Eur J Nutr 38:84–89 (1999)

ORIGINAL CONTRIBUTION

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Plant sterols in vegetables and fruits commonly consumed in Sweden

Received: 25 September 1998
Accepted: 10 February 1999

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Summary Plant sterols are known to have serum cholesterol lowering effects. A high dietary intake might therefore have a positive impact on health. All food items of vegetable origin contain some amount of plant sterols. The aim of this study was to analyse the plant sterol content of vegetables and fruits commonly consumed in Sweden, and to compare fresh and cooked samples of the same items.

Altogether 20 different vegetables and 14 fruits were analysed. All vegetables and fruits were purchased in two shops in the city of Gothenburg, Sweden. Lyophilization was performed within one month of the items being purchased. The samples were frozen at -20 (C and analysed within six months, with a GLC method after acid hydrolysis, alkaline hydrolysis and silylation with tri-methylsilylether. The acid hydrolysis was done in order to detect the fraction of glycosylated plant sterols, which are split during boiling with HCl.

The median plant sterol content of vegetables was 14 (3.8-50) mg/100 g edible portion. The highest concentrations were found in broccoli, Brussels sprouts, cauliflower and olives. The median plant sterol content of fruits was 16 (3-44) mg/100 g edible portion. The highest concentrations were found in oranges and passion fruits.

The plant sterol concentrations were thus low in vegetables and fruits commonly consumed in Sweden. A serum cholesterol lowering effect attributed to the plant sterols in vegetables and fruits would therefore be of limited significance.

Key words Plant sterols – phytosterols – vegetables – fruits – GLC-analysis

Introduction

Plant sterols are found in all food items of plant origin. The most common plant sterols are β-sitosterol, campesterol, and stigmasterol, classified as 4-desmethylsterols of the cholestane series (1). Their structures are similar to that of cholesterol with an extra methyl or ethyl group and a double bond in the side chain. Saturated plant sterols, referred to as stanols have no double bound in the ring structure (2). Sitostanol and campestanol exist in quantifiable amounts in cereals, fruits and vegetables, but generally of less concentration than the unsaturated plant sterols (3). Plant sterols can exist as free plant sterols, esterified plant sterols, plant steryl glycosides and acylated plant steryl glycosides (4). The different fractions are assumed to exist in different parts of the plant cell. Free plant sterols are, similar to cholesterol in animal cells, part of the cell wall with a structural property (5). Plant steryl esters are generally believed to be storage products. They can be found in the cytosol of plant cells, in droplets or vesicles (6). The largest amount of plant steryl gly-

cosides has been found in the microsomal fraction of the plant cell and acylated steryl glycosides are believed to exist in mitochondria (7).

Plant sterols are assumed to be hydrolysed in the gut by sterolester hydrolase, and therefore the different fractions should have the same effects on the cholesterol absorption in the human small bowel (8). Plant sterols have long been known to have serum cholesterol lowering properties. There has been increased interest in these natural compounds after a rapeseed oil margarine, enriched by sitostanol ester, was introduced commercially in Finland (9). The enrichment of this margarine by plant sterols has given the product serum cholesterol lowering properties, considered beneficial in the reduction of coronary heart disease (10). Plant sterols are also suggested to have an anti-tumourigenic effect (11, 12).

In 1978, a report from the Department of Agriculture in the USA on the sterol content of foods of plant origin, was published (13). The aim of the report was to aid in the evaluation of mixed diets. The authors concluded that the data were to be considered provisional as they were based on only a few observations. The gas-liquid chromatography (GLC) technique has developed since the end of the 70´s, raising the assumption that analyses of today are more accurate for assessing sterol content. Due to their possible implications for public health, there has been an increased interest in analysing the content of plant sterols in modern food items, for application in epidemiological studies.

The aim of this study was to investigate the content and variation of the most common isomers of plant sterols in fresh vegetables and fruits, often consumed in Gothenburg, Sweden, and to compare data of this study with earlier published results, particularly by Weihrauch (13). In addition, the effect of cooking on the content of sterols in vegetables and fruits was investigated. Plant sterol content of cereals and fats was also investigated, but will be published elsewhere.

Materials and methods

Sources and preparation of samples

The analysed vegetables and fruits were selected from food items commonly consumed in a cohort of Swedish women in the city of Gothenburg (14). A total of 1 361 women gave 24-h recalls of dietary intake at baseline examination in 1968-69 for the population study of women in Gothenburg (15). Follow-up studies, including new interviews, were done in 1974-75, 1980-81 and 1992-93 (14, 15). The most frequently reported food items were analysed for plant sterols.

Altogether 22 vegetables and 14 fruits were analysed. All vegetables and fruits were bought in two shops in the Gothenburg area, in September 1996. The shops represented two major Swedish food chains. The country of origin of all items was recorded. Seasonal variation was however not taken into account. Samples of the vegetables and fruits from the two shops were weighed in equal amounts and mixed together to minimise the number of analyses. The samples were frozen at -20 ºC. Lyophilization was performed within one month after the items were bought. The samples were then frozen again at -20 ºC and analysed within six months.

Comparison of raw and cooked vegetables and fruits

Vegetables and fruits were boiled in water according to general Swedish recipes. The boiled vegetables and fruits were freeze-dried as described above. Vegetables included in the comparison were broccoli, carrots, cauliflower, celeriac, fennel, onions, parsnips, Swedes (turnips) and white cabbage. The selected fruits were apples, pears, peaches and pineapple, which are sometimes boiled before being eaten as a dessert in Sweden.

Plant sterol analysis

Unsaponifiables were prepared from the vegetable and fruit samples according to the Jonker method, with some minor alterations (16). Briefly, 0.250 mg of the dissolved internal standard 5α-cholestane was added to a glass flask. The solvent was evaporated under a stream of nitrogen. Approximately 0.5 g of the freeze-dried food sample was weighed into the flask. Acid hydrolysis was performed by addition of 15 ml 6M HCL and the reaction mixture was heated for 30 minutes in a boiling water bath. After cooling the samples on ice, 6.72 g KOH pellets and 100 ml 2M KOH in 96 % ethanol were added and the flasks were thereafter refluxed for 30 minutes in a boiling water bath. Samples were left to cool to room temperature. Toluene, 100 ml, was added to the flask and shaken vigorously for two minutes. The upper phase was transferred to a 500 ml separating funnel, which contained 100 ml 2M KOH. The funnel was shaken for two minutes. The mixture was left to separate and the alkaline phase was discarded. The toluene phase was washed with portions of 100 ml de-ionised distilled water until the aqueous phase tested neutral. Water free Na_2SO_4 was used to remove water in the toluene phase. Evaporation of the toluene was performed at 50 ºC under vacuum. The residue was dissolved in 1.5-ml chloroform. The solvent was transferred to a 2-ml vial. Prepared samples were stored at -20 ºC. Tri-methylsilylether (TMS) derivatives were prepared the same day as the analysis was performed. This took place by evaporating the chloroform under a stream of nitrogen and adding 100 µl of Tri-Sil reagent (Pierce Chemical Co., Rockford, IL). The sample was incubated in an oven with an internal temperature of 60 ºC for 45 minutes and evaporated with nitrogen. The TMS-ether derivatives were dissolved in 1 ml Hexane. Details of the GLC method are described elsewhere (17).

Analytical control and calculation of plant sterol content

All chemicals and formulas were of analytical grade. A standard containing 5α-cholestane, cholesterol, β-sitosterol, sitostanol, campesterol, campestanol and stigmasterol (Fluka Chemie AG, Buchs, Switzerland) was analysed whenever the GLC was used. The relative retention times of the standard were used to identify the plant sterols. The peak areas were computed by a Vectra Hewlett Packard VL2 integrator (Hewlett Packard, USA). The contents were calculated as mg/100 g edible portion. Total plant sterols were calculated as the sum of the major fractions, β-sitosterol, campesterol, stigmasterol, sitostanol and campestanol. All samples were analysed in duplicate. Duplicate samples were not allowed to differ more than 10 % or less than 1mg/100 g in cases of a lower content than 10 mg /100 g. If samples differed more than 10 %, the samples were prepared and analysed again. Between run variation was performed to compare variation of the standard.

Results

Plant sterol content of vegetables

The method was successful in separating the unsaturated plant sterols from the saturated sterols (Figure 1).

The analysis showed a considerable variation of plant sterol content in different vegetables. Results are presented as median values in mg/100 g edible portion (mg/100 g e.p.) with minimum and maximum values in brackets. The median total amount of plant sterols in the vegetables was 16 mg/100 mg e.p. (3.8-50) (Table 1). Concentrations of the upper range, i.e. more than 30 mg/100 mg e.p., were found for broccoli, 39 mg/100 mg e.p., Brussels sprouts, 43 mg/100 mg e.p., cauliflower, 40 mg/100mg e.p., green olives, 35 mg/100mg e.p., and black olives, 49 mg/100 mg e.p.. (-sitosterol was the plant sterol found in the highest concentration, 8.2 (0-48) mg/100 mg e.p. Only mushrooms had no detectable amount of β-sitosterol. The campesterol fraction consisted of 2.1 (0-18) mg/100mg e.p., with the radish as the sole vegetable without detectable amounts. Mushrooms had in contrast the highest concentration of campesterol, 18 mg/100 e.p.. The median content of stigmasterol was only 0.38 (0-8.6) mg/100 g e.p.. Saturated plant sterols were of much lower concentration. Campestanol was only detectable in seven items, while sitostanol was detectable in ten items. The campesterol concentrations were however lower than 0.04 mg/100 g e.p., and the β-sitostanol conctrations were lower than 0.6 mg /100 g e.p.

Verification of the plant sterol analysis with gas chromatography-mass spectrometry has been described elsewhere (17).

Table 1 Plant sterols in vegetables, mg/100 g edible portion

Vegetables	Campesterol	Campestanol	Stigmasterol	β-Sitosterol	β-Sitostanol	Total plant sterols
Broccoli	6.9	0.10	1.1	31	0.08	39
Brussels sprouts	8.0	-	0.38	34	-	43
Carrot	2.2	-	2.8	11	0.08	16
Cauliflower	9.5	-	3.7	26	0.06	40
Celeriac	2.7	-	8.6	8.9	-	20
Celery	2.7	-	7.0	7.3	0.13	17
Chinese cabbage	1.6	0.03	0.03	6.8	0.06	8.5
Fennel	0.35	-	4.3	5.1	0.02	9.8
Kale*	0.91	0.07	0.38	7.4	0.11	8.8
Leek	0.61	0.09	0.06	7.3	-	8.1
Mushrooms	18	-	-	-	-	18
Olives. green	1.1	0.09	0.29	34	-	35
Olives. black	1.4	0.35	-	48	-	50
Onion	0.82	-	0.57	7.0	-	8.4
Parsnip	2.8	0	7.3	18	0.19	27
Pepper. green	2.0	-	0.33	4.9	-	7.2
Potato*	0.23	-	0.38	2.7	0.56	3.8
Radish	-	-	-	4.4	-	9.0
Sauerkraut*	3.2	-	0.09	11	-	15
Swedish turnip	3.3	0	0.26	14	0	17
Tomato	0.28	0.05	1.7	2.4	0.23	4.7
White cabbage	2.8	-	0.2	9.4	0.35	13
Median (min-max)	2.1 (0-18)	0 (0-0.35)	0.38 (0-8.6)	8.2 (0-48)	0.01 (0-0.56)	16 (3.8-50)

* Analysed as eaten, i.e. boiled.

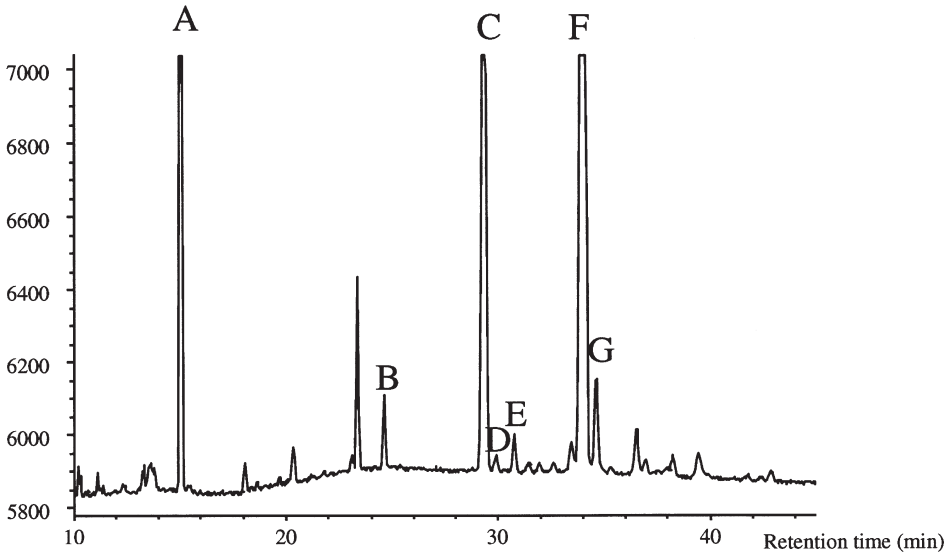

Fig. 1 Chromatogram of Swedish turnip. A -5 -α-cholestane. B-cholesterol. C-campesterol. D-campestanol. E-stigmasterol. F-β-sistosterol. G-β-sistostanol

Plant sterol content of fruits

Total plant sterols in the fourteen fruits had a median concentration of 16 (1.3-44) mg/100 g e.p., a figure quite similar to that for vegetables (Table 2). Only passion fruit had a content of more than 30 mg /100 g e.p., with a value of 44 mg/100 g e.p. The median concentration of β-sitosterol was 12 (0.9-34) mg/100 g e.p.. All fourteen samples had detectable amounts. The highest concentrations were found in oranges, 20 mg/100 g e.p., and passion fruit, 34 mg/100 g e.p.. Campesterol concentration was much lower, 1.2 (0.18-8.8) mg/100 g e.p. Passion fruit was again the best source with 8.8 mg/100 g e.p. Stigmasterol had a similar median concentration as campesterol but showed less variation (0-1.8). Detectable amounts of β-sitostanol and campestanol were only found in pineapple, 0.8 and 0.7 mg /100 g e.p. respectively.

Plant sterol content of cooked samples

The plant sterol concentrations in the boiled samples were: broccoli 68 mg/100 g e.p., carrots 15 mg/100 g e.p., cauliflower 34 mg/100 g e.p., celeriac 22 mg/100 g e.p., fennel 10 mg/100 g e.p., onion 7.2 mg/100 g e.p., parsnip 21 mg/100 g e.p., Swedes 13 mg/100 g e.p., white cabbage 11 mg/100 g e.p., apple 13 mg/100 g e.p., peach 13 mg/100 g e.p., pear 12 mg/100 g e.p., and finally pineapple 15 mg/100 g e.p. Concentrations given in mg/100 g dry mass were 130 (55-695) for raw items and 128 (64-839) for cooked samples. No significant difference was found between raw and cooked samples at a group level, either when compared in mg/100 g e.p or in mg/100 g dry mass.

Table 2 Plant sterols in fruit, mg/100 g edible portion

Fruit	Campesterol	Campestanol	Stigmasterol	β-Sitosterol	(-Sitostanol	Total plant sterols
Apple	0.36	-	0.10	13	-	13
Banana	1.5	-	1.8	11	-	14
Clementine	4.0	-	0.78	12	-	16
Fig	0.93	-	1.2	20	-	22
Grapefruit	2.5	-	1.0	15	-	18
Honeydew melon	0.17	-	0.49	1.2	-	1.8
Kiwi	0.44	-	1.4	7.2	-	9.1
Lemon	3.3	-	1.3	13	-	18
Orange	3.0	-	1.0	20	-	24
Passion-fruit	8.8	-	0.62	34	-	44
Peach	0.58	-	1.8	13	-	15
Pear	0.27	-	-	12	-	12
Pineapple	3.8	0.74	0.44	11	0.79	17
Watermelon	0.18	-	0.26	0.91	-	1.3
Median (min-max)	1.2 (0.18-8.8)	0 (0-0.74)	0.89 (0-1.8)	12 (0.9-34)	0 (0-0.8)	16 (1.3-44)

Discussion

Values of plant sterol concentration in vegetables and fruits are low compared with mixed diets. Studies have shown that ileostomists on low fibre diets excrete 120-220 mg plant sterols per day (18). A typical high fibre diet with the addition of brown bread, vegetables and fruit gave an excretion of 350 mg/day (19). Excretion in this case might be regarded as a close estimate of the intake as most plant sterols have a very limited absorption (20). Beveridge et al. tested the effect of different amounts of β-sitosterol on serum cholesterol levels in 85 students on a diet rich in butterfat. With intakes of 300 mg/950 kcal a decreasing effect on plasma cholesterol was seen (21). The intake corresponded to a total intake of 1 g per day, which is three times the intake from a diet high in vegetables and fruits. It is obvious after review of the highest concentrations of plant sterols found in this paper, that large amounts of vegetables and fruits must be consumed in order to increase the intake of plant sterols.

The present results are similar to that found in the report by Weihrauch (13). The median concentrations in the ten vegetables found in both reports were 12 (5-24) mg/100 g e.p. in the American report and 10.9 (3.8-43) mg/100 g e.p. in the present report. Similar results were found for the ten fruits, 12 (2-34) mg/100 g e.p. in the American report and 16 (1.3-24) mg/100 g e.p. in the present report. It appears that even though new and improved methods have been developed, the new method still gave results in the same range as the older methods. It is, however, of utmost importance to use the same method when data are compared in epidemiological studies. This was not taken into account in the Weihrauch report, in which results of plant sterols from 1963 to 1977 were compiled. Comparing the sparse data on the same food items in both the Weihrauch and Jonker reports (13, 16), the range was about the same and no statistically significant differences were found.

The recovery of plant sterol during analysis is higher if an acid hydrolysis is included in the preparation of the samples (16, 22). Jonker reported a 22-42 % increase of sitosterol in analyses of mixed diets (16). Piironen reported a 10 % increase in plant sterols when an acid hydrolysis step was added before the alkaline hydrolysis during preparation of various foods (20). The lack of difference between the Weihrauch report and the present paper might be explained by the fact that the AOAC method (23) also included an acid hydrolysis.

Extraction techniques often vary with different investigators due to tradition (5). The most common solvents are toluene, chloroform, acetone, methylene chloride, petroleum ether, and ethanol. The AOAC method (23) recommended the use of ether as the extraction solvent. Jonker investigated the differences between ether and toluene but found no significant statistical difference (16). Extraction solvent has been regarded as a possible confounder in comparisons of data on plant sterol concentrations (24). Sterols differ in hydrophobicity (25), which might have an impact on the sterol recovery by extraction. Hubbard et al. showed that an extraction procedure of chloroform and methanol significantly improved sterol recovery compared to a HCl:ethyl ether method (24). Choice of extraction solvent seemed to give varied results, and it might therefore be of importance to compare toluene with chloroform and methanol in efficiency.

Plant sterols are known to have structural properties, but also to exist in the cytosol (4, 26). The authors have considered the possibility that due to rupture of cells and tissues, part of the sterols may be lost. The opposite may also be true, i.e., the water loss might increase the plant sterol concentration. Any of the mentioned effects by boiling seemed not to be a major problem when samples were compared statistically at a group level, as there were no significant differences between the fresh and the cooked samples. The only food item with a major difference between raw and boiled samples, was broccoli, with 39 mg/100 g e.p. in the fresh sample and 68 mg/100 g e.p. in the boiled sample. Given in mg/100 g dry mass, the total plant sterol concentrations were 522 for raw broccoli, and 839 for cooked broccoli. As broccoli had an analogue increase in dry weight, the reason for the higher concentration in the boiled broccoli was probably not water loss during cooking. Losses of carbohydrates, proteins or minerals might have caused it. On the other hand, cauliflower had only a small decrease when total plant sterol concentration was compared in mg/100 g edible portion. Concentrations in dry mass were 695 mg/100 g dry mass before boiling, and 453 mg/100 g dry mass after boiling. In this case, boiling might have caused a leakage of the plant sterols. As the remaining food items had similar amounts of plant sterols whether they were boiled or not, compared both as wet or dry weights, changes of total plant sterol concentrations as a result of cooking, did not seem to be a major problem. The results were similar for vegetables and fruits.

Almost no data are available on the impact of plant variety, geographic location, agricultural practices and processing. Most vegetables and fruits selected in this study were from Sweden and other European countries, but also from China, New Zealand and Chile. The effect of the above mentioned factors should be carefully evaluated.

In conclusion, plant sterol concentrations in vegetables and fruits were low and would require consumption of large volumes of vegetables and fruits to increase the total intake. The finding that the data for vegetables and fruits were comparable with the data in the Weihrauch report indicates that the older methods were accurate in measuring plant sterol concentrations for vegetables and fruits.

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