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Phytosterol oxidation in oil-in-water emulsions and bulk oil

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Abstract

Dietary plants sterols (phytosterols) have been shown to lower plasma cholesterol level in humans. Since phytosterols may protect against coronary heart diseases, they are being incorporated into functional foods. However, phytosterols are susceptible to oxidative degradation. The purpose of this study was to evaluate the formation of phytosterols oxidation products (POPs) in oil-in-water emulsions and bulk corn oil. The extent of lipid oxidation was monitored by measuring the lipid hydroperoxides and hexanal, whereas 7-keto derivatives of phytosterols were determined by gas chromatography to follow sterol oxidation. A higher POPs level and formation rate was found in the oil-in-water (o/w) emulsion than in the bulk oil. Interfacial tension measurements showed that phytosterols had a high degree of surface activity, which would allow them to migrate to the oil–water interface of the emulsion droplets where oxidative stress is high.

Keywords: Lipid oxidation; Phytosterols; Emulsions; Interfacial tension

1. Introduction

Dietary plant sterols (phytosterols) have shown to decrease total and low density lipoprotein cholesterol in humans by inhibiting the absorption of dietary cholesterol. The cholesterol lowering activity of phytosterols suggests that they may protect against heart disease and, therefore, they have been recently incorporated into a growing spectrum of functional foods (Kritchevsky & Chen, 2005). However, phytosterols are susceptible to oxidation; this phenomenon has been evaluated in model systems, as well as in some oils and food products (Dutta, 1997; Bortolomeazzi, Cordano, Pizzale, & Conte, 2003; Lambelet et al., 2003; Grandgirard, Martine, Joffre, Juaneda, & Berdeaux, 2004; Louter, 2004; Soupas, Juntunen, Lampi, & Piironen, 2004). The resulting phytosterols oxidation products (POPs) could have toxic effects on human and animal organisms similar to those of cholesterol oxidation products (COPs) (Hodis, Crawford, & Sevanian, 1991; Rong et al., 1998; García-Cruset, Carpenter, Codony, & Guardiola, 2002; Osada, 2002), however, several aspects of the possible toxic effects of phytosterols are still to be elucidated (Adcox, Boyd, Oehrl, Allen, & Fenner, 2001; Maguire, Konoplyannikov, Ford, Maguire, & O'Brien, 2003; Tomoyori et al., 2004; Lea, Hepburn, Wolfreys, & Baldrick, 2004). Recently, the biological effects of COPs and POPs were compared and it was concluded that POPs have qualitatively similar toxic effects to COPs, but higher concentrations of POPs are required to achieve comparable levels of toxicity (Ryan, Chopra, McCarthy, Maguire, & O'Brien, 2005). Therefore, oxidation of phytosterols would be detrimental in foods, because it would lead to a reduction in the concentration of these beneficial compounds, generating POPs that are potentially cytotoxic.

Food lipids exist either in a bulk phase or a dispersion. Food dispersions include oil-in-water and water-in-oil emulsions that are represented by products such as butter, margarine, soups beverages, salad dressings and ice cream. Since phytosterols are insoluble in water, they have to be

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incorporated into most functional foods as an emulsion. Oxidative reactions in emulsions are mechanistically different than bulk oils, due to the presence of an interfacial region between the oil and water phases that strongly influences oxidation chemistry. One of the most common mechanisms of lipid oxidation in emulsions is the interaction between lipid hydroperoxides located at the emulsion droplet interface and transition metals originating in the aqueous phase (McClements & Decker, 2000). Transition metals accelerate lipid oxidation by promoting the decomposition of lipid hydroperoxides into free radicals that can in turn cause further oxidation of lipids. Lipid oxidation rates in oil-in-water emulsions can be affected by many factors, such as prooxidant and antioxidants concentrations, pH, and interfacial membrane properties (Frankel, 1998; McClements & Decker, 2000).

While much progress has been made on gaining a better understanding of oxidation of unsaturated fatty acids in bulk oils and oil-in-water emulsions, few studies have been focused on the factors that influence phytosterols oxidation in bulk and emulsified oils. Therefore, the objective of this research was to measure the oxidation of endogenous phytosterols in refined corn oil that was stored in a bulk phase or an oil-in-water emulsion. These studies should be useful for achieving a better understanding of the oxidative stability of phytosterols when they are dispersed into functional food products, so that technology can be developed to ensure their stability and minimize their potential cytotoxicity.

2. Materials and methods

2.1. Materials

Commercial corn oil was obtained from a local retailer. Chloroform, *n*-hexane, diethyl ether, methanol, acetone, potassium hydroxide and anhydrous sodium sulfate, were purchased from Fisher Scientific (Pittsburg, PA, USA). Large silica thin-layer chromatography (TLC) plates (20 cm × 20 cm × 0.25 mm film thickness) were supplied by Merck (Whitehouse Station, NY, USA). Tri-Sil® Reagent (silylating agents) was purchased from Pierce (Rockford, IL, USA). Cholest-5-en-3β,19-diol (19-hydroxycholesterol) (purity: 99%), 5α-cholestane (purity: 96%), cholest-5-en-3β-ol (cholesterol) (purity: 99) and cholest-5-en-3β,7α-diol (7α-hydroxycholesterol) (purity: 99%), were purchased from Steraloids (Newport, RI, USA). Standards of (24*R*)-ethylcholest-5-en-3β-ol (β-sitosterol) (purity: 78% β-sitosterol, 11% (24*R*)-ethylcholestan-3β-ol (sitostanol) and 8% (24*R*)-methylcholest-5-en-3β-ol (campesterol)), cholest-5-en-3β,7β-diol (7β-hydroxycholesterol) (purity: 90%), 5α,6α-epoxy-cholestan-3β-ol (α-epoxycholesterol) (purity: 87%), 5β,6β-epoxy-cholestan-3β-ol (β-epoxycholesterol) (purity: 80%), cholestan-3β,5α,6β-triol (cholestanetriol) (purity: 99%), cholest-5-en-3β-ol-7-one (7-ketocholesterol) (purity: 99%), (24*S*)-ethylcholest-5,22-dien-3β-ol (stigmasterol) (purity: 98%), cumene hydroperoxide and hexadecane, were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). The purity of the standards was established by gas chromatography (GC). Silica solid-phase extraction (SPE) cartridges (Supelclean LC-Si SPE, 500 mg/3 mL) from Supelco (Bellefonte, PA, USA) were used for sterol oxides purification. All the other chemicals were reagent grade or better and were obtained from Sigma–Aldrich Co. (St. Louis, MO, USA) or Fisher Scientific (Pittsburgh, PA, USA).

2.2. Preparation of bulk oil and oil-in-water emulsions for lipid oxidation studies

Oil-in-water emulsions were prepared by blending corn oil with a polyoxyethylene 23 lauryl ether solution (Brij 35; 1.5% wt/wt in 10 mM acetate–imidazole buffer, pH 7.0) at a final lipid concentration of 15% (wt/wt). Thimerosal (Sigma Chemical Co., St. Louis, MO, USA; 1 mM) was added to the emulsion to inhibit microbial growth.

A coarse emulsion was made first by homogenizing the lipid and aqueous phases for 2 min, using a two-speed hand held homogenizer (Biospec Products, Inc., Bartlesville, OK, USA) at the highest speed setting. The coarse emulsion was then passed through a two-stage high-pressure valve homogenizer (APV-Gaulin, Wilmington, MA, USA) at 34 MPa for 3 passes. Particle size distributions were measured using laser light scattering (Mastersizer, Malvern Instruments Ltd., Worchester, UK). The average droplet diameters (volume-surface mean diameter; d_{32}) ranged from 0.2 to 0.3 μm and did not change during the course of the experiment.

Both bulk oil and oil-in-water emulsion samples (2.0 mL) were placed in 10-mL headspace vials, sealed with polytetrafluoroethylene (PTFE)/butyl rubber septa using a crimper and aluminum seals, and then incubated at 55 °C in the dark.

2.3. Determination of headspace hexanal

Headspace hexanal was determined using a Shimadzu 17A Gas Chromatograph equipped with a Hewlett Packard 19395A headspace sampler (Nuchi, McClements, & Decker, 2001). The headspace conditions were as follows: sample temperature, 55 °C; sample loop and transfer line temperature, 110 °C; pressurization, 10 s; venting, 10 s; injection, 1.0 min. The aldehydes were separated isothermally at 65 °C on a methyl silicone (DB-1, J&W Scientific, Folsom, CA, USA) fused silica capillary column (50 m × 0.31 mm i.d. × 1.03 μm film thickness). The splitless injector temperature was 180 °C, and the eluted compounds were detected with a flame ionization detector at 250 °C. Concentrations were determined from peak areas using a standard curve made from hexanal standard.

Following headspace analysis, vials were opened and the contents were used for hydroperoxide, total sterol and POPs analysis.

2.4. Determination of lipid hydroperoxides

Lipid hydroperoxides were determined using a modified method of Shantha and Decker (1994). Oil or emulsions were mixed with 2.8 mL of methanol/1-butanol (2:1, v/v) and 30 μL of thiocyanate/Fe^{2+} solution and then vortexed. The thiocyanate/Fe^{2+} solution was made by mixing one part of a 3.94 M thiocyanate solution with one part of a 0.072 M Fe^{2+} solution (obtained from the supernatant of a mixture of one part of 0.144 M $FeSO_4$ and one part of 0.132 M $BaCl_2$ in 0.4 M HCl). After 20 min, the absorbance was measured at 510 nm using a UV–VIS scanning spectrophotometer (Shimadzu UV-2101PC, Kyoto, Japan). Lipid hydroperoxide concentrations were determined using a cumene hydroperoxide standard curve.

2.5. Synthesis of phytosterol oxides

Standards of phytosterol oxides are not commercially available, so they were produced using thermo-oxidation and chemical synthesis (Conchillo et al., 2005), depending on the type of oxides to be obtained. In brief, 8 mL of a β-sitosterol solution (1 mg/mL *n*-hexane:isopropanol (3:2, v/v)) were placed in a 200-mL open vial and the solvent was evaporated with nitrogen. Afterwards, it was thermo-oxidized by heating at 150 °C for 2 h in an oven, in order to form the 7α-hydroxy, 7β-hydroxy, α-epoxy, β-epoxy and 7-keto derivatives (mixture A). The resulting thermo-oxidized mixture was dissolved in 8 mL of *n*-hexane:isopropanol (3:2, v/v). This process was repeated with a solution of stigmasterol, obtaining mixture B.

Epoxy and triol derivatives of phytosterols were also synthesized in the laboratory, as suggested by Dzeletovic, Breuer, Lund, and Diczfalusy (1995). Phytosterol standards were stirred for 2 h with *m*-chloroperbenzoic acid to form the epoxy derivatives, which were extracted with diethyl ether and purified by SPE. The epoxy derivatives were then refluxed with methanol:6M HCl (5:1, v/v) for 2 h, giving rise to the triol derivatives. The latter were extracted with diethyl ether and purified by SPE, thus obtaining solutions C (containing sitostanetriol and campestanetriol) and D (containing stigmastentriol).

To separate the β-sitosterol oxides, about 250 μL of mixture A and 100 μL of mixture C were loaded on a 20 × 20 cm TLC plate. A COP solution (30 μL of a standard mixture containing 0.15 mg of each COP/mL of *n*-hexane:isopropanol (3:2, v/v)) was also loaded in the same TLC plate as reference for identification of analogous derivatives of phytosterols. The mobile phase was a mixture of diethyl ether:*n*-hexane:ethanol (70:30:3, v/v/v). The TLC bands of the oxides were visualized by spraying with a 0.2% ethanolic solution of 2′,7′-dichlorofluorescein sodium salt, under UV light (254 nm). The bands were then scrapped off and the extraction of pure oxide derivatives from the silica was carried out twice with diethyl ether. The solvent was finally evaporated under nitrogen flow at room temperature and pure derivatives were then dissolved in *n*-hexane-isopropanol (3:2, v/v). The same procedure was followed for the identification of stigmasterol oxides (mixture B and D). The different phytosterol oxide fractions were then subjected to silylation by using Tri-Sil® Reagent (Pierce, Rockford, IL, USA) according to the procedure of Sweeley et al. (Sweeley, Bentley, Makita, & Wells, 1963), dissolved in *n*-hexane and injected into GC and GC–MS for identification.

2.6. Determination of total phytosterols and phytosterol oxides

19-Hydroxycholesterol (0.020 mg in *n*-hexane:isopropanol (3:2, v/v); internal standard for the quantification of sterol oxides) and 5α-cholestane (0.2 mg in *n*-hexane:isopropanol (3:2, v/v); internal standard for the quantification of sterols) were placed in 15-mL vials. The solvent was then evaporated with nitrogen and approximately 165 mg of the corn oil or oil-in-water emulsion was added. Ten milliliters of 1N KOH solution in methanol were added to perform a cold saponification at room temperature for 18 h in darkness and under continuous agitation (Sander, Addis, Park, & Smith, 1989). The unsaponifiable material was extracted with diethyl ether.

For the determination of sterols, 10% of the unsaponifiable matter was subjected to silylation (Sweeley et al., 1963), dried under nitrogen and dissolved in 100 μL of *n*-hexane. One microliter of the silylated sterols were injected into GC and GC–MS for quantification and identification purposes, respectively.

For determination of POPs, the remaining 90% of the unsaponifiable matter was purified by silica SPE according to Guardiola, Codony, Rafecas, and Boatella (1995). POPs were eluted with acetone. The purified fraction was then silylated, dried under nitrogen stream and dissolved in 50 μL of *n*-hexane. One μL of the silylated sterol oxides was injected into GC and GC–MS for quantification and identification purposes, respectively, under the same analytical conditions used for the determination of sterols.

A GC Hewlett-Packard (HP) Model 5890 (Agilent Technologies, CA, USA) coupled with HP 3396 Series II integrator was equipped with a split–splitless injector and a flame ionization detector (FID). A fused silica capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) coated with 5% phenyl–95% dimethylpolysiloxane (SPB-5, Supelco, Bellefonte, PA, USA) was used. The oven temperature was programmed from 280 °C (kept for 10 min) to 300 °C at a rate of 0.8 °C/min and then raised to 320 °C at a rate of 10 °C/min and held for 10 min. The injector and detector temperatures were both set at 325 °C. Helium was used as a carrier gas at a flow rate of 2.5 mL/min; the split ratio was 1:20. Under the analytical condition used, not all POP peaks were completely resolved. Despite this fact, the GC method used was able to separate the 7-keto derivatives of sterols, without having interferences from other POPs.

Quantification of sterols was performed by comparing the peak areas of the internal standard (5α-cholestane)

and its concentration, with the peak areas of the sample sterols; the same procedure was followed for the quantitation of POPs, using the peak area and concentration of 19-hydroxycholesterol (internal standard). The GC response factors of sterols and POPs were considered equal to 1. The limit of quantitation (LOQ) of POPs was 0.5 mg/kg sample, which was calculated as a signal to noise ratio equal to 6:1.

Identification of sterols and the synthesized phytosterol oxides was performed by GC–MS. A GC Hewlett-Packard 6890 coupled to a 5973 mass selective detector (Agilent Technologies, CA, USA), was used. The system was fitted with a capillary SPB-5 column coated with 5% phenyl-95% dimethylpolysiloxane (30 m × 0.25 mm i.d. × 0.25 μm film thickness; Supelco, Bellefonte, PA, USA) and helium was used as carrier gas (1 mL/min). The oven temperature was programmed from 250 °C to 310 °C at rate of 0.8 °C/min. The injector and transfer line temperatures were set at 310 °C and 280 °C, respectively. Manual injection of 1 μL of the solution of standard phytosterol oxides and those obtained from oil samples, was performed in the split mode at 1:10 split ratio. The filament emission current was 70 eV. A mass range from 40 to 650 *m/z* was scanned at a rate of 1500 amu/s. Identification of phytosterols was performed by comparing the retention time and mass spectra with those of the TLC bands of the synthesized compounds, as well as with those reported in literature (Dutta, 2002).

2.7. Measurement of interfacial tension

Interfacial tension was determined using a digital tensiometer K 10 ST (Kruss USA, Charlotte, NC, USA) equipped with a platinum–iridium Du Noüy ring (Nuchi, Hernandez, McClements, & Decker, 2002). High purity standards of cholesterol, phytosterols and 5α-cholestane at different concentrations (0–5 mmol/kg) were dissolved in hexadecane (40 g) prior to being layer over double distilled water (40 g) followed by equilibration at 30 °C for 24 h. Preliminary measurements performed by using the digital tensiometer showed that the interfacial tension decreased during the first 24 h and then it reached a plateau indicating equilibrium; therefore, measurements were taken after 24 h.

2.8. Statistical analysis

Statistic analysis was performed using one-way analysis of variance (ANOVA). Mean multiple comparison were achieved using Duncan's multiple range test (Snedecor & Cochran, 1989). All experiments were conducted on triplicate samples.

3. Results and discussion

The phytosterol concentration in the corn oil was 6481 ± 220 mg/kg oil with β-sitosterol (59.7%), campesterol (19.0%) and stigmasterol (7.2%) being the most abundant phytosterols. These data are similar to those reported by Lercker, Frega, Conte and Capella (1981) and Serani and Piacenti (1992).

Oxidation of phytosterols can give rise to a number of products including ketones, alcohols, epoxides and dienes. The amount of phytosterol oxides and their distribution depends on several factors, such as sterol structure, matrix composition and reaction temperature (Lampi, Juntunen, Toivo, & Piironen, 2002; Soupas et al., 2004). The 7-keto derivative is the major phytosterol oxidation product in emulsified spreads (Conchillo et al., 2005), thus 7-keto derivatives represent a simple, reliable marker of the extent of phytosterol oxidation in food (Cercaci et al., 2006). Although the distribution of POP may depend on the oxidation phase/status as well (Kemmo, Soupas, Lampi, & Piironen, 2005), the 7-keto derivatives were the most abundant POPs during the whole experiment and, therefore, 7-keto derivatives of β-sitosterol, campesterol and stigmasterol were used in this study to follow phytosterol oxidation.

The formation of lipid hydroperoxides in bulk oil was detected after 10 days of storage at 55 °C (Fig. 1). Headspace hexanal concentrations increased after 30 days of storage (Fig. 1). The earlier appearance of hydroperoxides compared to hexanal is likely due to the fact that primary lipid oxidation products, such as hydroperoxides, must be produced and decomposed before secondary lipid oxidation products, such as hexanal, can be formed. Increases in the 7-keto derivatives of phytosterols were detected after 30 days of storage of the bulk oil (Fig. 2).

In the oil-in-water emulsion, lipid hydroperoxides increased after 0.6 days ($p \leqslant 0.01$) of storage at 55 °C (Fig. 3). Hydroperoxide concentrations increased rapidly until 3 days of storage after which they decreased rapidly. The decrease in hydroperoxide concentrations is likely due to rapid hydroperoxide breakdown during the later stages of oxidation. This trend of a fast increase followed by a rapid decrease in lipid hydroperoxide concentrations has

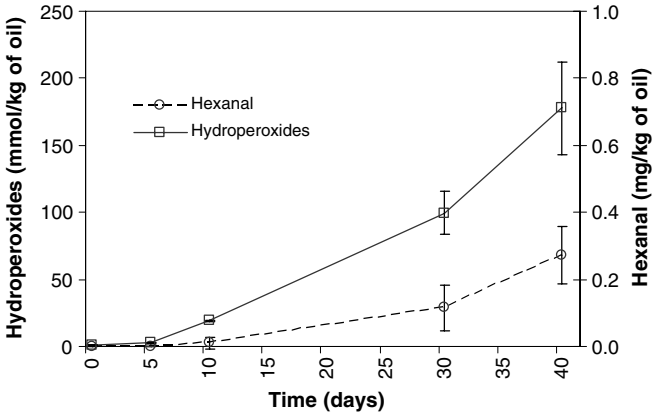

Fig. 1. Lipid hydroperoxide and headspace hexanal concentrations in bulk corn oil stored at 55 °C. Data points represent means ($n = 3$) ± standard deviations (some error bars may lie within the data points).

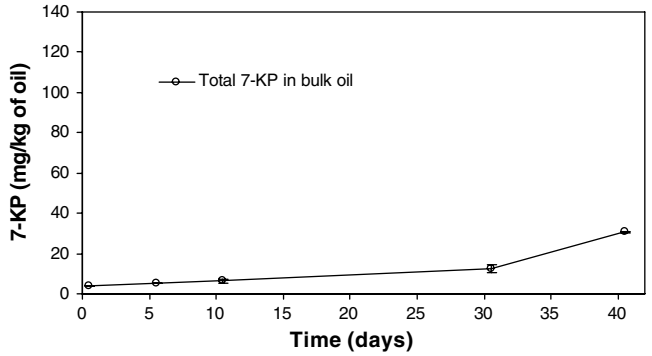

Fig. 2. Concentrations of 7-keto derivatives of phytosterols (7-KP) in bulk corn oil stored at 55 °C. Data points represent means ($n = 3$) ± standard deviations (some error bars may lie within the data points).

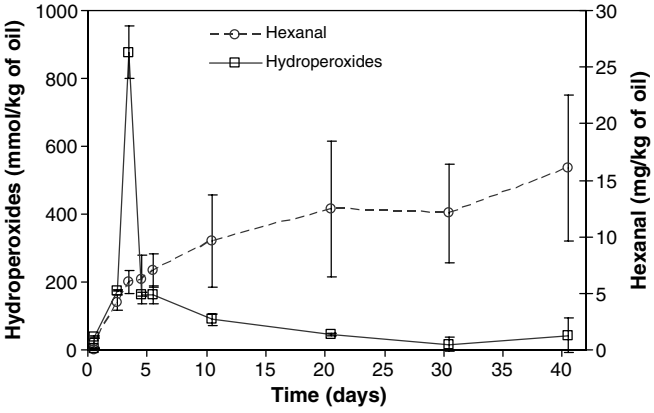

Fig. 3. Lipid hydroperoxide and headspace hexanal concentrations in corn oil-in-water emulsions stored at 55 °C. Data points represent means ($n = 3$) ± standard deviations (some error bars may lie within the data points).

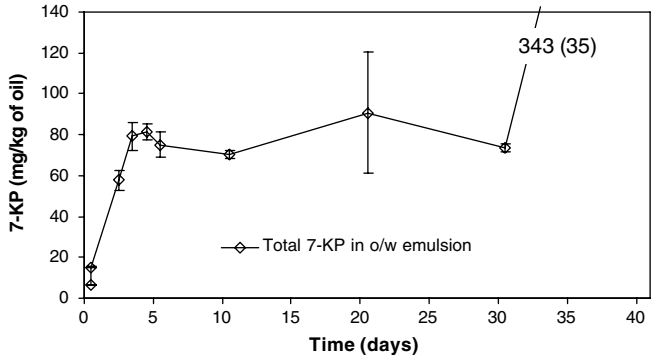

Fig. 4. Concentrations of 7-keto derivatives of phytosterols (7-KP) in corn oil-in-water emulsions stored at 55 °C. Data points represent means ($n = 3$) ± standard deviations (some error bars may lie within the data points). The 7-keto derivative concentration in the upper right hand portion of the graph is the concentration at 40 days of storage with the standard deviation in parenthesis.

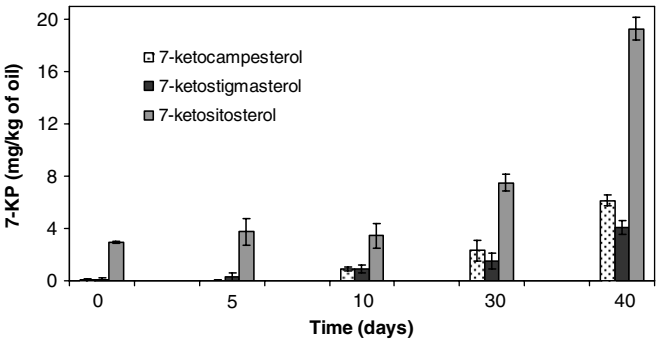

Fig. 5. Concentrations of 7-keto derivatives (7-KP) of campesterol, stigmasterol and β-sitosterol in bulk corn oil stored at 55 °C. Data points represent means ($n = 3$) ± standard deviations (some error bars may lie within the data points).

been previously reported in other oil-in-water emulsion systems (Mei, Decker, & McClements, 1998; Mancuso, McClements, & Decker, 1999). Headspace hexanal was also found to rise after 0.6 days of storage, reaching a maximum at 40 days of storage (Fig. 3). The 7-keto phytosterols increased from 0.6 till 3 days of storage ($p \leqslant 0.001$), after which concentrations remained roughly constant for about 30 days, followed by a sharp increase at the end of the storage period (Fig. 4). The cause of the rapid increase in POPs concentrations at the end of the experiment is not known. Hypothetically this behavior may be due to a bimolecular phase of the reaction, where the 7-hydroperoxide derivatives of phytosterols have to reach a certain concentration in the interface region of the oil-in-water emulsion after which they are rapidly decomposed into secondary oxidation products, such as 7-keto derivatives. Alternatively, this behavior could be due to co-oxidation, where accumulation of high concentrations of fatty acid oxidation products during the later stages of storage caused a rapid oxidation of the phytosterols.

Figs. 5 and 6 show the concentrations of β-sitosterol, campesterol and stigmasterol 7-keto derivatives in the bulk

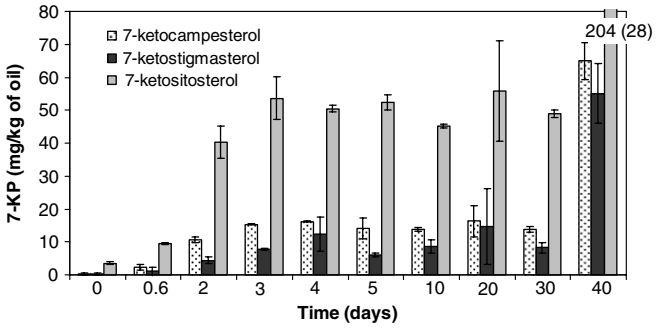

Fig. 6. Concentrations of 7-keto derivatives (7-KP) of campesterol, stigmasterol and β-sitosterol in corn oil-in-water emulsions stored at 55 °C. Data points represent means ($n = 3$) ± standard deviations (some error bars may lie within the data points). The 7-keto derivative concentration in the upper right hand portion of the graph is the concentration at 40 days of storage with the standard deviation in parenthesis.

oil and oil-in-water emulsion, respectively. The formation patterns of the 7-keto derivatives were calculated for the phase of oxidation where the amounts of POP remained roughly constant in the oil-in-water emulsion (2–30 days)

and the bulk oil (5–30 days). In general, the 7-keto distribution was similar, except for stigmasterol; in fact, 7-keto derivatives of β-sitosterol and campesterol in the emulsion were 1.04% and 1.12% of total phytosterols, respectively, whereas the bulk oil had 0.09% and 0.12% of 7-ketositosterol and 7-ketocampesterol. It must be noted, however, that the 7-keto derivatives of stigmasterol in the oil-in-water emulsion and bulk oil were higher (1.70% and 0.20%, respectively).

Both lipid hydroperoxides and hexanal formation was faster in the emulsified oil than in the bulk oil. This is not surprising since the surface area of the oil in the emulsion is high. Hexanal is a useful marker for the oxidation of omega-6 fatty acids. In corn oil, the major omega-6 fatty acid is linoleic acid, representing 55.9% of the total fatty acids [followed by oleic acid (26.7%), palmitic acid (14.0%), stearic acid (1.5%) and linolenic acid (0.8%)]. Polyunsaturated fatty acids, such as linoleic acid, are generally 10–40 times more susceptible to oxidation than monounsaturated ones (Frankel, 1998). Therefore, it was somewhat surprising that the length of the lag phase for the oxidation of the monounsaturated phytosterols was similar to polyunsaturated fatty acids in the oil-in-water emulsions.

In oil-in-water emulsions, oxidation has been postulated to occur at the emulsion droplet interface, due to the surface activity of lipid hydroperoxides that can be decomposed into free radicals by iron at the emulsion droplet surface or in the aqueous phase (Mei et al., 1998). Oxidation reactions in bulk oils could also depend on surface chemistry, since all oils contain small amounts of water dispersed by surface active agents (e.g. free fatty acids, phospholipids and mono or diacylglycerols). Therefore, one explanation for the higher than expected oxidation rate of the monounsaturated phytosterols would be that they concentrate at the lipid–water interface where oxidative stress is high. Phytosterols at the emulsion droplet interface could be especially susceptible to oxidation, since their oxidatively sensitive hydrophilic hydroxyl group and the double bond would be oriented towards the aqueous phase. To determine this possibility, the surface activity of phytosterols was determined by measuring interfacial tension (Fig. 7). 5α-Cholestane did not decrease the interfacial tension of hexadecane/water, suggesting that either the double bonds or the hydroxyl groups are necessary for surface activity. The ability of the other sterols to reduce interfacial tension was in the order of stigmasterol > cholesterol > β-sitosterol.

Differences in the ability of these sterols to reduce interfacial tension are related to their surface activity, as well as their physical structure that would allow them to pack tightly at the oil–water interface. Sterols have a planar, rigid structure that allows them to pack together tightly. The combination of surface activity and the ability to pack together tightly could allow the phytosterols to concentrate at oil–water interfaces. Differences in the surface activity of the phytosterols could influence their susceptibility to oxidation, since the more surface active (stigmasterol) oxidized to a greater extent than the less surface active (β-sitosterol). More research is needed to determine if this is true in complex food oils, where the concentration of the phytosterols at an oil–water or oil–air interface would not only be dependent on their surface activity but also on their concentrations. For instance, the corn oil used in this study contained 9.3 mM β-sitosterol, 3.1 mM campesterol and 1.1 mM stigmasterol. Thus, even though stigmasterol had a higher surface activity than the other sterols, its low concentration could result in its displacement from the oil–water or oil–air interface by high concentrations of other surface active compounds, such as other phytosterols, free fatty acids, phospholipids and mono- or diacylglycerols.

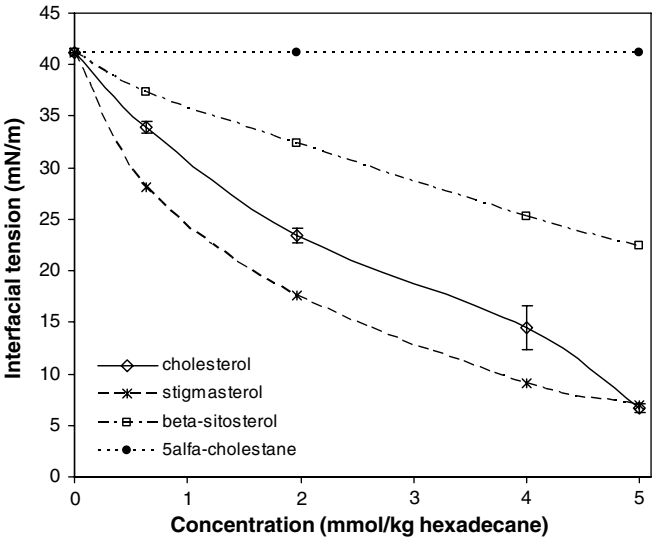

Fig. 7. Interfacial tension of various sterols in a water/hexadecane bilayer.

4. Conclusions

Phytosterols were found to oxidize during the storage of oil or oil-in-water emulsions. Phytosterol and unsaturated fatty acid oxidation was faster in the emulsions than bulk oil presumably due to the high surface area of the oil that would allow more interactions with aqueous phase prooxidants. The length of the lag phase for the oxidation of the monounsaturated phytosterols was similar to that of polyunsaturated fatty acids in the oil-in-water emulsion. Since the phytosterols were found to be surface active, this suggests that the phytosterols could be more susceptible to oxidation, since they can accumulate at oil–water interfaces where oxidative stress is high.

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