6, unknown; 7, 7-ketositosterol.

oxidized, 7-ketocholesterol, 7α-hydroxycholesterol, and 7β-hydroxycholesterol were the major COPs, with 5,6α-epoxycholesterol and 5,6β-epoxycholesterol being the minor ones. Oxidation of β-sitosterol produced similar oxidation products, with 7-ketositosterol, 7α-hydroxysitosterol, and 7β-hydroxysitosterol being relatively major and 5,6α-epoxysitosterol and 5,6β-epoxysitosterol being relatively minor (**Figure 2**).

Oxidation of Cholesterol and β-Sitosterol Alone. Both cholesterol and β-sitosterol were oxidized at 180 °C (**Figure 3**). The data showed that oxidative degradation of cholesterol and the production of COPs were similar to that of β-sitosterol. However, the production of 7β-hydroxysitosterol from β-sitosterol appeared to be lesser compared with its corresponding oxidation product, 7β-hydroxycholesterol, from cholesterol (**Figure 3**).

Oxidation of Cholesterol and β-Sitosterol in Corn Oil. No difference was seen in the oxidations of 2% cholesterol and 2% β-sitosterol in corn oil (**Figure 4**). With regard to the individual oxidation products, oxidation of β-sitosterol produced lesser amounts of 5,6α-epoxysitosterol and 7-ketositosterol compared with 5,6α-epoxycholesterol and 7-ketocholesterol from the oxidation of cholesterol (**Figure 4**).

Oxidation of Cholesterol and β-Sitosterol in Lard. Cholesterol and β-sitosterol (2% each) were oxidized in lard at 180 °C for 30, 60, 90, and 120 min (**Figure 5**). The time course changes in COPs, SOPs, and individual sterol oxidation products clearly demonstrated that the oxidative rate of cholesterol was similar to that of β-sitosterol in lard. A similar trend was seen for the production of COPs and SOPs between cholesterol and β-sitosterol. With regard to the individual oxidation products, production of 7-ketositosterol appeared to be less than that of 7-ketocholesterol (**Figure 5**).

Oxidation of Cholesterol and β-Sitosterol in Olive Oil. When 2% cholesterol and 2% β-sitosterol were oxidized in olive oil at 180 °C for 30, 60, and 90 min, no difference in degradation pattern and production of oxidative products was seen between cholesterol and β-sitosterol (**Figure 6**). However, β-sitosterol appeared to produce greater amounts of 5,6α-epoxysitosterol, 5,6β-epoxysitosterol, and 7-ketositosterol than of 5,6α-epoxycholesterol, 5,6β-epoxycholesterol, and 7-ketocholesterol from oxidation of cholesterol (**Figure 6**).

Oxidation of Cholesterol or β-Sitosterol in Lard, Corn Oil, and Olive Oil. The results clearly demonstrated that pure cholesterol oxidized more quickly than cholesterol in corn oil, lard, and olive oil when oxidation was conducted at 180 °C for 30, 60, and 90 min (**Figure 7**). Cholesterol in corn oil, lard, and olive oil appeared to oxidize at a similar rate. Similarly, pure β-sitosterol oxidized more quickly than that in corn oil, lard, and olive oil for the first 90 min at 180 °C. Thereafter, no difference was seen between pure β-sitosterol and that in corn oil, lard, and olive oil (**Figure 7**). Similar results were seen when cholesterol or β-sitosterol was oxidized at 120, 150, and 180 °C. Pure cholesterol and β-sitosterol oxidized more quickly than those in corn oil, lard, and olive oil (**Figure 8**). Consistently, greater amounts of COPs and SOPs were produced from pure cholesterol and β-sitosterol than from those in corn oil, lard, and olive oil (**Figure 8**).

Effect of Unsaponified Substances from Corn Oil, Lard, and Olive Oil on Oxidation of Cholesterol or β-Sitosterol. The unsaponified substances from corn oil, lard, and olive oil were effective in preventing the oxidation of both cholesterol and β-sitosterol (**Figure 9**). It appeared that the unsaponified substances from corn oil were more effective than those from lard and

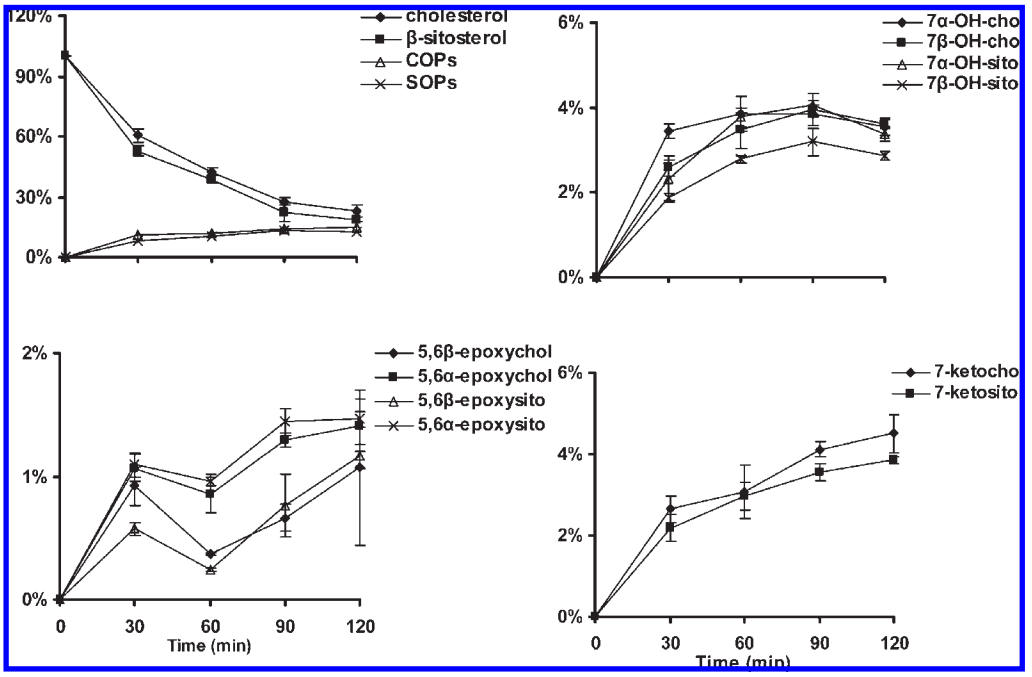

Figure 3. Time course changes (%) in cholesterol (Chol), β-sitosterol (Sito), cholesterol oxidation products (COPs), and β-sitosterol oxidation products (SOPs) when Chol and Sito were oxidized at 180 °C for 30, 60, 90, and 120 min.

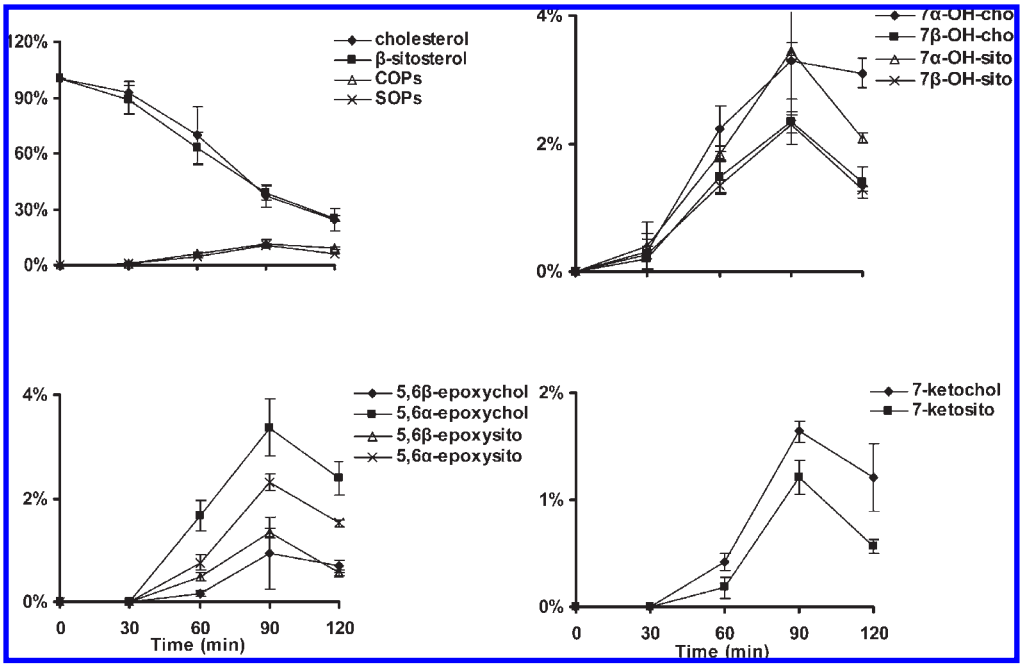

Figure 4. Time course changes (%) in cholesterol (Chol), β-sitosterol (Sito), cholesterol oxidation products (COPs), β-sitosterol oxidation products (SOPs), and individual sterol oxidation products when Chol and Sito were oxidized in corn oil at 180 °C for 30, 60, 90, and 120 min.

olive oil in preventing the oxidation of cholesterol and β-sitosterol and reducing the formation of the oxidation products (**Figure 9**).

Inhibition of BHT and Natural Antioxidants on Oxidation of Cholesterol and β-Sitosterol. BHT and natural antioxidants were able to prevent partially the oxidation of cholesterol (**Figure 10**). GTC, α-tocopherol, and quercetin were more effective than BHT in preventing the oxidation of cholesterol when cholesterol was oxidized alone or in lard.

Similarly, BHT and three natural antioxidants inhibited the oxidation of β-sitosterol. The results demonstrated quercetin, α-tocopherol, and GTC were more effective antioxidants than BHT in preventing β-sitosterol oxidation (**Figure 11**). However, it appeared that GTC, α-tocopherol, and quercetin were much more effective when β-sitosterol was oxidized alone compared with that in corn oil or olive oil (**Figure 11**).

DISCUSSION

The present study is the first report on the comparison of cholesterol oxidation with β-sitosterol oxidation. Mammals are able to synthesize cholesterol, whereas plants are capable of synthesizing β-sitosterol. Cholesterol is structurally similar to β-sitosterol except for the side chain (**Figure 1**). The present study clearly demonstrated that the oxidation behavior of β-sitosterol was similar to that of cholesterol in terms of oxidative rate and

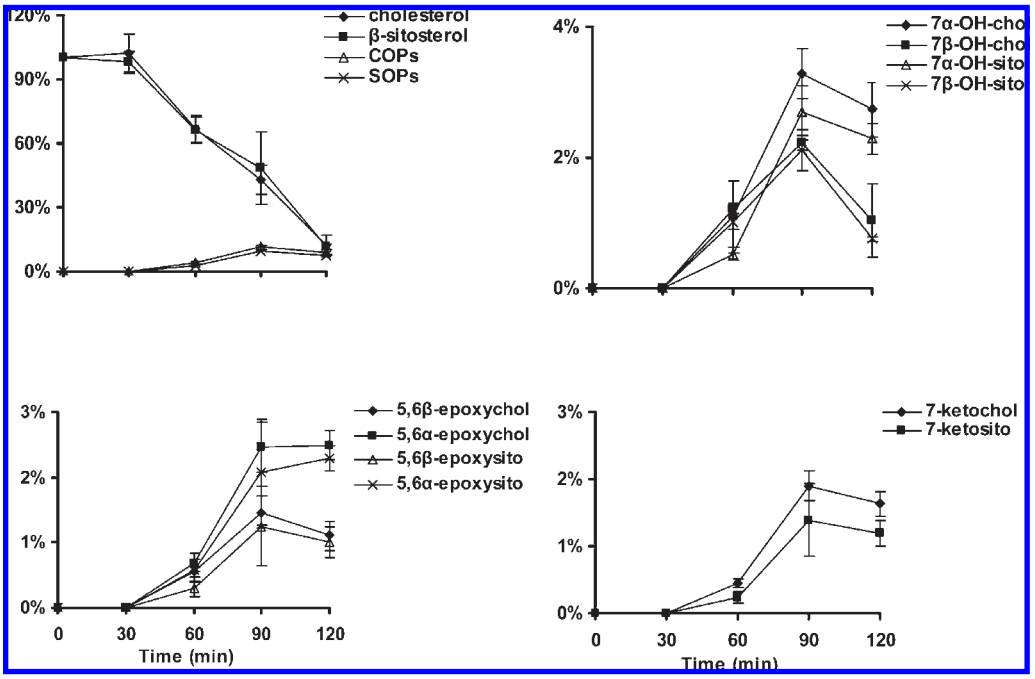

Figure 5. Time course changes (%) in cholesterol (Chol), β-sitosterol (Sito), cholesterol oxidation products (COPs), β-sitosterol oxidation products (SOPs), and individual sterol oxidation products when Chol and Sito were oxidized in lard at 180 °C for 30, 60, 90, and 120 min.

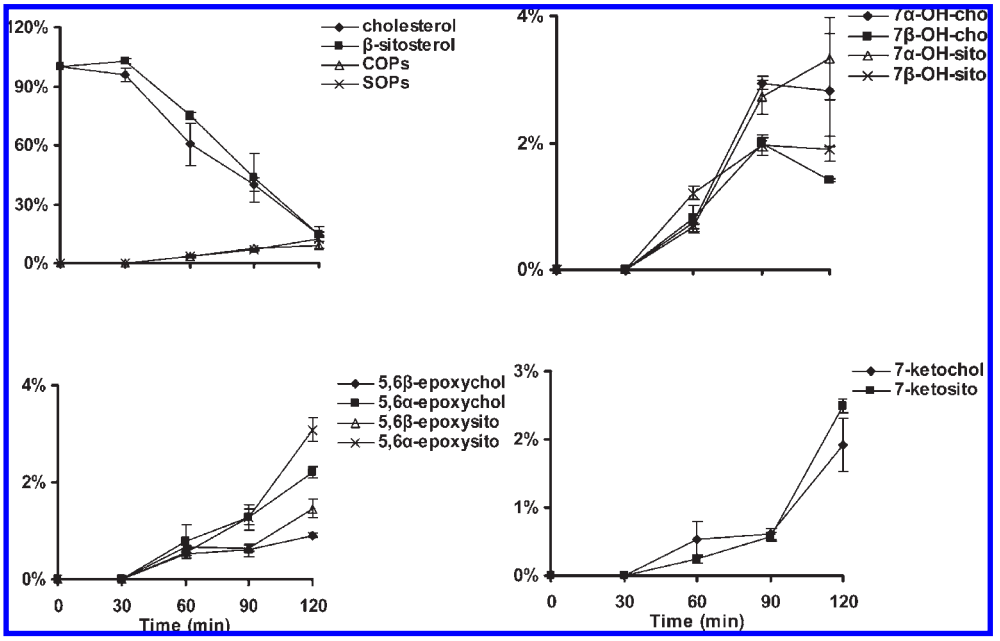

Figure 6. Time course changes (%) in cholesterol (Chol), β-sitosterol (Sito), cholesterol oxidation products (COPs), β-sitosterol oxidation products (SOPs), and individual sterol oxidation products when Chol and Sito were oxidized in olive oil at 180 °C for 30, 60, 90, and 120 min.

oxidation products. Oxidation of cholesterol led to the production of 7-ketocholesterol, 7α-hydroxycholesterol, 7β-hydroxycholesterol, 5,6α-epoxycholesterol, and 5,6β-epoxycholesterol, whereas oxidation of β-sitosterol generated similar oxidation products, namely, 7-ketositosterol, 7α-hydroxysitosterol, 7β-hydroxysitosterol, 5,6α-epoxysitosterol, and 5,6β-epoxysitosterol. The present result on the formation of individual COPs and SOPs was in agreement with that of Grandgirard et al. (*16*), who used GC-MS to identify the oxidation products of oxyphytosterol and oxycholesterol in spreads. Chien et al. (*17*) had studied the formation mechanism of COPs during heating, finding that in the early stage of oxidation, cholesterol underwent oxidation at position 7 to produce 7-hydroperoxycholesterol (7-OOH) followed by epoxidation, dehydration, reduction, and dehydrogenation, leading to the formation of various forms of COPs. It is expected that the formation mechanism of SOPs is similar to that of COPs as the degradation rate of β-sitosterol and its profile of SOPs were similar to those of cholesterol.

Cholesterol and β-sitosterol are basically stable compounds at room temperature; however, they are susceptible to thermal oxidation. In the present study, up to 75% of cholesterol and β-sitosterol was thermally degraded when they were oxidized alone at 180 °C for 120 min (**Figure 3**). Concomitantly, about 20% COPs and SOPs was produced under the same experimental

conditions. It was found that the changes in concentrations of individual COPs or SOPs did not show a consistent trend; that is, they rose over a heating period of 30−90 min and then declined thereafter (**Figures 3−5**). This phenomenon, probably due to further thermal degradation and evaporation of the oxides formed, has been well documented (*14*). We chose 180 °C as a heating temperature simply because it is a typical frying temperature. In this regard, Menendez-Carreno et al. (*18*) studied the oxidation of phytosterols in commercially available phytosterol-enriched milk subjected to usual and drastic heating conditions and found that Schaal oven conditions (24 h/65 °C, equivalent to 1 month of storage at room temperature) reduced the phytosterol content by only 4% while drastic heating treatments (2 min of microwave heating at 900 W or 15 min of electrical heating at 90 °C) led to a 60% decrease of total phytosterol content with a significant increase of thiobarbituric acid-reactive substances. Another study found that no significant oxidation of phytosterols could occur as a result of heating at 100 °C for 1 h or during storage at 50 °C for several weeks, whereas heating at 200 °C resulted in > 50% phytosterol degradation (*19*). Like β-sitosterol, cholesterol could degrade by 27% after 30 min of heating at 125 °C, whereas it degraded by 70 and 90% after 30 min at 150 and 175 °C, respectively (*20*). To predict the reaction of cholesterol oxidation, Chien et al. (*17*) analyzed COPs during the heating of cholesterol and found that oxidation of cholesterol or formation of individual COPS could fit either first-order or second-order reactions. Our major finding was that both cholesterol and β-sitosterol were prone to thermal oxidation and have similar oxidative degradation patterns when they were oxidized under the same experimental conditions.

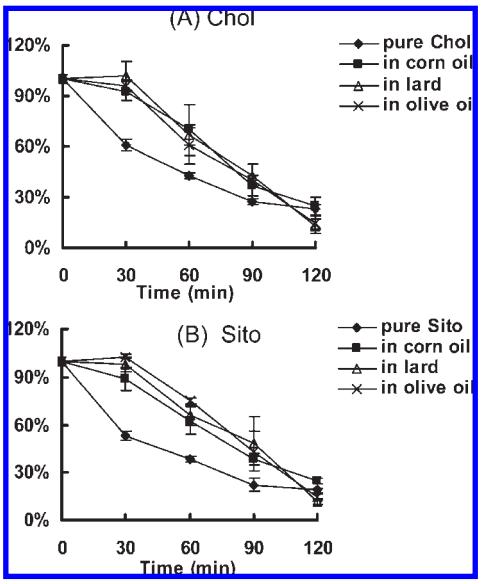

Figure 7. Time course changes (%) in cholesterol (**A**, Chol) and β-sitosterol (**B**, Sito) when Chol and Sito were oxidized alone or in corn oil, lard, and olive oil at 180 °C for 30, 60, 90, and 120 min.

It was noteworthy that both cholesterol and β-sitosterol were oxidized more slowly in the presence of corn oil, lard, and olive oil for the first 90 min at 180 °C (**Figure 7**). This was consistent with the observation that the pure cholesterol and β-sitosterol produced greater amounts of COPs and SOPs compared with those in corn oil, lard, and olive oil when they were oxidized at 120, 150, and 180 °C for 60 min (**Figure 8**). These cooking oils and fat were chosen as the frying media mainly because corn oil is a typical n-6 vegetable oil; olive oil is a typical n-9 vegetable oil, whereas lard is a typical animal fat. The present results clearly demonstrated that the frying oil/fat increased the oxidative stability of cholesterol and β-sitosterol regardless of their types. When the oxidation of cholesterol in the presence of peanut oil during heating was studied in comparison with the oxidation of pure cholesterol, it was found that COPs decreased as amounts of added peanut oil increased (*21*). In the study conducted by Xu et al. (*19*), cholesterol mixed with corn, canola, soybean, or olive oil had significantly improved thermal stability compared with pure cholesterol, suggesting that these oils may increase the thermal

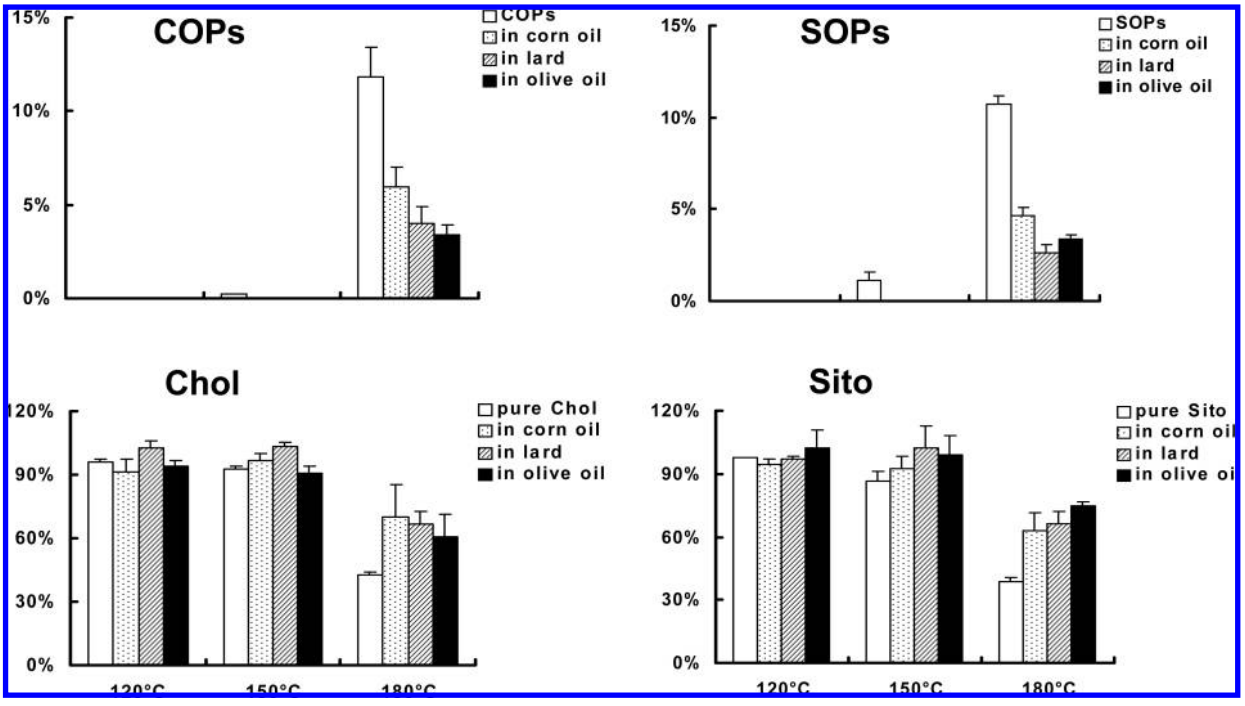

Figure 8. Time course changes in cholesterol (Chol), β-sitosterol (Sito), cholesterol oxidation products (COPs), and β-sitosterol oxidation products (SOPs) when Chol and Sito were oxidized alone or in corn oil, lard, and olive oil at 120, 150, and 180 °C for 60 min.

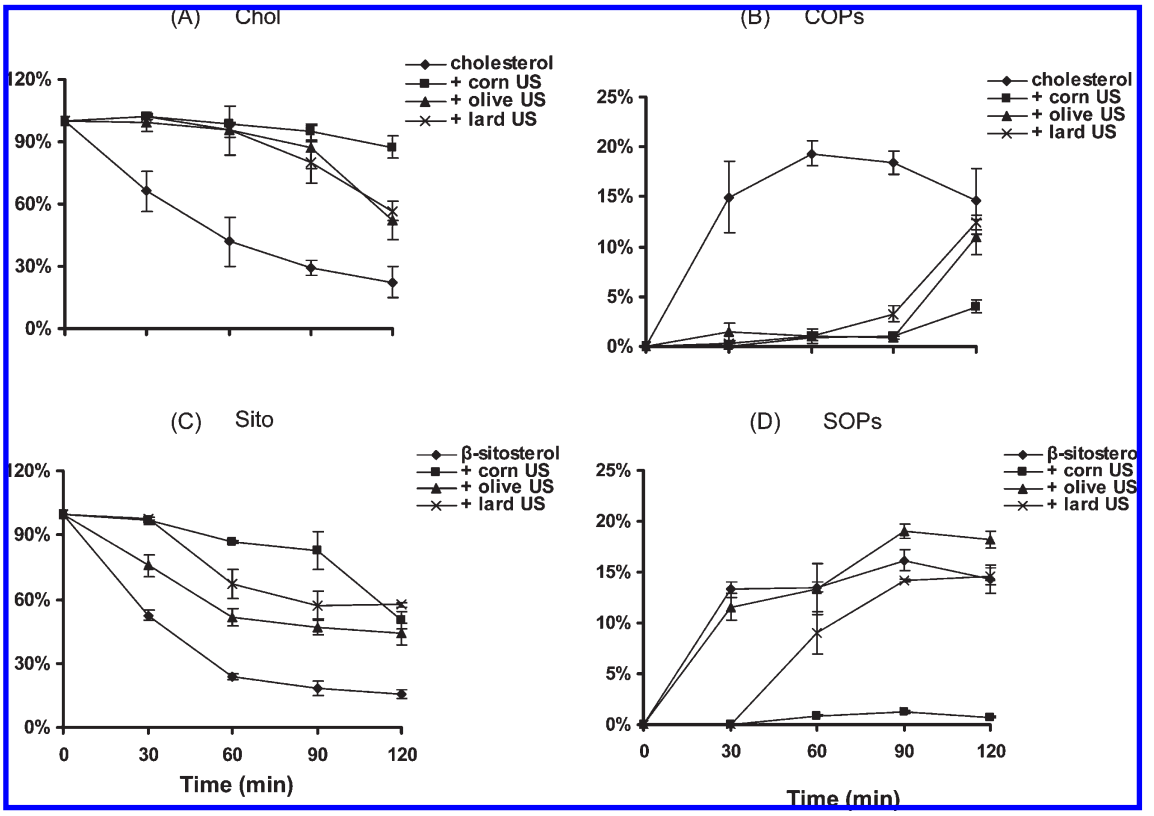

Figure 9. Oxidation of cholesterol and β-sitosterol with and without addition of unsaponified substances derived from corn oil (corn US), lard (lard US), and olive oil (olive US): (**A**) remaining cholesterol (Chol); (**B**) cholesterol oxidation products (COPs); (**C**) remaining β-sitosterol (Sito); (**D**) β-sitosterol oxidation products (SOPs).

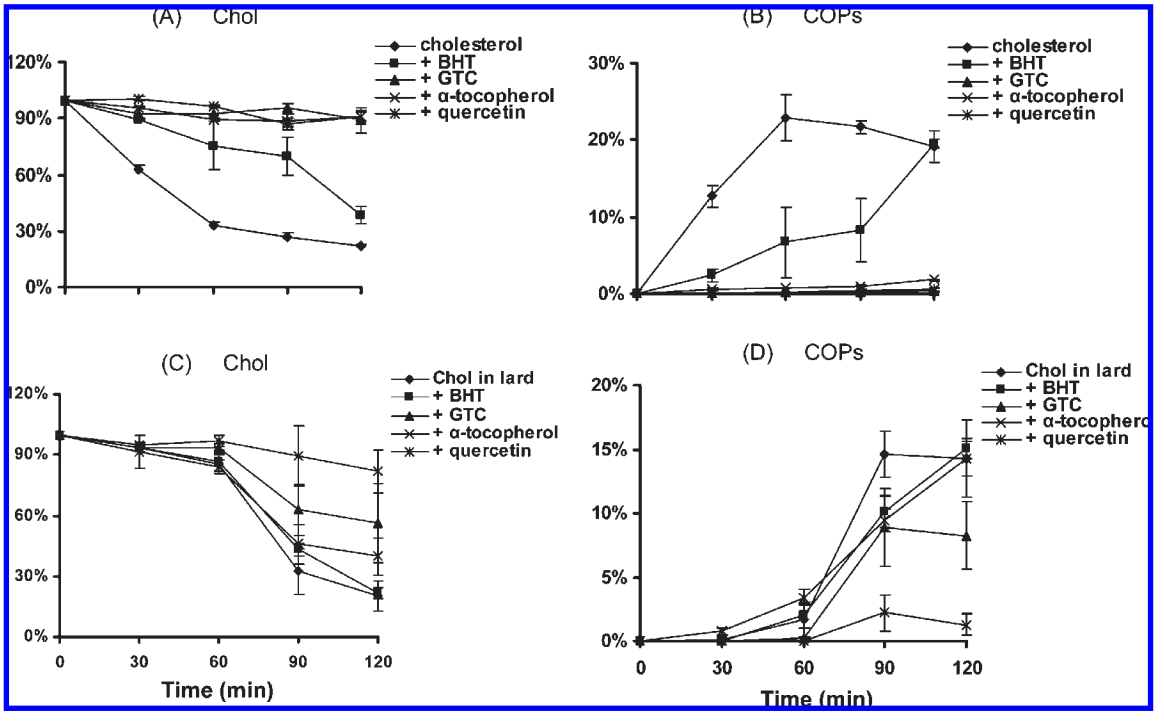

Figure 10. Oxidation of cholesterol (Chol) alone or in lard and formation of cholesterol oxidation products (COPs) in the presence of 200 ppm butylated hydroxytoluene (BHT), green tea catechins (GTC), α-tocopherol, and quercetin at 180 °C: (**A**) percent remaining cholesterol; (**B**) percent COPs formed; (**C**) percent remaining cholesterol in lard; (**D**) percent COPs formed in lard.

stability of cholesterol and retard its degradation rate. However, no study to date has addressed the oxidative stability of pure β-sitosterol with that in the presence of cooking oils. Therefore, we have no data available to be compared with the present study. In this regard, the research did show that phytosterol oxide could decrease by 50% in the rapeseed/palm oil blend, sunflower oil,

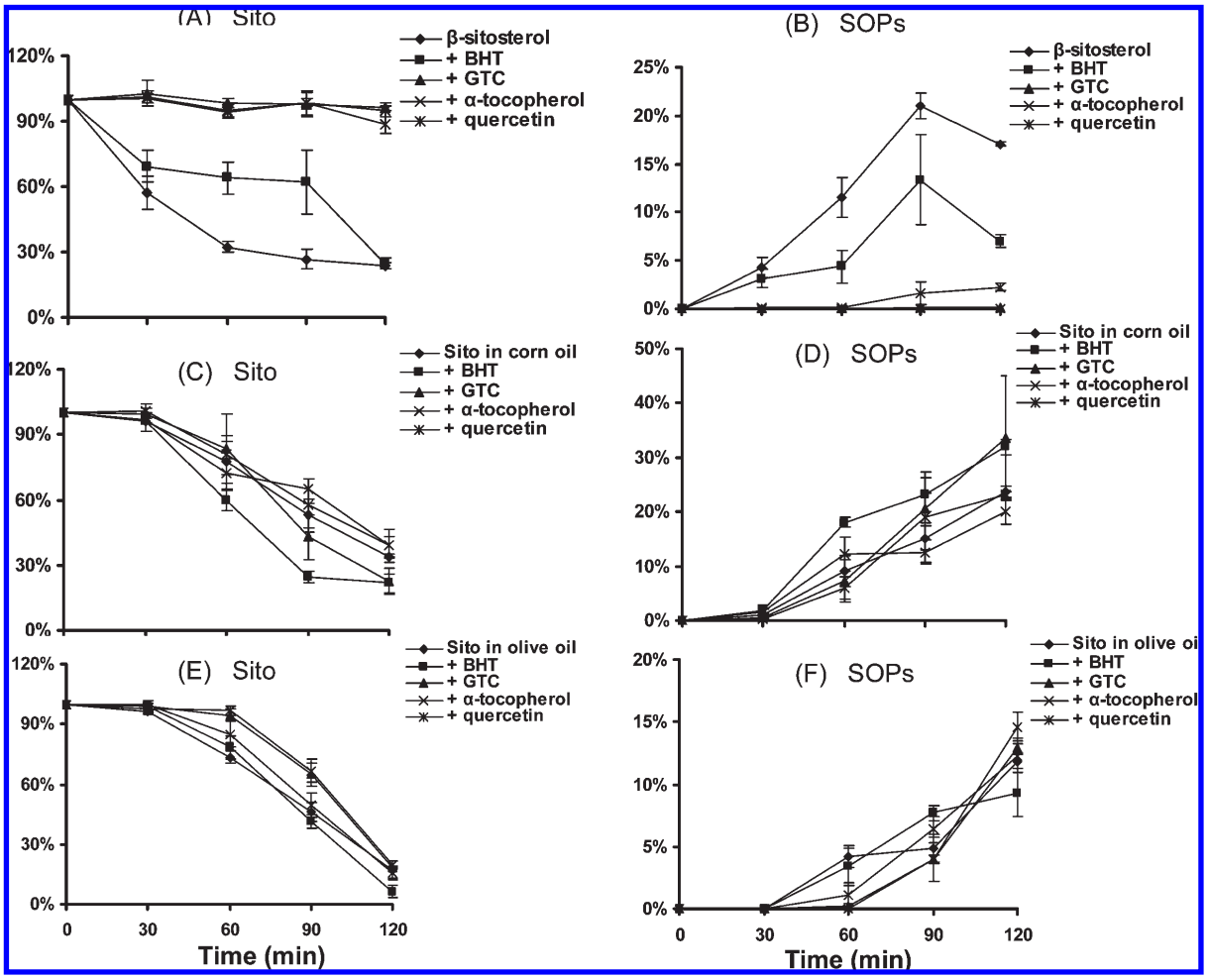

Figure 11. Oxidation of β-sitosterol (Sito) alone or in corn oil or olive oil and formation of β-sitosterol oxidation products (SOPs) in the presence of 200 ppm butylated hydroxytoluene (BHT), green tea catechins (GTC), α-tocopherol, and quercetin at 180 °C: (**A**) percent remaining Sito; (**B**) percent SOPs formed; (**C**) percent remaining Sito in corn oil; (**D**) % SOPs formed in corn oil; (**E**) percent remaining Sito in olive oil; (**F**) percent SOPs formed in olive oil.

and high-oleic sunflower oil after frying for 2 days at 200 °C (*22*). The mechanism by which cholesterol and β-sitosterol exhibit greater stability in vegetable oils/fat remains poorly understood. It has been suggested that the antioxidants present in vegetable oils are responsible for enhanced oxidative stability of sterols, particularly in corn oil and olive oil, which contain α-tocopherol and γ-tocopherol (*19*). To prove this hypothesis, we measured α-, β-, and γ-tocopherols, finding that corn oil contained 0.34 mg/g and olive oil contained 0.16 mg/g in total, whereas lard had no detectable amount of tocopherols. In addition, the unsaturated fatty acids in corn oil, lard, and olive oil were also susceptible to oxidation, competed for oxygen, and therefore could reduce the oxidation of cholesterol and β-sitosterol during heating.

The formation of both COPs and SOPs in frying oils/fat cannot be ignored as it is a growing health concern (*23*−*25*). Oxidation of cholesterol and β-sitosterol occurs in not only oils/fat but also foods. COPs are detected in foods such as heated milk (*17*), processed beef (*26*), butter (*27*), lard (*28*), cheese, salami, whole egg powder, biscuits, egg-containing snack foods, and egg noodles (*29*). SOPs exist mainly in foods of plant origin or those fried in vegetable oils (*30*). It is deemed necessary to prevent the oxidation of cholesterol and phytosterol in foods. In this study, GTC, α-tocopherol, and quercetin were chosen as antioxidants to prevent the oxidation of cholesterol and β-sitosterol. The results revealed that the addition of these natural antioxidants at 200 ppm significantly inhibited the oxidation of cholesterol and β-oxidation with the protective effect being stronger than that of BHT under the same conditions.

In summary, β-sitosterol and cholesterol were oxidized in a similar manner. Under the same experimental conditions, both cholesterol and β-sitosterol were oxidized more slowly in the frying corn oil, lard, and olive oil. GTC, α-tocopherol, and quercetin were more effective than BHT in preventing the oxidation of cholesterol and β-sitosterol. COPs have been known for being cytotoxic, atherogenic, mutagenic, and carcinogenic, whereas little information is available on the biological effects of SOPs. We have studied the effect of COPs on blood cholesterol and the functioning of arteries, finding that COPs are highly hypercholesterolemic and cause atherosclerosis and endothelial dysfunction (*10*). We are currently studying the effect of SOPs on blood cholesterol level and cardiovascular functions.

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