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Review

Recent advances in Phytosterol Oxidation Products

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ABSTRACT

Phytosterols and their oxidation products have become increasingly investigated in recent years with respect to their roles in diet and nutrition. We present a comprehensive review of recent literature on Phytosterol Oxidation Products (POP) identifying critical areas for future investigation. It is evident that POP are formed on food storage/preparation; are absorbed and found in human serum; do not directly affect cholesterol absorption; have evidence of atherogenicity and inflammation; have distinct levels of cytotoxicity; are implicated with high levels of oxidative stress, glutathione depletion, mitochondrial dysfunction and elevated caspase activity.

Contents

1. Introduction

Phytosterols are natural products which the past decade has seen a marked increase in their incorporation into nutraceutical formulations and diet. Phytosterols are commonly added to fortified foods as a phytosterol blend for economic reasons containing (but not limited to) β-sitosterol, stigmasterol, campesterol and dihydrobrassicasterol (Fig. 1). Although proven to exert health benefits via the lowering of low density lipoprotein cholesterol concentrations, the phytosterols are not without their problems due to their oxidative susceptibility.

Because of their inherent molecular structure, and close structural similarity to cholesterol, phytosterols are susceptible to

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oxidation to form hydroxy, epoxy, keto and triol derivatives which are collectively known as Phytosterol Oxidation Products (POP, Fig. 2). These derivatives have diverse biological functions of eminent interest to clinicians and this review will focus on the most recent developments in the POP field since the excellent review by Garcia-Llatas. [1]

2. Oxidation of phytosterols

Sterol autoxidation is oxidation of the steroid nucleus in an adventitious manner through storage, processing and preparation of foodstuffs whereas enzymatic oxidation is the biotransformation of phytosterols to their oxides via enzymatic pathways *in vivo*. POP may be generated *in vivo* through non-enzymatic oxidation of phytosterols as has been demonstrated for cholesterol [2] or by enzymatic oxidation [3]. Enzymatic activity of the intestinal microflora has been shown to result in the formation of cholesterol oxidation products (COP) [4] and could also result in POP formation *in vivo*. However, Grandgirard et al. [5] demonstrated that the plasma levels of sitostanetriol and campestanetriol were not increased following a 4 week feeding trial with a diet containing 1% phytosterol in rats. The authors concluded that the triol derivatives of phytosterols are not synthesised *in vivo* and it is highly probable that the presence of these compounds *in vivo* results from dietary origin. Similarly, there was no increase observed in the plasma levels of 7α-hydroxy or 7β-hydroxy derivatives in hamsters, following the consumption of stigmasterol or β-sitosterol-enriched diets [6].

3. Synthesis of POP

Significant steps have been taken towards the development of pure standards with a number of routes now published. A total synthesis approach was used in order to explore the chemistry of dihydrobrassicasterol and campesterol with concomitant synthesis of their oxides (Scheme 1) [7–9]. A similar route was chosen for the stigmasterol oxides from commercially available stigmasterol [10]. A different strategy for the synthesis of POP from a phytosterol mixture was recently described using semi-preparative HPLC as the purification method [11]. To this end, a mixture of phytosterols including avenasterol, brassicasterol, campesterol and β-sitosterol was converted to a complete mixture of POP which were assessed by chromatography and separated into their individual components. Four individual phytosterol oxides are reported as proof of this strategy: 7-ketocampesterol, 7-keto-β-sitosterol, 7-β-OH-campesterol and 7-β-OH-β-sitosterol.

4. Content of POP in foods

Phytosterol enriched foods are increasingly common in marketed products; analysis of the phytosterol content and stability over time is an essential measure to ensure product safety. A recent assessment of phytosterol enriched dark chocolate over a 5 month period of storage using sensory analysis and GCMS compared a formulation with palm oil, one with phytosterol esters and one with phytosterol esters and antioxidants [12]. Chemical analysis of the samples revealed the following: hydroperoxide concentrations peaked at 60 days for the samples held at 20 °C whereas in the samples held at 30 °C, the POP peaked at 30 days; antioxidants used (ascorbic acid and α-tocopherol) had little effect on storage outcome; the most commonly identified were 7-hydroxy, 7-keto, epoxides and triols with campesterol appearing the most susceptible.

Another recent study identified the degradation of phytosterols on storage over an 18 week period in an open environment. Rudzinska et al. [13] used GC and GCMS in order to quantify the phytosterol content of enriched margarines and identified that the phytosterol/phytostanol content dropped on storage at 4 and 20 °C from 7.9% to 6.3 and 5.5% respectively. In addition an increase in POP was seen for both temperatures with twice the original levels seen after storage at 20 °C. The most common oxidation product was seen to be the 7-hydroxy, followed by 7-keto and 5,6-epoxide. There is an obvious trend for loss of sterol/stanol content and formation of POP on storage at higher temperatures.

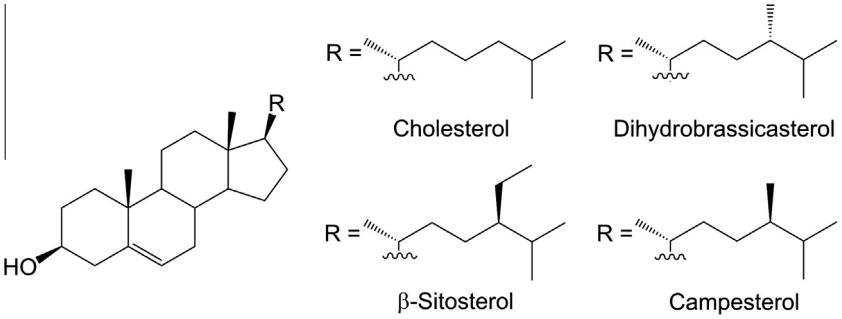


Fig. 1. Structures of cholesterol and common phytosterols.

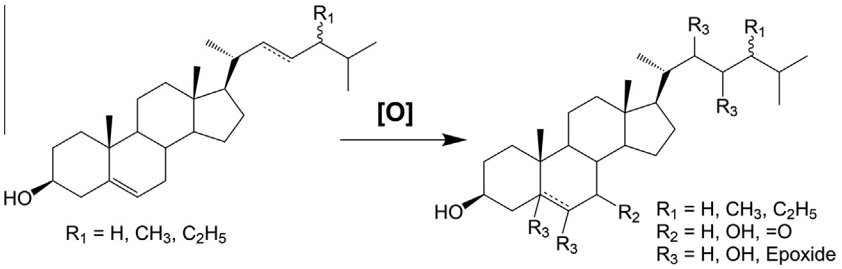
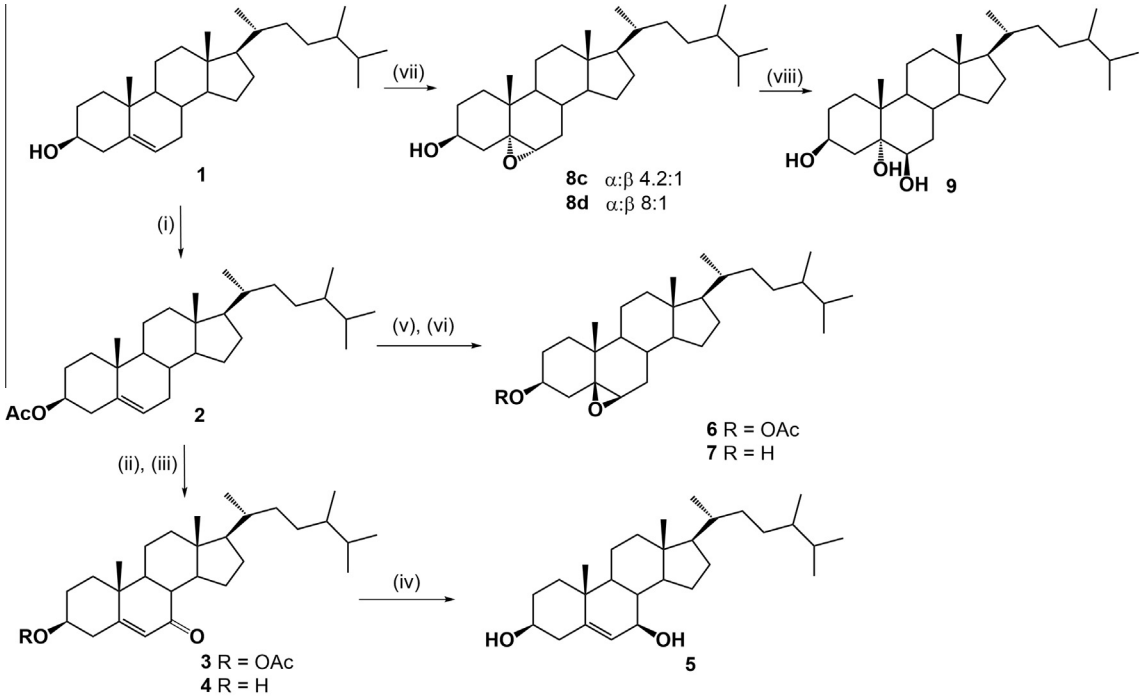


Fig. 2. Formation and general structure of the common oxides of cholesterol and phytosterols.



(i) Ac_2O, pyridine (ii) CrO_3, dimethylpyrazole, CH_2Cl_2 -20 to 5 °C (iii) K_2CO_3, MeOH, H_2O (iv) $CeCl_3.7H_2O$, $NaBH_4$, MeOH
(v) $KMnO_4.CuSO_4.5H_2O$, *t*-BuOH, H_2O, CH_2Cl_2 (vi) Na_2CO_3, MeOH (vii) *m*CPBA, CH_2Cl_2 (viii) H_2SO_4, $THF.H_2O$.

Scheme 1. A common route to the synthesis of phytosterol oxides.

5. Content of POP detected in human serum

A validated method for the analysis of 7-oxyphytosterols has been described via isotope dilution GCMS [14]. The limits of detection and quantification reported (7 and 23 pg/mL) are a significant improvement and allows for studies of minute quantities of POP. Use of this method (which incorporates sample preparation with BHT to avoid auto-oxidation) has allowed for analysis of oxyphytosterols in sixteen subjects consuming a plant sterol enriched margarine for 1 month. The study shows an increase of 7-β-OH-β-sitosterol and correlates the levels of campesterol with the total 7-oxycampesterol levels and β-sitosterol with the total 7-oxy-β-sitosterol levels.

In a study designed to establish the factors affecting human plasma oxysterol variations, Baumgartner et al. [15] set out to measure oxyphytosterols in forty-three subjects during a randomized trial for 4 weeks while consuming a control, plant sterol or plant stanol margarine. Plasma concentrations measured by GCMS indicated that despite consumption profile, β-sitosterol and campesterol concentrations did not correlate with oxyphytosterol concentration and that the concentration of oxyphytosterols remained stable over time. A key finding however is that concentration of oxycampesterols is reduced on consumption of a stanol diet, in particular 7-β-OH-campesterol and 7-ketocampesterol.

Mechanism of oxidation did not directly correlate with oxyphytosterol concentration but there appears to be a distinctive profile for individual subjects with a number of low and high oxidizers classified [16]. This oxidizing profile is not attributed to levels of α-tocopherol, TEAC, iron, ferritin, transferrin, copper or ceruloplasmin despite an evident correlation with oxLDL concentrations suggesting an as yet undefined relationship with oxidative stress.

Development of a 2D GCMS method for the analysis of ten POP (7-α, 7-β OH and 7-keto phytosterols) present at low levels in plasma has enabled some other POP derivatives to be uncovered (6-OH, epoxy and triols). The use of GC × GC separation allows for better selectivity of analysis with pg/mL limits of detection [17].

6. Absorption and distribution of POP

The absorption and distribution of POP from the diet has been assessed in a number of rodent models and the findings were previously reviewed [1,18,19]. Generally, the absorption of POP is higher than that of unoxidised phytosterol [20,21]. The oxides with the highest recovery in lymph were found to be the 7α-hydroxy and 7β-hydroxy derivatives and the absorption of epoxy and 7-keto derivatives was significantly lower. Importantly, studies show that campesterol oxides were absorbed better than sitosterol oxides possibly due to structural differences [21].

POP distribution following prolonged feeding trials (2–9 weeks) has been investigated in mice and hamsters. Hamsters fed a diet containing β-sitosterol and campesterol oxides (0.01%, 0.05% or 0.25%) over a 2 week period demonstrated a dose dependent increase in the POP content of each of the plasma, aorta, liver, kidney and heart at the two higher POP concentrations [22]. 7-Ketositosterol was the POP present at highest concentrations in the diet but was not detected at highest concentrations in the tissues indicating either a low uptake or rapid metabolism [5,20]. With the exception of plasma, the POP detected at highest concentrations in each of the tissues was sitostanetriol and authors postulated that the metabolism of sitostanetriol is slow.

An increase in the POP content of the serum, liver and aorta was also observed in apolipoprotein-E deficient mice fed a 0.02% POP diet for 9 weeks. The POP at highest concentrations in the diet were 7-keto derivatives of campesterol and β-sitosterol and the POP at highest concentrations in tissues were 7β-hydroxy derivatives [21]. Bang et al. [23] fed a diet containing 0.02% POP to C57BL/6J mice for 4 weeks. Again, 7-keto derivatives were present at highest concentrations in the feed while the POP present at highest

concentrations in the plasma and liver were 7α-hydroxy and 7β-hydroxy.

7. Effect of POP on cholesterol uptake and metabolism

Elevated plasma cholesterol levels are a contributing factor to the development of atherosclerosis. The widely accepted explanation for the cholesterol lowering effects of phytosterols is their competition for incorporation into micelles, however they may also reduce cholesterol absorption through regulation of the proteins and enzymes involved in cholesterol uptake and metabolism [24]. Recent studies have investigated the ability of phytosterols and POP to alter the expression of several cholesterol metabolising proteins and enzymes in hamsters fed a diet containing 0.1% of either β-sitosterol, β-sitosterol oxides, stigmasterol or stigmasterol oxides [6] and in Caco-2 intestinal epithelial cells exposed to 7-ketostigmasterol or 7-ketocholesterol [25].

Liang et al. [6] found that cholesterol absorption was decreased and that plasma levels of low-density lipoprotein cholesterol and triglycerides were reduced in hamsters fed the phytosterol diets but not in hamsters fed the POP diets. Further investigation revealed that in the former there was a reduction in the expression of intestinal acyl CoA: cholesterol acyl transferase (ACAT, esterifies cholesterol within the enterocyte), and microsomal triglyceride protein (MTP, facilitates the packaging of cholesterol into chylomicrons). The expression of ABCG5, which controls the efflux of cholesterol from the enterocyte, was reduced in hamsters fed both diets. No alteration was observed in the expression of Niemann-Pick C1-like 1 (NPC-1L1), a transporter protein which facilitates the absorption of micellular cholesterol and other sterols from the intestinal lumen, in hamsters fed either the phytosterol or POP diets. Alemany et al. [25] confirmed that POP do not alter the expression of NPC-1L1, observing no change in the expression of NPC-1L1 in Caco-2 cells incubated with 60 μM 7-ketostigmasterol. A decrease in the expression of cholesterol efflux transporters, ABCG5 and ABCG8, was observed.

The consumption of a POP enriched diet in hamsters (0.1% β-sitosterol oxides or 0.1% stigmasterol oxides) resulted in a down-regulation of sterol element binding protein 2 (SREBP-2) which is activated in response to low sterol status and promotes genes including low-density lipoprotein receptor (LDLR), responsible for the removal of LDL-cholesterol from blood, and 3-hydroxy-3-methylglutary-CoA (HMG-CoA) reductase, which is the rate limiting enzyme in cholesterol synthesis [6]. A decrease in LDLR and HMG-CoA reductase expression was also observed in hamsters consuming the stigmasterol oxide enriched diet but not the β-sitosterol oxide enriched diet. In contrast, Alemany et al. [25] found the expression of HMG-CoA reductase was increased in Caco-2 intestinal cells exposed to 7-ketostigmasterol which could result in increased cholesterol synthesis. Overall, it would appear that phytosterols regulate cholesterol absorption by decreasing esterification within the enterocyte, reducing the packaging of cholesterol into chylomicrons and that POP do not possess any cholesterol-lowering activity.

Liver x receptor α (LXRα) has been highlighted as a target for the reduction of cholesterol absorption and proposed benefits of LXR agonists include potential therapies for atherosclerosis, diabetes and cancer [26]. LXRα upregulates ABCG5 and ABCG8 and causes an efflux of cholesterol and phytosterols to the intestinal lumen. Oxysterols and phytosterols but not POP have previously demonstrated the capacity to activate LXRα [27].

8. POP and atherosclerosis

Studies have investigated if POP, similar to COP, have a role in the formation of atherosclerotic plaques. Tomoyori et al. [21] found that the size of atherosclerotic lesions did not increase in apolipoprotein E-deficient mice after a 9 week consumption of a diet enriched with POP and concluded that POP are not involved in the pathogenesis of atherosclerosis. Plat et al. [28] also found that following a 35 week consumption of a POP enriched diet there was no increase in lesion size but the proportion of severe atherosclerotic lesions was increased in LDLR +/- mice, a model for human mild hypercholesterolemia. A phytosterol enriched diet reduced atherosclerotic plaque formation and improved the functionality of the aorta in hamsters but these benefits were not observed for hamsters fed a POP enriched diet [6]. Yang et al. [29] found that a mixed oxide derivative of β-sitosterol attenuated vasorelaxation, a marker of vascular endothelial health, in isolated rat aorta indicating that POP may impair the functionality of the aorta. The attenuation of vasorelaxation was associated with an increase in the production of reactive oxygen species (ROS) and an upregulation of COX-2. Therefore, some evidence exists for the potential atherogenicity of POP.

9. POP and inflammation

Inflammation, which is characterised by elevated levels of inflammatory cytokines, is associated with diseases including Alzheimer's disease and cardiovascular disease. The 7β-epimers of 7-hydroxysitosterol were found to have a greater inhibitory effect on 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced inflammation than the 7α-epimer when topically applied to the mouse ear [30]. Vejux et al. [31] found that 7β-hydroxysitosterol did not affect the secretion of pro-inflammatory cytokines, monocyte chemotactic protein-1 (MCP-1) or interleukin-8 (IL-8) and 7-ketositosterol caused a decrease in IL-8 secretion, in U937 cells. Similarly, Plat et al. [28] found no change in serum concentrations of MCP-1 and tumour necrosis factor-α (TNF-α) in response to the consumption of a POP enriched diet, in mice. However, an increase in the production of the pro-inflammatory cytokines, TNF-α and IL-8 was observed in human colon adenocarcinoma (Caco-2) cells exposed to 60 μM 7-ketostigmasterol [25]. The authors suggested that the increase in TNF-α may have important consequences as it affects the integrity of the intestinal epithelium [32].

10. POP and cytotoxicity

Adcox et al. [33] first demonstrated that POP were cytotoxic to cells in culture with higher concentrations of POP required to elicit similar effects to COP. Vejux et al. [31] found that POP had no COP-like side effects in U937 cells, when similar concentrations were compared. Recent advances have allowed for the generation of pure oxides of phytosterols and the cytotoxicity of β-sitosterol oxides [19]; stigmasterol oxides [10]; dihydrobrassicasterol (95% purity) oxides [7]; and campesterol/dihydrobrassicasterol (67/33% mixture) oxides [8] have been investigated. Several POP have demonstrated cytotoxicity in a number of cell lines and the mechanism of cell death induced by POP included apoptosis.

The highest levels of apoptosis were found in cells exposed to β-sitosterol oxides, followed by campesterol oxides, stigmasterol oxides and dihydrobrassicasterol oxides, where equivalent oxides were compared. The triol derivatives were found to be the most cytotoxic but, in general, the 7β-hydroxy and 7-keto derivatives induced the highest levels of apoptosis, in U937 cells [7,8,10,19]. Novel derivatives of stigmasterol, 5,6,22,23-diepoxystigmastane and 5,6-epoxystigmasta-22*S*,23*S*-diol were notable for the high levels of apoptosis induced in U937 cells at relatively low (30 μM) concentrations [10].

The ability of a compound to induce apoptosis selectively in cancer cell lines is a useful indicator of the compounds potential

chemotherapeutic effects. The naturally occurring POP, 7α-hydroxysitosterol, extracted from the tropical plant *Chisocheton tomentosus* was found to be selectively cytotoxic, inducing cell death in three cancer cell lines (MCF-7; HSC-4; HepG2 cells) but not in a normal cell line (HMEC). Once triggered, apoptosis may progress via a number of pathways. The pathway of POP-induced apoptosis has not been widely studied, although the early stages of apoptosis have been linked to the generation of an oxidative stress [34].

11. Oxidative stress

An increase in superoxide production was found in response to all the β-sitosterol oxides investigated (7-keto, 7β-hydroxy, 7α-hydroxy, 6α-hydroxy-3-keto/6β-hydroxy-3-keto) in HepG2 cells and malondialdehyde was increased in response to exposure to two of the oxides (7-keto and 7α-hydroxy). Although all oxides were found to be cytotoxic only the 7-keto derivative induced apoptosis [35]. Ryan et al. [19] also demonstrated that although β-sitosterol oxides were cytotoxic to HepG2 cells they did not induce apoptosis. Maguire et al. [36] did not observe any alteration in the activity of the cellular antioxidant enzyme catalase in response to incubation with β-sitosterol oxides in U937 cells but apoptosis was induced. Apoptosis induced by 7β-hydroxysitosterol was not associated with the generation of ROS in Caco-2 cells, however cytotoxicity was reduced in the presence of the antioxidant vitamin C [37]. In contrast, Ryan et al. [19] found that the antioxidants α-tocopherol, γ-tocopherol and β-carotene afforded no protection against the apoptosis induced by β-sitosterol oxides. These antioxidants also did not protect against apoptosis induced by stigmasterol oxides with the exception of epoxydiol–stigmasterol [10].

A depletion of the antioxidant protein, glutathione, has been identified as one of the early events in COP induced apoptosis [38]. Maguire et al. [36] observed a decrease in glutathione at the 12hr timepoint following incubation of U937 cells with a mixed β-sitosterol oxide. Ryan et al. [19] found that of the five oxidised derivatives of β-sitosterol investigated, only 7β-hydroxysitosterol induced a depletion of glutathione and correspondingly, this oxide also induced the highest level of apoptosis. All of the stigmasterol oxides capable of inducing apoptosis (7β-hydroxy, epoxydiol and diepoxide derivatives) also caused a depletion of glutathione following a 24 h incubation in U937 cells [10].

12. Mitochondrial dysfunction in POP induced apoptosis

The loss of mitochondrial transmembrane potential, which may occur as a result of glutathione depletion and oxidative stress [39], results in the release of cytochrome *c* to the cytosol and the activation of proteases and enzymes which execute the apoptotic process [40]. Roussi et al. [37] observed a loss of mitochondrial transmembrane potential and a release of cytochrome *c* but no change in the apoptotic regulating proteins, Bcl-2 and Bax, during the incubation of Caco-2 cells with 7β-hydroxysitosterol. Tasyriq et al. [41] observed a downregulation of the extracellular signal-regulated kinase (ERK1/2) pathway, an increase in Bax protein and a decrease in Bcl-2 protein in MCF-7 cells exposed to 7α-hydroxysitosterol. Alemany et al. [25] also found a decrease in mitochondrial transmembrane potential in Caco-2 cells exposed to 60 μM 7-ketostigmasterol and there was a decrease in the Bcl-2 content of U937 cells incubated with stigmasterol oxides [10].

13. Caspases

Caspase-3, a cysteine protease, is a point of convergence for most apoptotic pathways and is generally regarded as the executioner of apoptosis. An increase in caspase-3 activity has been observed in U937 cells following incubation with three apoptotic stigmasterol oxides [10] and dihydrobrassicasterol oxides [7]. Tasyriq et al. [41] demonstrated that 7α-hydroxysitosterol caused a reduction of procaspase-9, procaspase-3 and procaspase-6 in MCF-7 cells. This investigation into the apoptotic pathway elicited by POP found that the pathway involved an increase in FasL, an inactivation of ERK1/2 and a dysregulation of the Bax/Bcl-2 ratio resulting in the cleavage of procaspase-9, -3 and -6 into their effector caspases. A depletion of glutathione and the loss of mitochondrial transmembrane potential also appear to be a common feature of POP-induced apoptosis [10,37]. Further study is required to determine the precise pathway of POP-induced apoptosis.

14. Current perspective

This review encapsulates the ongoing scientific quest to ascribe biological properties to phytosterol oxides. Though progress is clearly evident, it is obvious that POP require substantial future investigation to (i) elucidate their specific effects on human health; (ii) develop effective methods to measure their provenance and; (iii) minimise their production in foods and *in vivo*.

References

[1] G. García-Llatas, M.T. Rodríguez-Estrada, Current and new insights on phytosterol oxides in plant sterol-enriched food, Chem. Phys. Lipids 164 (6) (2011) 607–624.
[2] G. Maerker, E.H. Nungesser, F.J. Bunick, Reaction of cholesterol 5,6-epoxides with simulated gastric juice, Lipids 23 (8) (1988) 1–5.
[3] L. Aringer, P. Eneroth, L. Nordström, Side chain hydroxylation of cholesterol, campesterol and beta-sitosterol in rat liver mitochondria, J. Lipid Res. 17 (3) (1976) 263–272.
[4] K.K. Hwang, M.I. Kelsey, Evidence of epoxide hydrase activity in human intestinal microflora, Cancer Biochem. Biophys. 3 (1) (1978) 31–35.
[5] A. Grandgirard, L. Martine, P. Juanéda, C. Cordelet, Sitostanetriol is not formed in vivo from sitosterol in the rat, Reprod. Nutr. Dev. 44 (6) (2004) 609–616.
[6] Y.T. Liang, W.T. Wong, L. Guan, X.Y. Tian, K.Y. Ma, Y. Huang, Z.Y. Chen, Effect of phytosterols and their oxidation products on lipoprotein profiles and vascular function in hamster fed a high cholesterol diet, Atherosclerosis 219 (1) (2011) 124–133.
[7] O. Kenny, Y. O'Callaghan, N.M. O'Connell, F.O. McCarthy, A.R. Maguire, N.M. O'Brien, Oxidized derivatives of dihydrobrassicasterol: cytotoxic and apoptotic potential in U937 and HepG2 cells, J. Agric. Food Chem. 60 (23) (2012) 5952–5961.
[8] Y. O'Callaghan, O. Kenny, N.M. O'Connell, A.R. Maguire, F.O. McCarthy, N.M. O'Brien, Synthesis and assessment of the relative toxicity of the oxidised derivatives of campesterol and dihydrobrassicasterol in U937 and HepG2 cells, Biochimie 95 (3) (2013) 496–503.
[9] N.M. O'Connell, Y.C. O'Callaghan, N.M. O'Brien, A.R. Maguire, F.O. McCarthy, Synthetic routes to campesterol and dihydrobrassicasterol: a first reported synthesis of the key phytosterol dihydrobrassicasterol, Tetrahedron 68 (25) (2012) 4995–5004.
[10] Y.C. O'Callaghan, D.A. Foley, N.M. O'Connell, F.O. McCarthy, A.R. Maguire, N.M. O'Brien, Cytotoxic and apoptotic effects of the oxidized derivatives of stigmasterol in the U937 human monocytic cell line, J. Agric. Food Chem. 58 (19) (2010) 10793–10798.
[11] J. Gao, Q. Yue, Y. Ji, B. Cheng, X. Zhang, Novel synthesis strategy for the preparation of individual phytosterol oxides, J. Agric. Food Chem. 61 (2013) 982–988.
[12] P.B. Botehlo, M. Galasso, V. Dias, M. Mandrioli, L.P. Lobato, M.T. Rodriguez-Estrada, I.A. Castro. Oxidative stability of functional phytosterol-enriched dark chocolate. LWT Food Sci. Technol. (in press), doi: 10.1016/j.lwt.2013.09.002.
[13] M. Rudzinska, R. Przybylski, E. Wasowicz, Degradation of phytosterols during storage of enriched margarines, Food Chem. 142 (2014) 294–298.
[14] C. Huche, O. Weingartner, H. Petterson, T. Vanmierlo, M. Bohm, U. Laufs, D. Lutjohann, Validation of an isotope dilution gas chromatography–mass spectrometry method for analysis of 7-oxygenated campesterol and sitosterol in human serum, Chem. Phys. Lipids 164 (2011) 425–431.
[15] S. Baumgartner, R.P. Mensink, G. den Hartog, A. Bast, O. Bekers, C. Huche, D. Lutjohann, J. Plat, Oxyphytosterol formation in humans: identification of high vs. low oxidizers, Biochem. Pharmacol. 86 (2013) 19–25.
[16] S. Baumgartner, R.P. Mensink, C. Huche, D. Lutjohann, J. Plat, Effects of plant sterol- or stanol-enriched margarine on fasting plasma oxyphytosterol concentrations in healthy subjects, Atherosclerosis 227 (2013) 414–419.
[17] M. Menedes-Carreno, H. Steenbergen, H.-G. Janssen, Development and validation of a comprehensive two-dimensional gas chromatography–mass spectrometry method for the analysis of phytosterol oxidation products in human plasma, Anal. Bioanal. Chem. 402 (2012) 2023–2032.

[18] E. Hovenkamp, I. Demonty, J. Plat, D. Lütjohann, R.P. Mensink, E.A. Trautwein, Biological effects of oxidized phytosterols: a review of the current knowledge, Prog. Lipid Res. 47 (1) (2008) 37–49.
[19] E. Ryan, J. Chopra, F.O. McCarthy, A.R. Maguire, N.M. O'Brien, Qualitative and quantitative comparison of the cytotoxic and apoptotic potential of phytosterol oxidation products with their corresponding cholesterol oxidation products, Br. J. Nutr. 94 (3) (2005) 443–451.
[20] A. Grandgirard, J.P. Sergiel, M. Nour, J. Demaison-Meloche, C. Giniès, Lymphatic absorption of phytosterol oxides in rats, Lipids 34 (6) (1999) 563–570.
[21] H. Tomoyori, Y. Kawata, T. Higuchi, I. Ichi, H. Sato, M. Sato, I. Ikeda, K. Imaizumi, Phytosterol oxidation products are absorbed in the intestinal lymphatics in rats but do not accelerate atherosclerosis in apolipoprotein E-deficient mice, J. Nutr. 134 (7) (2004) 1690–1696. Erratum in: J. Nutr. 2004 Oct; 134(10):2738.
[22] A. Grandgirard, J. Demaison-Meloche, C. Cordelet, L. Demaison, Incorporation of oxyphytosterols in tissues of hamster, Reprod. Nutr. Dev. 44 (6) (2004) 599–608.
[23] H.J. Bang, C. Arakawa, M. Takada, M. Sato, K. Imaizumi, A comparison of the potential unfavorable effects of oxycholesterol and oxyphytosterol in mice: different effects, on cerebral 24S-hydroxychoelsterol and serum triacylglycerols levels, Biosci. Biotechnol. Biochem. 72 (12) (2008) 3128–3133.
[24] E. De Smet, R.P. Mensink, J. Plat, Effects of plant sterols and stanols on intestinal cholesterol metabolism: suggested mechanisms from past to present, Mol. Nutr. Food Res. 56 (7) (2012) 1058–1072.
[25] L. Alemany, J.M. Laparra, R. Barberá, A. Alegría, Relative expression of cholesterol transport-related proteins and inflammation markers through the induction of 7-ketosterol-mediated stress in Caco-2 cells, Food Chem. Toxicol. 56 (2013) 247–253, http://dx.doi.org/10.1016/j.fct.2013.02.040. Epub 2013 Feb 27.
[26] C.-P. Chuu, Modulation of liver X receptor signaling as a prevention and therapy for colon cancer, Med. Hypotheses 76 (5) (2011) 697–699.
[27] J. Plat, J.A. Nichols, R.P. Mensink, Plant sterols and stanols: effects on mixed micellar composition and LXR (target gene) activation, J. Lipid Res. 46 (11) (2005) 2468–2476.
[28] J. Plat, E. Theuwissen, C. Husche, D. Lütjohann, M.J. Gijbels, M. Jeurissen, R. Shiri-Sverdlov, I. van der Made, R.P. Mensink. Oxidised plant sterols as well as oxycholesterol increase the proportion of severe atherosclerotic lesions in female LDL receptor+/− mice. Br. J. Nutr. (in press), doi: 10.1017/S0007114513002018.
[29] C. Yang, Z.Y. Chen, S.L. Wong, J. Liu, Y.T. Liang, C.W. Lau, H.K. Lee, Y. Huang, S.Y. Tsang, β-Sitosterol oxidation products attenuate vasorelaxation by increasing reactive oxygen species and cyclooxygenase-2, Cardiovasc. Res. 97 (3) (2013) 520–532, http://dx.doi.org/10.1093/cvr/cvs370.
[30] Y. Kimura, K. Yasukawa, M. Takido, T. Akihisa, T. Tamura, Inhibitory effect of some oxygenated stigmastane-type sterols on 12-O-tetradecanoylphorbol-13-acetate-induced inflammation in mice, Biol. Pharm. Bull. 18 (11) (1995) 1617–1619.
[31] A. Vejux, T. Montange, L. Martine, A. Zarrouk, J.M. Riedinger, G. Lizard, Absence of oxysterol-like side effects in human monocytic cells treated with phytosterols and oxyphytosterols, J. Agric. Food Chem. 60 (16) (2012) 4060–4066.
[32] A. Fischer, M. Gluth, U.F. Pape, B. Wiedenmann, F. Theuring, D.C. Baumgart, Adalimumab prevents barrier dysfunction and antagonizes distinct effects of TNF-α on tight junction proteins and signaling pathways in intestinal epithelial cells, Am. J. Physiol. Gastrointest. Liver Physiol. 304 (11) (2013) G970–G979, http://dx.doi.org/10.1152/ajpgi.00183.2012.
[33] C. Adcox, L. Boyd, L. Oehrl, J. Allen, G. Fenner, Comparative effects of phytosterol oxides and cholesterol oxides in cultured macrophage-derived cell lines, J. Agric. Food Chem. 49 (4) (2001) 2090–2095.
[34] A. Samali, A.M. Gorman, T.G. Cotter, Apoptosis-the story so far, Experientia 52 (1996) 933–941.
[35] K. Koschutnig, S. Heikkinen, S. Kemmo, A.M. Lampi, V. Piironen, K.H. Wagner, Cytotoxic and apoptotic effects of single and mixed oxides of beta-sitosterol on HepG2-cells, Toxicol. In Vitro 23 (5) (2009) 755–762.
[36] L.S. Maguire, S.M. O'Sullivan, K. Galvin, T.P. O'Connor, N.M. O'Brien, Fatty acid profile, tocopherol, squalene and phytosterol content of walnuts, almonds, peanuts, hazelnuts and the macadamia nut, Int. J. Food Sci. Nutr. 55 (3) (2004) 171–178.
[37] S. Roussi, F. Gossé, D. Aoudé-Werner, X. Zhang, E. Marchioni, P. Geoffroy, M. Miesch, F. Raul, Mitochondrial perturbation, oxidative stress and lysosomal destabilization are involved in 7beta-hydroxysitosterol and 7beta-hydroxycholesterol triggered apoptosis in human colon cancer cells, Apoptosis 12 (1) (2007) 87–96.
[38] G. Lizard, S. Gueldry, O. Sordet, S. Monier, A. Athias, C. Miguet, G. Bessede, S. Lemaire, E. Solary, P. Gambert, Glutathione is implied in the control of 7-ketocholesterol-induced apoptosis, which is associated with radical oxygen species production, FASEB J. 12 (15) (1998) 1651–1663.
[39] B.V. Chernyak, P. Bernardi, The mitochondrial permeability transition pore is modulated by oxidative agents through both pyridine nucleotides and glutathione at two separate sites, Eur. J. Biochem. 238 (3) (1996) 623–630.
[40] S. Coppola, L. Ghibelli, GSH extrusion and and the mitochondrial pathway of apoptotic signalling, Biochem. Soc. Trans. 28 (2) (2000) 56–61.
[41] M. Tasyriq, I.A. Najmuldeen, L.L. In, K. Mohamad, K. Awang, N. Hasima, 7α-Hydroxy-β-sitosterol from chisocheton tomentosus induces apoptosis via dysregulation of cellular Bax/Bcl-2 ratio and cell cycle arrest by downregulating ERK1/2 activation, Evid. Based Complement Alternat. Med. (2012), http://dx.doi.org/10.1155/2012/765316. Article ID 765316.