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**Phytosterol oxidation products in foods: Analysis,
occurrence, exposure and biological effects**

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Phytosterols/-stanols and their fatty acid esters are able to reduce the serum cholesterol level and are therefore added to an increasing number of products claiming cholesterol lowering properties. An undesirable reaction that may be expected in these products is the formation of so-called phytosterol oxidation products, i.e. keto-, hydroxy- and epoxy derivatives of phytosterols/-stanols. The Senate Commission on Food Safety (SKLM) of the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) summarized and evaluated the current knowledge on the formation, intake and biological effects of phytosterol oxidation products and identified gaps in knowledge as well as research needs for a safety assessment, particularly in light of the potentially increasing dietary exposure to such compounds via the consumption of foods enriched with phytosterols/-stanols and their esters. The following opinion was adopted on the 5th of December 2014.

Phytosterol oxidation products in foods: Analysis, occurrence, exposure and biological effects

1 Introduction

Hypercholesterolemia is an important risk factor for the development of cardiovascular diseases. A daily dietary intake of 2 g phytosterols/-stanols results in a reduction of LDL- and total plasma cholesterol of approximately 10%.¹⁻³ Owing to these cholesterol-lowering properties, phytosterols/-stanols and their fatty acid esters were among the first ingredients used to enrich foods and thus to obtain an additional beneficial effect. On the basis of a safety assessment by the Scientific Committee on Food in 2000, a yellow fat spread enriched with specified amounts of phytosteryl fatty acid esters was the first product authorized by the EU Commission as a novel food according to Regulation (EC) No 258/97.^{4,5,6} In the meantime, a broad spectrum of other foods with added phytosteryl/-stanyl fatty acid esters has been placed on the market in the European Union. They comprise milk-type products, yoghurt-type products, milk-based fruit drinks, soy-based drinks, cheese-type products, salad dressings, spice sauces, rye bread, rice drinks, and oils;⁷ regularly updated lists of the respective authorizations and notifications are available.^{8,9} Phytosterols/-stanols and their fatty acid esters are among those food ingredients for which health claims referring to the reduction of disease risk have been permitted.^{10,11}

There is no evidence of additional cholesterol-lowering benefits at intakes of phytosterols higher than 3 g/d;³ in addition, high intakes may induce undesirable effects, such as a reduction of plasma levels of β -carotene.^{1,12,13} Therefore, in its

general view on the long-term effects of the intake of elevated levels of phytosterols from multiple dietary sources, the Scientific Committee on Foods (SCF) considered it prudent to avoid intakes exceeding a range of 1-3 g/d.¹⁴ This precautionary limit is in line with the group ADI of 0-40 mg/kg bw for the group of phytosterols, phytostanols and their esters, expressed as the sum of phytosterols and phytostanols in their free form, later derived by JECFA.¹⁵

The DFG Senate Commission on Food Safety (SKLM) previously published two scientific opinions regarding the use of phytosteryl/-stanyl fatty acid esters in foods.^{16,17} They focused on the need for assessment of individual phytosteryl/-stanyl ester preparations and the importance of the respective specifications. In addition, they drew particular attention to the challenges arising from the broad spectrum of enriched food categories and the uncertainties in ensuring that an intake of 1-3 g/d is not exceeded. The need for current and reliable consumption data and for measures to ensure that the products are only consumed by the target groups was emphasized. In order to allow users to restrict their consumption to a maximum of 3 g of phytosterols/-stanols per day and to ensure that the product reaches its target group, specific provisions regarding the labelling of foods and food ingredients with added phytosterols/-stanols and their esters have been implemented; for example, an indication on the label that the consumption of more than 3 g/d of added plant sterols/stanols should be avoided is required.¹⁸ However, a consumer awareness study performed in Germany revealed that 45% of the consumers did not belong to the target group, 3.5% were children and only 1% were aware that an intake of 3 g phytosterols/d should not be exceeded.¹⁹ Data on the actual exposure of consumers to phytosterols via the multiple sources of enriched foods are also inconsistent. According to a post-launch monitoring (PLM) survey on consumer purchases of foods (spreads, salad dressings, milk- and yoghurt-type products) with added phytosterols in five European countries, the mean phytosterol intakes per household were 0.35-0.86 g/d. In the 95th percentile of the population, intakes ranged from 1.0 g/d in France to 3.7 g/d; The Netherlands were the only country in which approximately 6% of households were identified as potential “over consumers”.²⁰ These data indicating that overconsumption of phytosterols seems unlikely are in agreement with the results obtained in the mandatory PLM performed by Unilever covering the first year of marketing of enriched vegetable oil spreads; in that survey the median intakes of phytosterols for regular purchasers were 1.2-1.4 g/d, the 95th percentile intakes

ranged from 2.2 g/d in France to 3.6 g/d in The Netherlands.²¹ On the other hand, a significantly higher mean phytosterol intake (2.45 g/d) was reported in a study performed on the Irish market. In total, 23% of the respondents had mean phytosterol intakes higher than 3 g/d and the majority of consumers (58%) had been consuming these products for more than one year.²² A study by Sioen *et al.* (2011)²³ investigating the consumption of phytosterol-enriched foods in Belgium also identified 16% of consumers to have a phytosterol intake above 3 g/d.

Another controversially discussed issue is the increased absorption of phytosterols, potentially resulting in their accumulation and subsequently the promotion of vascular diseases.²⁴ Examples are the presence of plant sterols in atherosclerotic plaques of patients undergoing carotid endarterectomy,²⁵ the accumulation of plant sterols in human stenotic aortic valves,^{26,27} and the effects of long term plant sterol and stanol consumption on the retinal vasculature.²⁸ The potential accumulation of phytosterols in the arterial wall versus the plasma cholesterol-reducing effect of dietary phytosterols/-stanols and, thus, their usefulness in preventing coronary heart disease are intensively being discussed.²⁹⁻³² One recent study showed the induction of intestinal adenoma formation in *Apc*^{Min} (*Adenomatous polyposis coli*) mice having been fed a plant stanol-enriched diet.³³

Phytosterols/-stanols are structurally very similar to cholesterol. This similarity is the molecular basis for the cholesterol-lowering properties of these substances; however, undesirable reactions known for cholesterol may also be expected in the case of phytosterols. A typical example is the formation of so-called cholesterol oxidation products, i.e. keto-, hydroxy- and epoxy derivatives of cholesterol, a well-known class of substances studied in detail for many years. On the one hand, cholesterol oxidation products are crucial intermediates in mammal metabolism which are enzymatically synthesized *in vivo*, and serve several regulatory purposes such as cholesterol homeostasis.^{34,35} On the other hand, they may be formed endogenously via non-enzymatic oxidation of cholesterol and may also be absorbed from the diet. In cholesterol-containing foods, cholesterol oxidation products can be formed via processing and storage.³⁵ Elevated plasma levels of cholesterol oxidation products have particularly been correlated to atherogenic effects, and are also thought to be involved in other inflammatory processes such as neurodegeneration.³⁶ Therefore, the occurrence in foods and the subsequent dietary intake not only of intact cholesterol but also of cholesterol oxidation products has been in the focus of recent

research activities. An increased intake of dietary cholesterol oxidation products was shown to be associated with impaired hepatic function and lipid metabolism and ultimately atherosclerotic progression in various animal models.³⁷⁻⁴⁵

Taking into account the structural similarities between cholesterol and phytosterol oxidation products and considering the growing number and the variety of products enriched with phytosteryl/-stanyl fatty acid esters, studies on the formation and intake of phytosterol oxidation products and the assessment of their potential adverse effects seem advisable. A present application to extend the use of phytosteryl esters to spreads and liquid vegetable fat-based emulsions specifically intended for cooking and baking underlines the relevance of such an evaluation.⁴⁶

In the last decade, research activities regarding phytosterol oxidation products significantly increased; the progress has been reflected in a series of reviews.^{36,47-52}

The objective of this opinion is to summarize and to evaluate the current knowledge, particularly in the light of the potentially increasing dietary exposure to phytosterol oxidation products via the consumption of foods enriched with phytosterols/-stanols and their esters, and to outline research needs for a safety assessment.

2 Analytical approaches

Thermooxidation of phytosterols occurring in foods encompasses a sequence of reactions resulting in primary (hydroperoxides), secondary (polar: ketones, alcohols, epoxides; unpolar: steradienes, steratrienes) and tertiary oxidation products (dimers, oligomers, polymers). Owing to the analytical capabilities, the focus has almost exclusively been put on the secondary polar phytosterol oxidation products. Accordingly, the term “phytosterol oxidation products” (POP) used in this opinion refers to the keto-, epoxy- and hydroxy-compounds derived from the respective sterols/stanols; Figure 1 shows exemplarily structures of phytosterol oxidation products obtained from β -sitosterol.

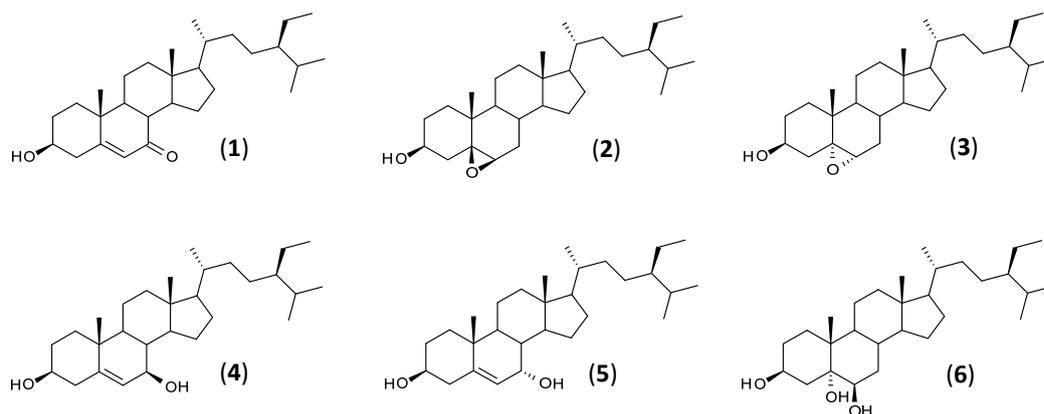


Figure 1. Structures of phytosterol oxidation products derived from β -sitosterol: 7-keto-sitosterol (1), 5,6 β -epoxy-sitosterol (2), 5,6 α -epoxy-sitosterol (3), 7 β -hydroxy-sitosterol (4), 7 α -hydroxy-sitosterol (5), sitostanetriol (6)

There are various analytical methodologies available based on (i) lipid extraction and saponification or transesterification, (ii) isolation and purification via thin layer chromatography or solid phase extraction, (iii) derivatization to trimethylsilyl ethers, and (iv) detection via HPLC- and GC-based techniques.^{53,54}

These methods have been employed to generate a broad spectrum of analytical data on model systems involving the thermal treatment of phytosterol standards.⁵⁵⁻⁵⁷

Thermooxidations under different time/temperature conditions revealed that some of the secondary phytosterol oxidation products constitute intermediates, which are further transformed or degraded in the course of the reaction.⁵⁸ First attempts to isolate fractions containing dimers, trimers and tetramers via size exclusion chromatography have been described, and structures for sterol dimers have been proposed.⁵⁹⁻⁶³

Heating of stigmasterol for 3h at 180°C resulted in a loss of the intact sterol of 61%. Polar, mid-polar and non-polar oxidation products accounted for 39% of this loss; the formation of dimers and polymers accounted for 30% of the loss. This means that there is a gap in the mass balance, leaving 31% of the stigmasterol loss unexplained.⁶⁴

There are data available indicating qualitative and quantitative differences in oxidation profiles between free and esterified phytosterols.⁶⁵⁻⁷⁰ Complex mixtures are to be expected owing to potential oxidations in the sterol as well as in the fatty acid moiety of phytosteryl esters of unsaturated fatty acids.⁷¹ Very recent studies

described first approaches to analyze intact oxidized phytosteryl fatty acid esters via HPLC-ESI-MS.⁷²

3 Occurrence in foods

Non-enriched foods

Data on phytosterol oxidation products exist for various foods containing phytosterols/-stanols or their esters as naturally occurring constituents. The presence of phytosterol oxidation products in crude vegetable oils and their fate during refining has been analyzed.⁷³ The effects of the heating of vegetable oils on the formation of phytosterol oxidation products have been studied in model experiments^{55,74} as well as under industrial frying conditions.⁷⁵ Commercial potato crisps,⁷⁶ potato chips prepared in different vegetable oils,⁷⁷ and French fries prepared in these oils⁷⁵ have been investigated. Sterol oxidation in infant milk formulas and milk cereals⁷⁸ and in ready-to-eat infant foods during storage⁷⁹ has also been studied. As examples, data on spread,⁸⁰ French fries,⁷⁵ and potato crisps⁷⁶ are summarized in Table 1. The average content of phytosterol oxidation products in the heat-treated products French fries and potato crisps was around 1 mg/kg (except for the French fries obtained as restaurant samples); this content corresponds to an oxidation rate of approximately 0.8%.

Table 1. Contents of phytosterol oxidation products (POP) determined in selected non-enriched foods and resulting intakes calculated on the basis of consumption data of the respective foods

Type of food	POP [mg/kg]	Oxidation rate [%] ^a	POP intake [mg/d] ^b		Ref
			Median	95 th Perc	
<i>Spread</i>					
63% fat	13.3	0.41	0.14 ^c	0.65 ^c	(80)
<i>French Fries</i>					
Oven, 225 °C, 15 min	1.2	0.5	0.08	0.19	(75)
Pre-fried samples	0.8	1.3	0.06	0.12	(75)
Restaurant samples	3.4	0.8	0.23	0.53	(75)
<i>Potato crisps</i>					
High fat (>25%)	1.1	0.6	0.02	0.06	(76)
Low fat (<25%)	1.2	0.8	0.03	0.06	(76)

^a calculated as percentage of POP with respect to the initial phytosterol content

^b calculated on the basis of consumption data for adults among consumers only across European countries,⁸¹ except for the data labelled by footnote ^c

^c calculated on the basis of consumption data for adults among consumers only in Germany⁸²

Enriched foods

Information on the contents of phytosterol oxidation products in foods enriched with phytosteryl/-stanyl fatty acid esters is limited; data available for the matrices milk, spreads, liquid spread for cooking and baking and chocolate are summarized in Table 2. The analyzed products differ regarding type (phytosteryl vs. phytostanyl esters) and degree of enrichment and regarding the employed treatments (heat, storage).

The contents of phytosterol oxidation products determined in conventionally pasteurized milk enriched with free phytosterols or phytosteryl esters were consistently around 2 mg/kg, corresponding to oxidation rates between 0.05 and 0.07%. The effect of thermal processing on the formation of phytosterol oxidation products in milk was investigated using different heating techniques.⁸³ The detected contents ranged from 3.5 to 6.4 mg/kg; microwave heating at 900 W for 1.5 minutes yielded the highest amounts of phytosterol oxidation products. However, the amounts of phytosterol oxidation products and the corresponding oxidation rates did not reflect the additionally determined losses of initial phytosterols. For example, heating in the Schaal oven resulted in similar amounts of phytosterols oxidation products as electric heating, both procedures leading to oxidation rates of 0.1%. At the same time, the determined loss of initial phytosterols was 4% after heating in the Schaal oven, but 60% after electric heating. This confirms the abovementioned gap in mass balances based on the currently employed analytical procedures focusing solely on the polar phytosterol oxidation products.

The oxidation rates determined in commercially produced, non-heated spreads enriched with phytosteryl esters^{74,80,84} were in the same order of magnitude (0.1%) as those in non-heated milk.^{83,85} The effect of heating has been investigated in a liquid spread enriched with phytosteryl esters; treatment at 205°C for 30 minutes resulted in a more than 10-fold higher content of phytosterol oxidation products compared to non-heated spreads, corresponding to an oxidation rate of 1.0%.⁸⁶ This is in the same order of magnitude as oxidation rates determined in experiments investigating the effect of pan-frying at 180 °C on the oxidation of sitosterol in rapeseed oil and liquid margarine enriched with phytosteryl esters.⁸⁷

The effects of storage were followed in a dark chocolate enriched with phytosteryl esters; after 5 months at 30°C, the additionally formed amount of phytosterol oxidation products was low and corresponded to an oxidation rate of only 0.003%.⁸⁸

On the other hand, storage of a phytostanyl ester-enriched spread resulted in the highest oxidation rates reported in non-heated enriched spreads.⁸⁹

The data obtained upon storage of spread enriched with phytostanyl esters are in contrast to the view that phytosterols and their fatty acid esters are less susceptible to oxidation reactions than phytosterols and the corresponding esters due to the completely saturated ring structure.^{56,67} This assumption was supported by the 10-fold lower oxidation rate observed in pasteurized milk enriched in phytostanyl fatty acid esters compared to milk enriched to the same extent with phytosteryl fatty acid esters.⁸⁵ However, intra- and intermolecular reactions, e.g. the promotion of oxidation of the stanol moieties by oxidized fatty acid moieties may influence the formation of phytosterol oxidation products.⁶⁷ Therefore, not only the initial phytosterol-/stanol composition, but also the composition of the fatty acid moieties has to be taken into account when assessing the oxidative potential of an enriched food and the potentially resulting loss of functional ingredients.

Table 2. Contents of phytosterol oxidation products (POP) determined in enriched foods and resulting intakes calculated on the basis of a consumption of enriched foods corresponding to 3 g phytosterols per day

Type of food	Treatment	POP [mg/kg]	Oxidation rate [%] ^a	POP intake [mg/d] ^b	Ref
<i>Milk</i>					
Free phytosterols (\triangleq 0.5% phytosterols)	Pasteurization (127 °C, 2 s)	2.2	0.05	1.32	(85)
Phytosteryl esters (\triangleq 0.5% phytosterols)	Pasteurization (127 °C, 2 s)	2.0	0.04	1.2	(85)
Phytostanyl esters (\triangleq 0.5% phytosterols)	Pasteurization (127 °C, 2 s)	0.2	0.004	0.1	(85)
Phytosteryl esters (\triangleq 0.3% phytosterols)	Pasteurization	2.2	0.07	2.2	(83)
Phytosteryl esters (\triangleq 0.3% phytosterols)	65 °C, 24 h	3.5	0.1	3.5	(83)
Phytosteryl esters (\triangleq 0.3% phytosterols)	Microwave (900 W, 1.5 min)	6.4	0.21	6.4	(83)
Phytosteryl esters (\triangleq 0.3% phytosterols)	Microwave (900 W, 2.0 min)	5.6	0.19	5.6	(83)
Phytosteryl esters (\triangleq 0.3% phytosterols)	Electric heating (15 min)	4.2	0.14	4.2	(83)
<i>Spread</i>					
Phytosteryl esters (\triangleq 8% phytosterols)	-	68	0.09	2.6	(84)
Phytosteryl esters (\triangleq 6% phytosterols)	-	46.5	0.07	2.3	(80)
Phytosteryl esters	-	12	-	-	(74)
Phytostanyl esters	-	255	-	9.6	(89)
Phytostanyl esters	Storage (6 weeks, 4 °C)	354	0.12	13.3	(89)
Phytostanyl esters	Storage (6 weeks, 20 °C)	734	0.61	27.5	(89)
<i>Liquid spread</i>					
Phytosteryl esters (\triangleq 7.5% phytosterols)	Heating (205 °C, 30 min)	740	0.99	29.6	(86)
Phytosteryl esters (\triangleq 5% phytosterols)	Pan frying (180 °C, 5 min)	291	0.6	10.9	(87)
Phytosteryl esters (\triangleq 5% phytosterols)	Pan frying (180 °C, 10 min)	668	1.3	25.1	(87)
<i>Dark chocolate</i>					
Phytosteryl esters	-	68.6	-	2.9	(88)
Phytosteryl esters	Storage (5 months, 30 °C)	71	0.003	3.0	(88)

^a calculated as percentage of POP with respect to the initial phytosterol content

^b calculated on the basis of consumptions corresponding to 3 g phytosterols per day

The few data available demonstrate the complexity of the processes underlying the oxidation of phytosterols/-stanols and their esters added to foods. The determined concentrations of phytosterol oxidation products are the sums of keto-, hydroxy-, and epoxy-compounds as shown exemplarily in Figure 1. The interpretation of the data is hampered by the fact that the employed analytical methods are not standardized; thus, the actually covered phytosterol oxidation products may differ not only quantitatively but also qualitatively. Analytical methods allowing the investigation of intact oxidized phytosteryl- and phytostanyl fatty acid esters, without hydrolytic cleavage of the ester bond, are at the beginning.⁷² Systematic studies on the impact of the food matrix on the oxidation of phytosterols/-stanols and their esters are lacking.

4 Estimation of dietary exposure to phytosterol oxidation products

There is only a limited data base for the estimation of the dietary exposure to phytosterol oxidation products via enriched foods. The available data on the occurrence of phytosterol oxidation products and the consumption of foods enriched with phytosteryl-/stanyl esters have been employed in two approaches:

One approach was based on (i) the use of the experimentally determined contents of phytosterol oxidation products in thermally treated enriched foods and (ii) the assumption that the upper daily intake of phytosterols/-stanols of 3 g is achieved by consuming one of these foods. The daily intakes of phytosterol oxidation products resulting from the consumption of the respective serving sizes corresponding to 3 g phytosterols are given for the different enriched foods in Table 2. For non-heated foods (spreads, milk and dark chocolate) the intakes of phytosterol oxidation products range from 1.2 to 2.9 mg/d. Upon heating, the intake is increased to 3.5 – 4.2 mg/d for milk and to 29.6 mg/d for liquid spread for cooking and baking.

Another approach was based on (i) the use of data on the dietary exposure to phytosterols estimated from surveys on the consumption of enriched foods²⁰⁻²³ and (ii) the assumption of a minimum (0.1%) and a maximum (1%) oxidation rate. As shown in Table 3, the mean intakes of phytosterol oxidation products resulting from the application of this approach (0.35 mg/d – 2.45 mg/d for a minimum and 3.5 mg/d – 24.5 mg/d for a maximum oxidation rate) are in a similar order of magnitude as those determined on the basis of the previously mentioned estimate.

Table 3. Intake of phytosterol oxidation products (POP) based on consumption of enriched foods

Phytosterol intake [g/d]		POP intake [mg/d]				Ref
Mean	95 th Perc	Oxidation rate 0.1%		Oxidation rate 1.0%		
		Mean	95 th Perc	Mean	95 th Perc	
0.35 – 0.86	1.06 – 3.70	0.35 – 0.86	1.06 – 3.70	3.5 – 8.6	10.0 – 37.0	(20) ^{a,b}
0.24 – 0.96 ^d	1.68 – 2.64	0.24 – 0.96	1.68 – 2.64	2.4 – 9.6	16.8 – 26.4	(21) ^{a,b}
2.45	5.48	2.45	5.48	24.5	54.8	(22) ^c
1.51	4.20	1.51	4.20	15.1	42.0	(23) ^c

^a intake calculated on the basis of purchases per household
^b data from the Netherlands, United Kingdom, France, Germany, and Belgium
^c intake calculated on the basis of purchases per consumer
^d data representing the median daily intake

A comparison of the estimated intakes of phytosterol oxidation products from enriched foods (Tables 2 and 3) to those resulting from non-enriched foods (Table 1) shows the significantly higher intakes to be expected from the consumption of foods with added phytosterols/-stanols and their esters. As shown in Table 2, this increase in intake is particularly pronounced for enriched foods subjected to heating processes.

In order to estimate the intake of phytosterols from multiple sources, a worst case model simulating prospective phytosterol intake has been developed,⁹⁰ thereby assuming that the consumer does not follow the recommendations on the label.¹⁸ Using the German National Food Consumption Study, 0.3 – 2 g phytosterols were hypothetically added to the usual daily servings of ten different food products, selected from the novel food applications; the prospective phytosterol intake was calculated by stepwise accumulation of different functional foods in three enrichment scenarios. According to the worst case in this model, an enrichment of 2 g phytosterols per proposed serving size would result in a maximum intake of 13 g/d. Assuming again oxidation rates of 0.1% and 1%, respectively, this would result in dietary exposures to phytosterol oxidation products of 13 mg/d and 130 mg/d, respectively.

5 Uptake of phytosterol oxidation products from the diet

Animal studies

Intragastric administration of two of the main classes of phytosterol oxidation products (7-keto- and epoxides; 5 mg in 1 mL of triolein) to mesenteric duct-cannulated adult male rats revealed that the lymphatic absorption rate of 7-keto-sitosterol (1.4%) was similar to that of sitosterol (1.2%). Epoxy-derivatives showed the highest lymphatic absorption rates (e.g. α -epoxy-sitostanol: 2.7% and β -epoxy-campestanol: 7.9%); campesterol oxidation products were generally better absorbed than the respective sitosterol derivatives.⁹¹

Administration of an AIN-93G-based diet to thoracic duct-cannulated rats (2.5 g cholesterol/kg diet or 2.5 g cholesterol/kg diet + 2.5 g phytosterols or phytosterol oxidation products/kg diet) confirmed the low lymphatic absorption rates of phytosterols (sitosterol: 2.2%, campesterol: 5.5%) when compared to cholesterol (37.3%). However, it revealed that the lymphatic absorption rates of oxidation products of sitosterol (9.1%) and campesterol (15.9%) were actually higher than those of the parent phytosterols.⁹²

A mixture of phytosterol oxidation products was fed to hamsters for 2 weeks and their concentrations were followed in plasma, aorta, liver, kidneys and heart.⁹³ At the two highest doses (2500 mg/kg diet and 500 mg/kg diet), phytosterol oxidation products were detectable in all investigated tissues. However, the proportion has changed after intake: The levels of campesterol oxidation products were higher than those of the sitosterol oxidation products in plasma, while the amount of 7-keto-sitosterol, which is the dominating phytosterol oxidation product in the diet, was very low in blood. In contrast to plasma, sitostanetriol was the major phytosterol oxidation product detected in the tissues.

Similar discriminations of administered phytosterol oxidation products (1 g/kg diet) were observed in a 6-weeks feeding study with hamsters.⁹⁴ In the employed dietary mixtures of sitosterol and stigmasterol oxidation products the 7-keto-derivatives dominated, whereas in plasma only the 7 α - and 7 β -hydroxy-derivatives and in liver 7 α - and 7 β -hydroxy- as well as the 5,6 α - and 5,6 β -epoxides were detected.

Human studies

The occurrence of oxidized plant sterols in human serum was first described for phytosterolemic patients.⁹⁵ In the meantime, several studies reporting the presence

of phytosterol oxidation products in plasma of healthy human subjects have been published. However, they differ significantly in the types and amounts of oxidation products detected (Table 4).

Table 4. Baseline levels of phytosterol oxidation products (POP) determined in human plasma

	POP in human plasma / serum [μM]				
	Year (Ref)				
	2013 (100)	2012 (98)	2011 (99)	2008 (97)	2004 (96)
7 α -OH-Brassicasterol	-	0.0007	-	-	-
7 α -OH-Campesterol	0.0002	0.006	0.0002	-	-
7 α -OH-Stigmasterol	-	0.008	-	-	-
7 α -OH-Sitosterol	0.0005	0.01	0.0004	0.11	-
7 β -OH-Brassicasterol	-	0.0006	-	-	-
7 β -OH-Campesterol	0.0008	0.004	0.0004	-	-
7 β -OH-Stigmasterol	-	0.003	-	0.11	-
7 β -OH-Sitosterol	0.003	0.008	0.003	-	-
α -Epoxy-Sitostanol	-	-	-	-	0.01
β -Epoxy-Sitostanol	-	-	-	-	0.13
Campestanetriol	-	-	-	-	0.01
Sitostanetriol	-	-	-	-	0.09
7-Keto-Campesterol	0.001	0.002	0.001	-	-
7-Keto-Stigmasterol	-	0.002	-	-	-
7-Keto-Sitosterol	0.006	0.004	0.007	-	0.01
Total POP	0.011	0.05	0.012	0.22	0.26

The earlier GC/MS-based studies only reported the presence of α - and β -epoxy- and triol-derivatives⁹⁶ or the presence of 7 α - and 7 β -hydroxy-derivatives.⁹⁷ The largest spectrum of phytosterol oxidation products (in total: 11) was detected by applying GCxGC/TOF.⁹⁸ Two studies based on isotope dilution GC/MS quantified 6 phytosterol oxidation products, 7-keto- and 7 β -hydroxy-sitosterol being the major representatives.^{99,100} These studies reported similar ranges of the detected phytosterol oxidation products in two panels of 16 and 43 healthy volunteers, respectively; the determined concentration ranges of individual phytosterol oxidation products were 0.07 - 3.01 ng/ml serum (0.0002 – 0.007 μM)⁹⁹ and 0.09 – 2.49 ng/ml plasma (0.0002 – 0.006 μM).¹⁰⁰

There are only two studies available providing comparative data on the levels of phytosterol oxidation products before and after consumption of phytosteryl ester-enriched margarine. In the first study involving 16 human subjects consuming 3g phytosterols/d via a margarine enriched with phytosteryl esters for 28 days, there

were significant increases in the serum concentrations of campesterol (from $2.82 \pm 1.44 \mu\text{g/mL}$ ($7.0 \pm 3.6 \mu\text{M}$) before to $4.19 \pm 1.55 \mu\text{g/mL}$ ($10.5 \pm 3.9 \mu\text{M}$) after the dietary intervention) and sitosterol ($2.06 \pm 1.27 \mu\text{g/mL}$ ($5.0 \pm 3.1 \mu\text{M}$) before and $4.30 \pm 1.89 \mu\text{g/mL}$ ($10.4 \pm 4.6 \mu\text{M}$) after).⁹⁹ Among the detected phytosterol oxidation products, 7 β -hydroxy-sitosterol was the major representative in the consumed margarine ($8.62 \pm 0.28 \text{ ng/mg}$). For this phytosterol oxidation product, a statistically significant increase (87%) of the serum concentration from $1.20 \pm 0.54 \text{ ng/mL}$ ($0.003 \pm 0.001 \mu\text{M}$) (before consumption of the margarine) to $2.24 \pm 1.25 \text{ ng/ml}$ ($0.005 \pm 0.003 \mu\text{M}$) (after consumption of the margarine) was observed. In addition, there was a highly significant correlation between the serum levels of campesterol and the sum of 7-oxygenated campesterol ($R^2=0.915$; $P<0.001$) and sitosterol and the sum of 7-oxygenated sitosterol ($R^2=0.915$; $P<0.001$).

In a second randomized, double-blind, cross-over study, 43 healthy subjects consumed a margarine enriched with phytosteryl esters, a margarine enriched with phytostanyl esters and a control margarine, each of them for 4 weeks, separated by wash-out periods of 4 weeks; the consumption of the enriched margarines corresponded to intakes of 3 g/d of sterols and stanols, respectively.¹⁰⁰ Compared to control, the serum LDL-cholesterol concentrations were reduced after consumption of the phytosteryl ester-enriched (-8.1%) and the phytostanyl ester-enriched (-7.8%) margarines. The consumption of the phytosteryl ester-enriched margarine did not result in changes of the concentrations of phytosterol oxidation products in plasma; the individual phytosterol oxidation products ranging from 0.09 to 2.49 ng/ml ($0.0002 - 0.006 \mu\text{M}$) plasma before and from 0.09 to 2.35 ng/mL ($0.0002 - 0.006 \mu\text{M}$) plasma after the dietary intervention. On the other hand, the intake of the phytostanyl ester-enriched margarine reduced the serum concentration of 7 β -hydroxy-campesterol by 0.07 ng/mL compared with the control (~14%) and the phytosteryl ester-enriched margarine (~15%).

The reason for the apparently inconclusive data regarding the concentrations of phytosterol oxidation products in plasma after consumption of enriched margarine obtained in the same laboratory using the same methods remains unclear.

The second study revealed large variations in the baseline plasma phytosterol oxidation product concentrations among the study subjects; however, they remained relatively stable over time. Serum concentrations of (non-oxidized) sitosterol and campesterol did not correlate with plasma concentrations of sitosterol and

campesterol oxidation products during any of the three interventions.¹⁰¹ Six subjects could be arbitrarily classified as having consistently low or high plasma phytosterol oxidation product concentrations. This differentiation into “low and high oxidizers” was also reflected in oxidized low-density lipoprotein concentrations. However, oxidative and anti-oxidative capacity markers, such as iron/copper status, α -tocopherol concentrations and TEAC values could not explain these differences.

In a recent study, the concentrations of the phytosterols campesterol and sitosterol and their oxidation products were determined in plasma and aortic valve cusps of patients with severe aortic stenosis.²⁷ The absolute and cholesterol corrected levels of campesterol and sitosterol in plasma, in the aortic valve cusps and between both compartments showed a strong correlation. In contrast, the correlation between the concentrations of the phytosterols and those of the corresponding phytosterol oxidation products in plasma and the correlation between the phytosterol oxidation product levels in plasma and those in aortic valve cusps were only weak. Moreover, the concentrations of plant sterols and those of their 7-oxidized metabolites in the tissue of aortic valve cusps significantly correlated. The authors speculated that the latter finding could relate to local inflammatory processes in atherosclerotic plaques and tissues, which generate free radicals and trigger oxidation processes.

Endogenous formation of phytosterol oxidation products

The endogenous formation of phytosterol oxidation products has been shown in various *in vitro* experiments. In rat liver mitochondria and fractions oxidations of both the sterol nucleus and the side chain of β -sitosterol were observed; however, the conversion rates of β -sitosterol were far below those of cholesterol.¹⁰²⁻¹⁰⁵ In rat liver mitochondria side chain hydroxylation of campesterol occurred at a rate similar to cholesterol.¹⁰⁴ In addition, as the microbial formation of cholesterol oxidation products in the gut has been observed in humans and rats, such transformation reactions are also being discussed for phytosterol oxidation products.^{106,107} The different routes of formation, i.e. enzyme-catalyzed vs. chemical oxidations, are expected to be reflected in differences between the spectra of endogenously formed phytosterol oxidation products and those ingested via the diet.

6 Biological effects of phytosterol oxidation products

Genotoxicity

Genotoxicity was assessed *in vitro* using a heat-treated mixture of phytosterols containing approximately 30% phytosterol oxidation products.¹⁰⁸ According to the results obtained from a bacterial mutation assay, a chromosome aberration assay and a micronucleus assay, phytosterol oxides are not considered to possess a genotoxic potential. A study employing fractions isolated from thermo-oxidized β -sitosterol confirmed that individual phytosterol oxidation products did not show mutagenic activity towards *Salmonella typhimurium* strains.¹⁰⁹ No evidence of genotoxic effects *in vivo* was observed in a flow cytometer-based micronucleus assay in murine erythrocytes after intraperitoneal injection of mixtures of phytosterol epoxides or phytosterol triols.¹¹⁰

Subchronic toxicity

A 90 day study feeding study in rats was performed using a heat-treated mixture of phytosterols containing approximately 30% phytosterol oxidation products.¹⁰⁸ Rats were fed a control diet without added sterols or diets with either steryl esters (5.6%) or steryl esters supplemented with 0.2, 0.6 or 1.6% of this mixture of phytosterol oxidation products. There were no effects on clinical observations, neurobehavioural tests, food and water consumption, ophthalmoscopy, urinalysis and renal concentrating ability, gross necropsy and histopathology. At the highest dose tested, there were some findings compared with the control and the diet containing only steryl esters; they comprised a slight reduction in bodyweight (females), slight increases in thrombocyte count (males) and decreases in haemoglobin, packed cell volume and mean corpuscular volume (females), slight decreases in glucose level and increases in albumin level plus albumin:globulin ratio (males), increases in gamma glutamyl transferase (females), reduced triglycerides and phospholipids (both sexes), and a slight increase in liver weight (females). None of these findings was supported by histopathological changes. According to these findings, a No Observed Effect Level (NOEL) based on the mid dose (0.6% of the mixture of phytosterol oxidation products in the diet) was established at an estimated dietary level of phytosterol oxidation products of 128 mg/kg/d for males and 144 mg/kg/d for females.

Cytotoxicity and pro-inflammatory potential

Numerous *in vitro*-experiments incubating various cell types and cell lines with mixtures of oxides or synthesized pure oxides showed that phytosterol oxidation products exert cytotoxic effects, which are qualitatively similar to those observed for cholesterol oxidation products, but higher concentrations were required ($> 60 \mu\text{M}$).¹¹¹⁻¹¹⁵ The assessment of markers indicative of inflammatory and/or apoptotic cellular mechanisms demonstrated a reduction of cell viability as well as the generation of oxidative stress and related processes thereof, 7- β -hydroxy- and 7-keto-derivatives exhibiting the highest cytotoxic potential among those oxides dominating in foods. One study investigated the release of the cytokines TNF α , IL-8 and IL-10 in the intestinal epithelial cell line Caco-2 upon addition of 7-ketocholesterol and 7-ketostigmasterol at a concentration of $60 \mu\text{M}$.¹¹⁶ It was shown that 7-ketostigmasterol significantly increased the release of the three abovementioned cytokines. Moreover, the amounts of the pro-inflammatory mediators TNF α and IL-8 released upon incubation with 7-ketostigmasterol were significantly higher than those secreted upon addition of 7-ketocholesterol. One *in vivo* study investigated the effect of mixtures of sitosterol oxidation products or stigmasterol oxidation products after being injected at a concentration of $5 \mu\text{M}$ into mealworms.¹¹⁷ In accordance with the *in vitro* observations, the administered phytosterol oxidation products were shown to be cytotoxic, thereby inducing an increase in mealworm mortality, but their activities were five times lower than those of cholesterol oxidation products.

Pro-atherogenic effects

In vivo-generated as well as dietary cholesterol oxidation products have been shown to be closely associated to atherosclerotic processes.^{35,36,39} Regarding potential pro-atherogenic effects of phytosterol oxides, Yang *et al.*¹¹⁸ analyzed *in vitro* the effect of sitosterol and sitosterol oxidation products on the acetylcholine (ACh)-induced relaxation of rat aortae, a marker of vascular health, by isometric tension measurements. Whereas sitosterol did not impair vasorelaxation, sitosterol oxidation products significantly attenuated ACh-mediated relaxation at a concentration of $1 \mu\text{g/mL}$ ($2.3 \mu\text{M}$). This effect observed *in vitro* was considered by the authors to be an indicator for a pro-atherogenic potential of phytosterol oxidation products and was ascribed to an increased production of reactive oxygen species.

However, data on this issue are inconclusive when considering the available *in vivo* experiments. An *in vivo* study in Apo-E-deficient mice could not establish a correlation between dietary administered phytosterol oxidation products (0.2 g/kg diet) for 9 weeks and serum cholesterol concentrations as well as the size of atherosclerotic plaques when compared to a phytosterol-supplemented diet (0.2 g/kg diet).⁹² A recent study by Plat *et al.*¹¹⁹ investigated the effects of a western-like diet containing 0.25% cholesterol compared to the same diet in which 10% of the cholesterol had been replaced by cholesterol oxidation products and dietary phytosterol oxidation products, respectively, in LDLR ^{+/+} mice for 35 weeks. Concerning the lesion size, no differences could be observed between the 3 groups, confirming the results obtained by the abovementioned study. However, there was a significantly higher proportion of severe atherosclerotic lesions not only in the mice having been fed the diet containing cholesterol oxidation products but also in the group having received the phytosterol oxidation products.

Loss of anti-atherogenic properties

Besides potential pro-atherogenic effects, a lower anti-atherogenic potency of phytosterols due to oxidation when compared to non-oxidized phytosterols is being discussed. Such an effect would be of particular relevance considering the functionality and efficacy of phytosterol-enriched foods.

Tomoyori *et al.*⁹² showed that Apo-E-deficient mice had elevated cholesterol oxidation product serum levels after having been fed a diet containing phytosterol oxidation products (0.2 g/kg diet) for 9 weeks in comparison to a phytosterol-fed control group (0.2 g/kg diet). Furthermore, Liang *et al.*⁹⁴ fed diets containing either 1 g/kg phytosterols (sitosterol/stigmasterol) or 1 g/kg of the corresponding oxidation products to male hamsters for 6 weeks. The aortic plaque size and aortic cholesterol levels were reduced in the animals having obtained the phytosterol diets when compared to a control group; these effects were not observed upon consumption of the diets containing phytosterol oxidation products. In addition, aortic contractions in response to ACh stimulation were significantly reduced in the phytosterol-fed group if compared to the animals fed a control diet. In turn, the aortae of the animals having consumed phytosterol oxidation products exhibited contractions as strong as the control group, indicating a loss of the cardio-protective properties of oxidized phytosterols.

Potential impact of phytosterol oxidation products on the hydrolysis of phytosteryl esters

The essential pre-condition for the cholesterol lowering properties of phytosterols added to foods as their fatty acid esters is the intestinal cleavage of the ester bonds. In a mechanistic study Julien-David *et al.*¹²⁰ determined the impact of oxidation on the *in vitro* activity of pancreatic cholesterol esterase using sitosteryl oleate and the oxidation products 7-keto-sitosteryl oleate and sitosteryl-9,10-dihydroxystearate as substrates. As shown for 7-keto-sitosteryl oleate, the oxidation of the sterol moiety led to an increased affinity to the cholesterol esterase and a faster conversion when compared to sitosteryl oleate. In contrast, the oxidative modification of the fatty acid moiety leading to sitosteryl-9,10-dihydroxystearate resulted in an almost complete loss of hydrolysis. In addition, in the presence of sitosteryl-9,10-dihydroxystearate the hydrolysis rate of sitosteryl oleate was significantly decreased.

The observations made in the above mentioned *in vitro* study illustrate the complexity of the aspects that need to be considered in the course of an assessment of phytosterol oxidation products. Commercially, foods are almost exclusively enriched with phytosteryl/-stanyl esters rather than with the free sterols/stanols. Owing to the analytical capabilities, the knowledge generated so far is focusing on the oxidized sterols/stanols. Alkaline hydrolysis or interesterification which are commonly applied in the course of the analysis, results in a loss of knowledge regarding the formation of oxidized esters. The demonstrated interactions between the various oxidized “species” that may be formed during the oxidation process and the impact on the hydrolysis rate of the intact steryl ester demonstrate that it may be insufficient to focus on an isolated consideration of phytosterol oxidation products when it comes to the assessment of foods enriched with various mixtures of fatty acid phytosteryl- and phytostanyl esters.

7 Summary and knowledge gaps

- Analysis, occurrence and dietary exposure
 - The presently employed analytical procedures almost exclusively focus on the polar phytosterol oxides. The calculation of mass balances demonstrates a gap: losses of intact phytosterols cannot be explained by the formed amounts of these polar phytosterol oxides.
 - Analytical approaches for both, the investigation of primary (hydroperoxides) and tertiary (dimers, oligomers, polymers) oxidation products as well as oxidized fatty acid esters are still at the beginning.
 - Systematic studies on the impact of the employed heating processes (including the respective time/temperature profiles), the food matrix and the presence of antioxidative compounds on the oxidation of phytosterols/ -stanols and their esters are lacking.
 - There are data on phytosterol oxidation products in several foods containing phytosterols/-stanols or their esters as naturally occurring constituents. However, the systematic assessment of intakes resulting from these background levels is lacking.
 - Data on the contents of phytosterol oxidation products in foods enriched with phytosteryl/-stanyl fatty acid esters are limited. However, heat-treatment of enriched liquid spreads or plant oils resulted in a more than 10-fold higher content of phytosterol oxidation products compared to non-heated spreads. Furthermore, storage of spread seems to increase the levels of phytosterol oxidation products as well. A systematic determination of phytosterol oxidation products in stored or heat-treated foods is lacking.
 - There is only a limited data base for the estimation of dietary exposure to phytosterol oxidation products via enriched foods.
- Absorption and endogenous formation
 - There is experimental evidence from animal studies for the absorption of dietary phytosterol oxidation products and their distribution to various tissues. The observed absorption rates differ depending on the type of phytosterol and the type of oxidation product. There are qualitative and quantitative differences between phytosterol oxidation products administered via the diet and those determined in plasma or tissues. On the basis of the presently available knowledge, predictions of the spectrum and the amounts of phytosterol

oxidation products upon absorption/metabolization of dietary mixtures of phytosterol oxidation products are not possible.

- Regarding the baseline levels of phytosterol oxidation product in humans arising from the natural occurrence of phytosterols, there are significant differences in the types of oxidation products and in the concentrations detected in the available studies. The data do not allow concluding whether the observed variability is due to methodological differences or to actual exposure/biological differences.
- There is a lack of knowledge regarding the contributions of phytosterol oxidation products ingested via the diet and those endogenously formed to levels in human tissues.
- Data on the additional contribution to the levels of phytosterol oxidation products in humans to be expected from the consumption of enriched foods are scarce.
- The few available data regarding the absorption of phytosterol oxides by humans upon consumption of foods enriched with phytosteryl/-stanyl fatty acid esters are conflicting.
- Phytosterol oxidation products have been detected in aortic valve cusps of patients with severe aortic stenosis. The observed weak correlations between the amounts of phytosterols and those of the corresponding oxidation products in plasma as well as the weak correlations between the concentrations of phytosterol oxidation products in plasma and those in the aortic valve tissue remain unexplained.
- Biological effects
 - There is substantial evidence (*in vitro* and animal studies) for reduced cholesterol-lowering properties of phytosterols upon oxidation. However, this effect has only been observed under experimental conditions involving the complete substitution of the non-oxidized phytosterol by the corresponding oxide. The data obtained from this experimental set-up do not allow to draw conclusions regarding these effects for more realistic scenarios, i.e. consumption of foods in which the phytosterols are oxidized at a much lower rate of 0.1%-1%.
 - *In vitro* experiments indicated a pro-atherogenic potential of phytosterol oxides. The available *in vivo* studies are inconclusive.

- The potential inflammatory effect of phytosterol oxidation products on intestinal epithelial cells indicated by one *in vitro* experiment remains unclear.
- Knowledge on biological effects of the non-identified phytosterol oxidation products is lacking.
- Knowledge on potentially adverse effects of oxidized phytosteryl/-stanyl esters (either oxidized at the sterol/stanol nucleus or in the fatty acid chain) is lacking.
- A risk-benefit analysis of the cholesterol-lowering effects of phytosterols/-stanols and the potential adverse effects of the phytosterol oxidation products inherently present in enriched foods is lacking.

8 Research needs

- Development of analytical approaches which cover tertiary phytosterol oxidation products (dimers, trimers, polymers) as well as oxidized steryl/-stanyl fatty acid esters.
- Systematic studies on the impact of the food matrix on the oxidation of phytosterols/-stanols and their esters upon processing and storage.
- Extension of the limited data base regarding the fate of dietary phytosterol oxidation products upon consumption of enriched foods (including the potential contribution of the gut microbiota).
- Determination of the dietary intake of phytosterol oxidation products via enriched foods in relation to the endogenous background resulting from the consumption of foods containing phytosterols/-stanols and their esters as naturally occurring constituents (for example by making use of isotope-labelled phytosterols).
- Further studies on the potential pro-atherogenic effects of phytosterol oxidation products observed in *in vitro* and animal experiments.
- Further studies on the presence of phytosterol oxidation products in aortic valve tissue observed in patients with severe aortic stenosis, in order to increase the understanding of a potential relationship between phytosterol oxidation products and cardiovascular risk.
- Verification of the evidence from *in vitro* and animal studies for a reduction of the cholesterol-lowering properties of phytosterols due to oxidation.

9 References

- (1) Katan, M. B., Grundy, S. M., Jones P., Law, M. et al., *Efficacy and safety of plant stanols and sterols in the management of blood cholesterol levels. Mayo Clin. Proc.* 2003, 78, 965–978.
- (2) Abumweis, S. S., Barake, R., Jones, P., *Plant sterols/stanols as cholesterol lowering agents: A meta-analysis of randomized controlled trials. Food Nutr. Res.* 2008, 52. doi:10.3402/fnr.v52i0.1811
- (3) Demonty, I., Ras, R. T., van der Knaap, H. C. M., Duchateau, G. S. M. J. E. et al., *Continuous Dose-Response Relationship of the LDL-Cholesterol-Lowering Effect of Phytosterol Intake. J Nutr* 2009, 139, 271–284.
- (4) *Regulation (EC) No 258/97 of the European Parliament and of the Council of 27 January 1997 concerning novel foods and novel food ingredients. O. J. L 43/1.*
- (5) SCF (Scientific Committee on Food). *Opinion of the Scientific Committee on Food on a request for the safety assessment of the use of phytosterol esters in yellow fat spreads. 2000. http://ec.europa.eu/food/fs/sc/scf/out56_en.pdf (accessed: Apr 22, 2014)*
- (6) *Commission Decision of 24 July 2000 on authorising the placing on the market of 'yellow fat spreads with added phytosterol esters' as a novel food or novel food ingredient under regulation (EC) No 258/97 of the European Parliament and of the Council. (2000/500/EC) O.J. L 200/59*
- (7) EFSA (European Food Safety Authority). *Consumption of food and beverages with added plant sterols in the European Union. EFSA J.* 2008, 133, 1-21.
- (8) *Bundesinstitut für Risikobewertung (BfR). Anträge auf Zulassung neuartiger Lebensmittel gemäß Artikel 4 der Verordnung (EG) Nr. 258/97; <http://www.bfr.bund.de/cm/343/140417-antraege-auf-zulassung-neuartiger-lebensmittel-gemaess-artikel-4-der-verordnung-eg-nr-258-97.pdf> (accessed Oct 16, 2014).*
- (9) *Bundesinstitut für Risikobewertung (BfR). Notifizierungen neuartiger Lebensmittel gemäß Artikel 5 der Verordnung (EG) Nr. 258/97; <http://www.bfr.bund.de/cm/343/notifizierungen-neuartiger-lebensmittel-gemaess-artikel-5-der-verordnung-eg-258-97.pdf> (accessed Oct 1, 2014)*
- (10) *Commission Regulation (EC) No 983/2009 of 21 October 2009 on the authorisation and refusal of authorisations of certain health claims made on food and referring to the reduction of disease risk and to children's development and health. O. J. L. 27/3*
- (11) *Commission Regulation (EU) No 376/2010 of 3 May 2010 amending Regulation (EC) No 983/2009 on the authorisation and refusal of authorisations of certain health*

- claims made on food and referring to the reduction of disease risk and to children's development and health. *O. J. L.* 111/3
- (12) *Derdemezis, C. S., Filippatos, T. D., Mikhailidis, D. P., Elisaf, M. S., Review Article: Effects of Plant Sterols and Stanols Beyond Low-Density Lipoprotein Cholesterol Lowering. J. Cardiovasc. Pharmacol. Ther.* 2010, 15, 120–134.
- (13) *Hendriks, H. F. J., Brink, E. J., Meijer, G. W., Princen, H. M. G., Ntanios, F. Y., Safety of long-term consumption of plant sterol esters-enriched spread. Eur. J. Clin. Nutr.* 2003, 57, 681–692.
- (14) *SCF (Scientific Committee on Food). General view of the Scientific Committee on Food on the long-term effects of the intake of elevated levels of phytosterols from multiple dietary sources, with particular attention to the effects on β -carotene. 2002. http://ec.europa.eu/food/fs/sc/scf/out143_en.pdf (accessed Oct 16, 2014)*
- (15) *WHO (World Health Organization). Evaluation of Certain Food Additives. Sixty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives. World Health Organization Technical Report Series 952, 2009.*
- (16) *SKLM (2005). Beschluss der SKLM zum Einsatz von Phytosterolen und Phytosterylestern in Lebensmitteln vom 21. September 2001, Deutsche Forschungsgemeinschaft, Lebensmittel und Gesundheit II, Sammlung der Beschlüsse und Stellungnahmen (1997-2004), Herausgegeben von der Senatskommission zur Beurteilung der gesundheitlichen Unbedenklichkeit Lebensmitteln, SKLM, Gerhard Eisenbrand (Vorsitzender), Mitteilung 7, pp 129-130, Wiley-VCH Verlag, Weinheim*
- (17) *SKLM (2007) Eisenbrand, G., Einsatz von Phytosterinen in Lebensmitteln http://www.dfg.de/download/pdf/dfg_im_profil/reden_stellungnahmen/2007/sklm_phytosterine_07.pdf (accessed Oct 16, 2014)*
- (18) *EC (2004) Commission Regulation No 608/2004 of 31 March 2004 concerning the labelling of foods and food ingredients with added phytosterols, phytosterol esters, phytostanols and/or phytostanol esters. O. J. L.* 97/44
- (19) *Niemann, B., Sommerfeld, C., Hembeck, A., Bergmann, C., Plant sterol enriched foods as perceived by consumers. Project report on a joint project of consumer advice centres and BfR. BfR-Wissenschaft 05/2007, 2007.*
- (20) *Willems, J. I., Blommaert, M. A., Trautwein, E. A., Results from a post-launch monitoring survey on consumer purchases of foods with added phytosterols in five European countries. Food Chem. Toxicol.* 2013, 62, 48–53.
- (21) *Lea, L., Hepburn, P., Safety evaluation of phytosterol-esters. Part 9: Results of a European post-launch monitoring programme. Food Chem. Toxicol.* 2006, 44, 1213–1222.

- (22) Hearty, Á., Duffy, E., Joyce, J., O'Connor, C., Gibney, M. J., *Phytosterol-enriched products on the Irish market: examination of intake and consumption patterns. Public Health Nutr.* 2009, 12, 51.
- (23) Sioen, I., Matthys, C., Huybrechts, I., van Camp, J., de Henauw, S., *Consumption of plant sterols in Belgium: consumption patterns of plant sterol-enriched foods in Flanders, Belgium. Br. J. Nutr.* 2011, 105, 911–918.
- (24) Weingärtner, O., Bohm, M., Laufs, U., *Controversial role of plant sterol esters in the management of hypercholesterolaemia. Eur. Heart J.* 2008, 30, 404–409.
- (25) Miettinen, T. A., Railo, M., Lepäntalo, M., Gylling, H., *Plant Sterols in Serum and in Atherosclerotic Plaques of Patients Undergoing Carotid Endarterectomy. J. Am. Coll. Cardiol.* 2005, 45, 1794–1801.
- (26) Helske, S., Miettinen, T., Gylling, H., Mayranpaa, M. et al., *Accumulation of cholesterol precursors and plant sterols in human stenotic aortic valves. J. Lipid Res.* 2008, 49, 1511–1518.
- (27) Schött, H.-F., Luister, A., Husche, C., Schäfers, H.-J. et al., *The relationships of phytosterols and oxyphytosterols in plasma and aortic valve cusps in patients with severe aortic stenosis. Biochem. Biophys. Res. Commun.* 2014, 446, 805–810.
- (28) Kelly, E. R., Plat, J., Mensink, R. P., Berendschot, T. T., *Effects of long term plant sterol and -stanol consumption on the retinal vasculature: A randomized controlled trial in statin users. Atherosclerosis* 2011, 214, 225–230.
- (29) Lottenberg, A. M., Bombo, R. P., Ilha, A., Nunes, V. S. et al., *Do clinical and experimental investigations support an antiatherogenic role for dietary phytosterols/stanols? IUBMB Life* 2012, 64, 296–306.
- (30) Bombo, R. P., Afonso, M. S., Machado, R. M., Lavrador, M. S. F. et al., *Dietary phytosterol does not accumulate in the arterial wall and prevents atherosclerosis of LDLr-KO mice. Atherosclerosis* 2013, 231, 442–447.
- (31) Lütjohann, D., Schött, H.-F., Plat, J., *Invited commentary on the paper published by Bombo et al.: Dietary phytosterol does not accumulate in the arterial wall and prevents atherosclerosis of LDLr-KO mice. Atherosclerosis* 2014, 233, 157–159.
- (32) Zampelas, A., *From the Maastricht meeting to the European Atherosclerosis Society Consensus on phytosterols/phytostanols: What is new of an old story?. Atherosclerosis* 2014, 233, 357–358.
- (33) Martinen, M., Päivärinta, E., Storvik, M., Huikko, L. et al., *Plant stanols induce intestinal tumor formation by up-regulating Wnt and EGFR signaling in Apc^{Min} mice. J. Nutr. Biochem.* 2013, 24, 343–352.
- (34) Bjorkhem, I., Diczfalusy, U., *Oxysterols: Friends, Foes, or Just Fellow Passengers?. Arterioscler. Thromb. Vasc. Biol.* 2002, 22, 734–742.

- (35) Leonarduzzi, G., Sottero, B., Poli, G., Oxidized products of cholesterol: dietary and metabolic origin, and proatherosclerotic effects (review). *J. Nutr. Biochem.* 2002, 13, 700–710.
- (36) Alemany, L., Barbera, R., Alegría, A., Laparra, J., Plant sterols from foods in inflammation and risk of cardiovascular disease: A real threat? *Food Chem. Toxicol.* 2014, 69, 140-9.
- (37) Brown, A. J., Jessup, W., Oxysterols and atherosclerosis. *Atherosclerosis* 1999, 142, 1–28.
- (38) Meynier, A., Andre, A., Lherminier, J., Grandgirard, A., Demaison, L., Dietary oxysterols induce in vivo toxicity of coronary endothelial and smooth muscle cells. *Eur.J. Nutr.* 2005, 44, 393–405.
- (39) Staprans, I., Pan, X.-M., Rapp, J. H., Feingold, K. R., The role of dietary oxidized cholesterol and oxidized fatty acids in the development of atherosclerosis. *Mol. Nutr. Food Res.* 2005, 49, 1075–1082.
- (40) Ng, C. H., Qiang Yao, X., Huang, Y., Chen, Z.-Y., Oxidised cholesterol is more hypercholesterolaemic and atherogenic than non-oxidised cholesterol in hamsters. *Br. J. Nutr.* 2008, 99, 749-755.
- (41) Soto-Rodríguez, I., Campillo-Velázquez, P. J., Alexander-Aguilera, A., Rodríguez-Estrada, M. T. et al., Biochemical and histopathological effects of dietary oxidized cholesterol in rats. *J. Appl. Toxicol.* 2009, 29, 715–723.
- (42) Sasaki, T., Fujikane, Y., Ogino, Y., Osada, K., Sugano, M., Hepatic Function and Lipid Metabolism Are Modulated by Short-term Feeding of Cholesterol Oxidation Products in Rats. *J. Oleo Sci.* 2010, 59, 503–507.
- (43) Sato, K., Nakano, K., Katsuki, S., Matoba, T. et al., Dietary Cholesterol Oxidation Products Accelerate Plaque Destabilization and Rupture Associated with Monocyte Infiltration/Activation via the MCP-1-CCR2 Pathway in Mouse Brachiocephalic Arteries: Therapeutic Effects of Ezetimibe. *J. Atheroscler. Thromb.* 2012, 19, 986–998.
- (44) Soto-Rodríguez, I., Alexander-Aguilera, A., Zamudio-Pérez, A., Camara-Contreras, M. et al., Alteration of Some Inflammatory Biomarkers by Dietary Oxysterols in Rats. *Inflammation* 2012, 35, 1302–1307.
- (45) Terunuma, S., Kumata, N., Osada, K., Ezetimibe Impairs Uptake of Dietary Cholesterol Oxidation Products and Reduces Alterations in Hepatic Cholesterol Metabolism and Antioxidant Function in Rats. *Lipids* 2013, 48, 587–595.
- (46) ACNFP (Advisory Committee on Novel Foods and Processes) Phytosterol esters: extension of use. June 2013: Application from Unilever to extend the use of phytosterol esters under the Novel Foods Regulation (EC) 258/97. Under evaluation.

<http://acnfp.food.gov.uk/assess/fullapplics/phytosterol> (accessed Oct 16, 2014)

- (47) Hovenkamp, E., Demonty, I., Plat, J., Lütjohann, D. et al., *Biological effects of oxidized phytosterols: A review of the current knowledge*. *Prog. Lipid Res.* 2008, 47, 37–49.
- (48) Ryan, E., McCarthy, F., Maguire, A., O'Brien, N., *Phytosterol Oxidation Products: Their Formation, Occurrence, and Biological Effects*. *Food Revs. Int.* 2009, 25, 157–174.
- (49) Otaegui-Arrazola, A., Menéndez-Carreño, M., Ansorena, D., Astiasarán, I., *Oxysterols: A world to explore*. *Food Chem. Toxicol.* 2010, 48, 3289–3303.
- (50) García-Llatas, G., Rodríguez-Estrada, M. T., *Current and new insights on phytosterol oxides in plant sterol-enriched food*. *Chem. Phys. Lipids* 2011, 164, 607–624.
- (51) Vanmierlo, T., Husche, C., Schött, H., Pettersson, H., Lütjohann, D., *Plant sterol oxidation products – Analogs to cholesterol oxidation products from plant origin?* *Biochimie* 2013, 95, 464–472.
- (52) O'Callaghan, Y., McCarthy, F. O., O'Brien, N. M., *Recent advances in Phytosterol Oxidation Products*. *Biochem. Biophys. Res. Commun.* 2014, 446, 786–791.
- (53) Dutta, P. C., in Dutta, P. C. (Ed.) *Chemistry, Analysis, and Occurrence of Phytosterol Oxidation Products in Foods*, Marcel Dekker, Inc.: New York; Basel, 2004, pp. 397–417.
- (54) Guardiola, F., Bou, R., Boatella, J., Codony, R., *Analysis of Sterol Oxidation Products in Foods*. *J AOAC Int* 2004, 87, 441–467.
- (55) Oehrl, L. L., Hansen, A. P., Rohrer, C. A., Fenner, G. P., Boyd, L. C., *Oxidation of phytosterols in a test food system*. *J Am Oil Chem Soc* 2001, 78, 1073–1078.
- (56) Soupas, L., Juntunen, L., Lampi, A.-M., Piironen, V., *Effects of Sterol Structure, Temperature, and Lipid Medium on Phytosterol Oxidation*. *J. Agric. Food Chem.* 2004, 52, 6485–6491.
- (57) Barriuso, B., Otaegui-Arrazola, A., Menéndez-Carreño, M., Astiasarán, I., Ansorena, D., *Sterols heating: Degradation and formation of their ring-structure polar oxidation products*. *Food Chem.* 2012, 135, 706–712.
- (58) Rudzińska, M., Przybylski, R., Wąsowicz, E., *Products Formed During Thermo-oxidative Degradation of Phytosterols*. *J Am Oil Chem Soc* 2009, 86, 651–662.
- (59) Lampi, A.-M., Kemmo, S., Mäkelä, A., Heikkinen, S., Piironen, V., *Distribution of monomeric, dimeric and polymeric products of stigmasterol during thermo-oxidation*. *Eur J Lipid Sci Technol* 2009, 111, 1027–1034.
- (60) Rudzińska, M., Przybylski, R., Zhao, Y. Y., Curtis, J. M., *Sitosterol Thermo-oxidative Degradation Leads to the Formation of Dimers, Trimers and Oligomers: A Study*

- Using Combined Size Exclusion Chromatography/Mass Spectrometry. Lipids* 2010, 45, 549–558.
- (61) Struijs, K., Lampi, A.-M., Ollilainen, V., Piironen, V., Dimer formation during the thermo-oxidation of stigmasterol. *Eur. Food Res. Technol.* 2010, 231, 853–863.
- (62) Sosińska, E., Przybylski, R., Hazendonk, P., Zhao, Y. Y., Curtis, J. M., Characterization of non-polar dimers formed during thermo-oxidative degradation of β -sitosterol. *Food Chem.* 2013, 139, 464–474.
- (63) Sosińska, E., Przybylski, R., Aladedunye, F., Hazendonk, P., Spectroscopic characterisation of dimeric oxidation products of phytosterols. *Food Chem.* 2014, 151, 404–414.
- (64) Menéndez-Carreño, M., Ansorena, D., Astiasarán, I., Piironen, V., Lampi, A.-M., Determination of non-polar and mid-polar monomeric oxidation products of stigmasterol during thermo-oxidation. *Food Chem.* 2010, 122, 277–284.
- (65) Yanishlieva, N., Marinova, E., Autoxidation of sitosterol. I: Kinetic studies on free and esterified sitosterol. *Riv. Ital. Sostanze Gr.* 1980, 57, 477–480.
- (66) Yanishlieva-Maslarova, N., Schiller, H., Seher, A., Die Autoxidation von Sitosterin III Sitosterylstearat. *Fett Wiss. Technol.* 1982, 84, 308–311.
- (67) Soupas, L., Huikko, L., Lampi, A.-M., Piironen, V., Esterification affects phytosterol oxidation. *Eur J Lipid Sci Technol* 2005, 107, 107–118.
- (68) Lehtonen, M., Kemmo, S., Lampi, A.-M., Piironen, V., Effects of esterification on the formation and decomposition of steryl hydroperoxides. *Eur. Food Res. Technol.* 2011, 232, 255–264.
- (69) Lehtonen, M., Lampi, A.-M., Agalga, F., Struijs, K., Piironen, V., The effects of acyl moiety and temperature on the polymerization of sterols. *Eur J Lipid Sci Technol* 2011, 114, 677–686.
- (70) Lehtonen, M., Lampi, A.-M., Ollilainen, V., Struijs, K., Piironen, V., The role of acyl moiety in the formation and reactions of steryl ester hydroperoxides. *Eur. Food Res. Technol.* 2011, 233, 51–61.
- (71) Lehtonen, M., Lampi, A.-M., Riuttamäki, M.-A., Piironen, V., Oxidation reactions of steryl esters in a saturated lipid matrix. *Food Chem.* 2012, 134, 2030–2039.
- (72) Julien-David, D., Zhao, M., Geoffroy, P., Miesch, M. et al., Analysis of sitosteryl oleate esters in phytosterols esters enriched foods by HPLC–ESI-MS². *Steroids* 2014, 84, 84–91.
- (73) Bortolomeazzi, R., Cordaro, F., Pizzale, L., Conte, L. S., Presence of Phytosterol Oxides in Crude Vegetable Oils and Their Fate during Refining. *J. Agric. Food Chem.* 2003, 51, 2394–2401.

- (74) Johnsson, L., Dutta, P. C., Determination of phytosterol oxides in some food products by using an optimized transesterification method. *Food Chem.* 2006, 97, 606–613.
- (75) Dutta, P. C., Studies on phytosterol oxides. II: Content in some vegetable oils and in French fries prepared in these oils. *J Am Oil Chem Soc* 1997, 74, 659–666.
- (76) Tabea, E., Jägerstad, M., Dutta, P. C., Lipids and phytosterol oxidation products in commercial potato crisps commonly consumed in Sweden. *Eur. Food Res. Technol.* 2008, 227, 745–755.
- (77) Dutta, P. C., Appelqvist, L.-Å., Studies on phytosterol oxides. I: Effect of storage on the content in potato chips prepared in different vegetable oils. *J Am Oil Chem Soc* 1997, 74, 647–657.
- (78) Zunin, P., Calcagno, C., Evangelisti, F., Sterol oxidation in infant milk formulas and milk cereals. *J. Dairy Res.* 1998, 65, 591–598.
- (79) García-Llata, G., Cercaci, L., Rodríguez-Estrada, M. T., Lagarda, M. J. et al., Sterol Oxidation in Ready-to-Eat Infant Foods During Storage. *J. Agric. Food Chem.* 2008, 56, 469–475.
- (80) Conchillo, A., Cercaci, L., Ansorena, D., Rodríguez-Estrada, M. T. et al., Levels of Phytosterol Oxides in Enriched and Nonenriched Spreads: Application of a Thin-Layer Chromatography–Gas Chromatography Methodology. *J. Agric. Food Chem.* 2005, 53, 7844–7850.
- (81) EFSA. Draft Scientific Opinion on Acrylamide in Food. EFSA Panel on Contaminants in the Food Chain (CONTAM)
<http://www.efsa.europa.eu/de/consultationsclosed/call/140701.pdf> (accessed Oct 16, 2014).
- (82) EFSA. The EFSA Comprehensive European Food Consumption Database
<http://www.efsa.europa.eu/en/datexfoodcdb/datexfooddb.htm> (accessed Oct 16, 2014).
- (83) Menéndez-Carreño, M., Ansorena, D., Astiasarán, I., Stability of Sterols in Phytosterol-Enriched Milk under Different Heating Conditions. *J. Agric. Food Chem.* 2008, 56, 9997–10002.
- (84) Grandgirard, A., Martine, L., Joffre, C., Juaneda, P., Berdeaux, O., Gas chromatographic separation and mass spectrometric identification of mixtures of oxyphytosterol and oxycholesterol derivatives. Application to a phytosterol-enriched food. *J Chromatogr A* 2004, 1040, 239–250.
- (85) Soupas, L., Huikko, L., Lampi, A.-M., Piironen, V., Oxidative stability of phytosterols in some food applications. *Eur. Food Res. Technol.* 2006, 222, 266–273.

- (86) *Unilever Research and Development. Multi-Purpose Vegetable Fat Spreads and Liquid Vegetable Fat Based Emulsions with Added Plant Sterol esters for Cooking and Baking Applications (PS-Spreads/Liquids for CB).*
<http://acnfp.food.gov.uk/assess/fullapplics/phytosterol> (accessed Oct 16, 2014)
- (87) *Soupas, L., Huikko, L., Lampi, A.-M., Piironen, V., Pan-frying may induce phytosterol oxidation. Food Chem. 2007, 101, 286-297.*
- (88) *Botelho, P. B., Galasso, M., Dias, V., Mandrioli, M. et al., Oxidative stability of functional phytosterol-enriched dark chocolate. LWT - Food Sci. Technol. 2014, 55, 444–451.*
- (89) *Rudzińska, M., Przybylski, R., Wąsowicz, E., Degradation of phytosterols during storage of enriched margarines. Food Chem. 2014, 142, 294–298.*
- (90) *Kuhlmann, K., Lindtner, O., Bauch, A., Ritter, G. et al., Simulation of prospective phytosterol intake in Germany by novel functional foods. Br. J. Nutr. 2005, 93, 377-385.*
- (91) *Grandgirard, A., Sergiel, J.-P., Nour, M., Demaison-Meloche, J., Ghiès, C., Lymphatic absorption of phytosterol oxides in rats. Lipids 1999, 34, 563–570.*
- (92) *Tomoyori, H., Kawata, Y., Higuchi, T., Ichi, I. et al., Phytosterol Oxidation Products Are Absorbed in the Intestinal Lymphatics in Rats but Do Not Accelerate Atherosclerosis in Apolipoprotein E-Deficient Mice. J. Nutr. Biochem. 2004, 134, 1690–1696.*
- (93) *Grandgirard, A., Demaison-Meloche, J., Cordelet, C., Demaison, L., Incorporation of oxyphytosterols in tissues of hamster. Reprod. Nutr. Dev. 2004, 44, 599–608.*
- (94) *Liang, Y. T., Wong, W. T., Guan, L., Tian, X. Y. et al., Effect of phytosterols and their oxidation products on lipoprotein profiles and vascular function in hamster fed a high cholesterol diet. Atherosclerosis 2011, 219, 124–133.*
- (95) *Plat, J., Brzezinka, H., Lütjohann, D., Mensink, R. P., von Bergmann, K., Oxidized plant sterols in human serum and lipid infusions as measured by combined gas-liquid chromatography-mass spectrometry. J. Lipid Res. 2001, 42, 2030–2038.*
- (96) *Grandgirard, A., Martine, L., Demaison, L., Cordelet, C. et al., Oxyphytosterols are present in plasma of healthy human subjects. Br. J. Nutr. 2004, 91, 101-106.*
- (97) *Menéndez-Carreño, M., García-Herreros, C., Astiasarán, I., Ansorena, D., Validation of a gas chromatography–mass spectrometry method for the analysis of sterol oxidation products in serum. J Chromatogr B 2008, 864, 61–68.*
- (98) *Menéndez-Carreño, M., Steenbergen, H., Janssen, H.-G., 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 2012, 402, 2023–2032.*

- (99) Husche, C., Weingärtner, O., Pettersson, H., Vanmierlo, T. et al., Validation of an isotope dilution gas chromatography–mass spectrometry method for analysis of 7-oxygenated campesterol and sitosterol in human serum. *Chem. Phys. Lipids* 2011, 164, 425–431.
- (100) Baumgartner, S., Mensink, R. P., Husche, C., Lütjohann, D., Plat, J., Effects of plant sterol- or stanol-enriched margarine on fasting plasma oxyphytosterol concentrations in healthy subjects. *Atherosclerosis* 2013, 227, 414–419.
- (101) Baumgartner, S., Mensink, R. P., Hartog, G. d., Bast, A. et al., Oxyphytosterol formation in humans: Identification of high vs. low oxidizers. *Biochem. Pharmacol.* 2013, 86, 19–25.
- (102) Aringer, L., Eneroth, P., Studies on the formation of C7-oxygenated cholesterol and β -sitosterol metabolites in cell-free preparations of rat liver. *J. Lipid Res.* 1973, 14, 563–572.
- (103) Aringer, L., Eneroth, P., Formation and metabolism in vitro of 5,6-epoxides of cholesterol and β -sitosterol. *J. Lipid Res.* 1974, 15, 389–398.
- (104) Aringer, L., Eneroth, P., Nordström, L., Side chain hydroxylation of cholesterol, campesterol, and β -sitosterol in rat liver mitochondria. *J. Lipid Res.* 1976, 17, 263–272.
- (105) Subbiah, M., Kuksis, A., Oxidation of 4-¹⁴C-Sitosterol by mitochondria of rat liver and testes. *Fed. Proc.* 1969, 28, 515.
- (106) Hwang, K., Kelsey, M., Evidence of epoxide hydrase activity in human intestinal microflora. *Cancer Biochem. Biophys.* 1978, 70, 31–35.
- (107) Fioriti, J. A., Kanuk, M. J., George, M., Sims, R. J., Metabolic Fate of Epoxycholesterol in the Rat. *Lipids* 1970, 5, 71–75.
- (108) Lea, L., Hepburn, P., Wolfreys, A., Baldrick, P., Safety evaluation of phytosterol esters. Part 8. Lack of genotoxicity and subchronic toxicity with phytosterol oxides. *Food Chem. Toxicol.* 2004, 42, 771–783.
- (109) Koschutnig, K., Kemmo, S., Lampi, A.-M., Piironen, V. et al., Separation and isolation of β -sitosterol oxides and their non-mutagenic potential in the *Salmonella* microsome assay. *Food Chem.* 2010, 118, 133–140.
- (110) Abramsson-Zetterberg, L., Svensson, M., Johnsson, L., No evidence of genotoxic effect in vivo of the phytosterol oxidation products triols and epoxides. *Toxicol. Lett.* 2007, 173, 132–139.
- (111) Adcox, C., Boyd, L., Oehrl, L., Allen, J., Fenner, G., Comparative Effects of Phytosterol Oxides and Cholesterol Oxides in Cultured Macrophage-Derived Cell Lines. *J. Agric. Food Chem.* 2001, 49, 2090–2095.

- (112) Maguire, L., Konoplyannikov, M., Ford, A., Maguire, A. R., O'Brien, N. M., Comparison of the cytotoxic effects of β -sitosterol oxides and a cholesterol oxide, 7 β -hydroxycholesterol, in cultured mammalian cells. *Br. J. Nutr.* 2003, 90, 767-775.
- (113) O'Callaghan, Y. C., Foley, D. A., O'Connell, N. M., McCarthy, F. O. et al., Cytotoxic and Apoptotic Effects of the Oxidized Derivatives of Stigmasterol in the U937 Human Monocytic Cell Line. *J. Agric. Food Chem.* 2010, 58, 10793–10798.
- (114) O'Callaghan, Y., Kenny, O., O'Connell, N. M., Maguire, A. R. et al., Synthesis and assessment of the relative toxicity of the oxidised derivatives of campesterol and dihydrobrassicasterol in U937 and HepG2 cells. *Biochimie* 2012, 95, 496-503.
- (115) Vejux, A., Montange, T., Martine, L., Zarrouk, A. et al., Absence of Oxysterol-like Side Effects in Human Monocytic Cells Treated with Phytosterols and Oxyphytosterols. *J. Agric. Food Chem.* 2012, 60, 4060–4066.
- (116) Alemany, L., Laparra, J., Barberá, R., Alegría, A., 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.* 2013, 56, 247-53.
- (117) Meyer, W., Jungnickel, H., Jandke, M., Dettner, K., Spiteller, G., On the cytotoxicity of oxidized phytosterols isolated from photoautotrophic cell cultures of *Chenopodium rubrum* tested on meal-worms *Tenebrio molitor*. *Phytochemistry* 1998, 47, 789–797.
- (118) Yang, C., Chen, Z. Y., Wong, S. L., Liu, J. et al., β -Sitosterol oxidation products attenuate vasorelaxation by increasing reactive oxygen species and cyclooxygenase-2. *Cardiovasc. Res.* 2013, 97, 520–532.
- (119) Plat, J., Theuwissen, E., Husche, C., Lütjohann, D. et al., Oxidised plant sterols as well as oxysterol increase the proportion of severe atherosclerotic lesions in female LDL receptor+/- mice. *Br. J. Nutr.* 2014, 111, 64–70.
- (120) Julien-David, D., Ennahar, S., Miesch, M., Geoffroy, P. et al., Effects of oxidation on the hydrolysis by cholesterol esterase of sitosteryl esters as compared to a cholesteryl ester. *Steroids* 2008, 74, 832–836.