# Common Sources and Estimated Intake of Plant Sterols in the Spanish Diet

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Plant sterols (PS) are minor lipid components of plants, which may have potential health benefits, mainly based in their cholesterol-lowering effect. The aim of this study was to determine the composition and content of PS in plant-based foods commonly consumed in Spain and to estimate the PS intake in the Spanish diet. For this purpose, the determination of PS content, using a modern methodology to measure free, esterified, and glycosidic sterol forms, was done. Second, an estimation of the intake of PS, using the Spanish National Food Consumption data, was made. The daily intake per person of PS—campesterol, $\beta$-sitosterol, stigmasterol, and stigmastanol—in the Spanish diet was estimated at 276 mg, the largest component being $\beta$-sitosterol (79.7%). Other unknown compounds, tentatively identified as PS, may constitute a considerable potential intake (99 mg). When the daily PS intake among European diets was compared in terms of campesterol, $\beta$-sitosterol, stigmasterol, and stigmastanol, the PS intake in the Spanish diet was in the same range of other countries such as Finland (15.7% higher) or The Netherlands (equal). However, some qualitative differences in the PS sources were detected, that is, the predominant brown bread and vegetable fat consumption in the northern diets versus the white bread and vegetable oil consumption in the Spanish diet. These differences may help to provide a link between the consumption of PS and healthy effects of the diet.

KEYWORDS:  Plant sterols; Spanish diet; dietary intakes; food composition table

## INTRODUCTION

Currently, there is a great interest in research involving bioactive compounds from plant food origin. This is because of their potential preventive effects on the chronic diseases of modern Western societies, especially cardiovascular disease and cancer (*1–3*). Plant-based foods contain a large number of plant sterols (PS) as minor lipid components. They can exist as free sterols and as bound conjugate sterols—fatty or phenolic acid esters, glycosides with $\beta$-D-glucose, or acylated glycosides with acyl-$\beta$-D-glucose—with these substitutions occurring at carbon 3 (*4*).

The nutritional role of these compounds is based on their cholesterol-lowering [total and low-density lipoprotein (LDL)] effect in human and animal blood, because PS competitively inhibit cholesterol intestinal uptake (*5, 6*). For healthy humans, the absorption rate of PS is usually <5% of dietary levels. Thus, ~95% of dietary PS enter the colon. A dietary intake of 1–3 g/day of PS from oils or functional foods usually produces a 10–15% fall in LDL cholesterol (*5, 7, 8*). As Phillips et al. (*9*) state, recent research (*10*) has evidenced that PS, in the level found in plant-based foods, may reduce cholesterol absorption.

An important barrier to phytonutrient research is the almost complete lack of availability of dietary bioactive compound tables (*2, 11–13*). Thus, the study of the common dietary contributors to PS intake may be of use in developing strategies to improve their intake, if necessary, and essential to facilitating epidemiological studies.

The most recent review of PS contents in foods of plant origin is the paper of Weihrauch and Gardner (*14*), stating that most of the analyses of sterols antedate 1970. The major advancement in the gas–liquid chromatography (GC) analysis of PS is made afterward:  first, with the introduction of polar liquid phases such as OV-17 allowing the separation of critical PS pairs—campesterol versus campestanol and $\Delta^5$-avenasterol versus stigmastanol—and, second, with the introduction of wall-coated capillary columns allowing better resolution and sensitivity. Nowadays, GC capillary column and combination of GC with mass spectroscopy are the most widely used analytical techniques for quantitative and qualitative analysis of sterols in plant foods (*15*). Specifically, with regard to oils, the method most frequently used in the determination of 4-desmethyl sterols in olive oil, proposed as the official method by European Union (EU) legislation (*16*), involves numerous sample manipulations (*17, 18*) such as the removal of triglycerides, the fractionation of the unsaponifiable matter into several classes of compounds,

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and their subsequent analysis by GC. Thus, the usual sample preparation of these methods involves a long procedure and, in some cases, the use of relatively high volumes of hazardous solvents.

The method used for us, which was performed by Toivo et al. (*19, 20*), on the one hand simplified the preparation step by using a solid-phase extraction procedure and, on the other, allowed the glycosylated sterols to be measured by using an acid hydrolysis step. Jonker et al. (*21*) and more recent research (*9, 22–28*) indicate that literature PS data may be underestimated, because the hydrolysis acid step, which allows the measurement of the glycosylated sterol fraction, is not used. The former method has been validated and optimized in different food matrices such as oils (*19*), wheat flour (*20*), diet composites representing an average American diet (*23*), certain fruits and vegetables (*22*), and different oat cultivars (*28*).

Recently, PS contents in some groups of foods commonly consumed in different European populations have been reported: it has been estimated in the British (*29*), the Dutch (*26*), and the Finnish (*13*) diets. In contrast, there is no updated estimation of PS intake in a Mediterranean country. The reliability of these estimated intake studies depends on a representative sampling of foods, quality assurance of analytical methods, selection of adequate food consumption data, and an adequate combination of food compound concentrations with food consumption figures (*30*).

The Mediterranean diet could be described as the dietary pattern found in the olive oil growing areas in the late 1950s and early 1960s, before the fast food culture began influencing nutritional habits. Beyond olives and olive oil it is also characterized by the consumption of fruits, vegetables, fish and seafood, legumes, and cereals (*31, 32*).

The aim of this study was to apply an analytical methodology that measures free, esterified, and glycosidic sterols to determine the composition and content of PS in plant-based foods commonly consumed in Spain. In addition, an estimation of the PS intake in the Spanish diet on the basis of the Spanish National Food Consumption (SNFC) data (*33*) was done.

## MATERIALS AND METHODS

**Plant Materials.** The foods selected were those presented in the SNFC study (*33*), and they represent the plant-based foods commonly consumed by the Spanish population. Several food items from different groups—cereals, fruits, legumes, nuts, oils, and vegetables—were analyzed. In each group, food items were selected to reflect intakes of >96% of the total plant-based food intake, except in the case of cereals, which reflect nearly 80%. Samples were purchased from a large store (El Corte Inglés), which is one of the most representative food chains in Spain. The collection was made in a way that approximately represented their quantitative market shares. Most samples were of domestic origin, and all of them were purchased at commercial maturity. In the case of manufactured samples such as oils and legumes, the most common brands were taken. A description of each food item is shown in **Table 1**.

The samples were determined as consumed (edible parts were analyzed). It is reported that differences in the separation of edible portions of vegetables may cause considerable variation in the PS contents of vegetables (*22*). In this sense, we have evidenced that the nonedible portions—peels and seeds—of certain fruits gave significantly higher content than the edible portions (data not shown). The preparation of the plant material includes several steps. A description of how each item was prepared is shown in **Table 1**. In a general way the preparation could be described as follows. Fruits and vegetables were washed with demineralized water. Then the skin or rind of fruits and vegetables was peeled when necessary. Samples were freeze-dried, powdered to 0.5 mm, and stored at −20 °C until analysis. In the case of grains, legumes, nuts, and oils the freeze-drying process was not performed.

All samples were analyzed without a cooking process. In this sense, no difference has been reported in the PS content between the fresh and cooked samples of vegetables and fruits commonly consumed in Sweden (*24*) and The Netherlands (*26*).

The water content of fresh and freeze-dried products was taken into account to calculate PS composition.

**Reagents.** The reference sterols (CAS Registry No., purity) campesterol (474-62-4, 65%), cholesterol (57-88-5, 99%), β-sitosterol (83-46-5, 98%), stigmastanol (19466-47-8, 96.7%), and stigmasterol (83-48-7, 95%) were obtained from Sigma-Aldrich Chemical Co. (Madrid, Spain). *N,O*-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) and trimethylchlorosilane (TMCS) (Fluka, Madrid, Spain) were used as silylating reagents. All other solvents and reagents were of analytical grade (Merck, Madrid, Spain) and were used without further purification.

**Extraction of PS.** The procedures followed for the preparation of the oil samples (*19*) and nonoil samples (*20*) were validated and reported previously.

*Preparation of Oil Samples.* Sample (0.25 g) was weighed in a 50 mL screw-capped glass tube; 8.0 mL of absolute ethanol and 0.5 mL of potassium hydroxide solution (saturated in water) were added, and the tubes were heated at 80 °C for 30 min in a shaken water bath. After the saponification, the tubes were cooled to room temperature, and 1 mL of internal standard solution (0.4 mg of cholesterol in 1 mL of *n*-hexane) was added. Then 20 mL of cyclohexane and 12 mL of distilled water were added to each sample, and the tubes were shaken thoroughly for 10 min. Afterward, we let the tubes stand for 15 min to separate the two phases. Finally, 10 mL of the unsaponifiable upper phase was recovered in a round-bottom flask and evaporated to dryness in a rotavapor at 50 °C. The residue was dissolved in 0.5 mL of dichloromethane.

*Preparation of Nonoil Samples.* A first step of acid hydrolysis and lipid extraction was performed as follows. Sample (0.5 g) was weighed in a 40 mL screw-capped glass tube, 4 mL of internal standard solution (0.02 mg of cholesterol in 1 mL of ethanol) and 5 mL of 6 M chlorhydric acid were added to each sample, and the tubes were heated at 80 °C for 1 h in a shaken water bath. After the acid hydrolyzation, the tubes were cooled to room temperature, 20 mL of hexane/diethyl ether (1:1) solvent mixture was added, and the tubes were shaken thoroughly for 10 min. After the shaking step, we let the tubes stand for 15 min to separate the two phases. Finally, the upper phase containing lipids was recovered in a round-bottom flask and evaporated to dryness in a rotavapor at 50 °C. Afterward, a second step of a saponification process was applied as follows: 8.0 mL of ethanol was added to the dry extracts, and the mixture was transferred to a 50 mL glass tube; then 0.5 mL of potassium hydroxide solution (saturated in water) was added, and the tubes were heated at 80 °C for 30 min in a shaken water bath. Then 20 mL of cyclohexane and 12 mL of distilled water were added to each sample, and the tubes were shaken thoroughly for 10 min. Afterward, the tubes were allowed to stand for 15 min to separate the two phases. Finally, 15 mL of the unsaponifiable upper phase was recovered in a round-bottom flask and evaporated to dryness in rotavapor at 50 °C. The residue was dissolved in 1 mL of chloroform. In the case of these nonoil samples a last purification step was applied, as follows: 1.0 mL of chloroform extract sample was passed through a 0.45 μm nylon membrane filter (101445C, Acrodisc, Gelman) to a C-18 solid-phase extraction cartridge (1225-6001, 6 g/mL, Megabond elut, Varian), which had been activated sequentially with 5 mL of methanol and 5 mL of deionized water. The cartridges could be attached to a vacuum manifold (5-7044, Visiprep, Supelco). Solutions were eluted freely for a couple of minutes, and afterward, we used a vacuum for 5 min. Afterward, clean flasks were placed to collect the sterol fractions. The sterol fraction in each sample was eluted with 15 mL of methanol/chloroform (5:95) and evaporated completely to dryness in a rotavapor at 50 °C; then each residue was dissolved in 0.5 mL of dichloromethane.

**Preparation of Trimethylsilyl (TMS) Ether Derivatives.** The silylation procedure of oil samples (*19*) was similar to that of nonoil samples (*20*). Fifty microliters in the case of oil samples and 100 μL in the case of nonoil samples of the PS fraction were placed in a presilanized screw-capped vial. The solvents were evaporated under nitrogen, and the TMS ether derivatives of the sterols were prepared

**Table 1.** Plant Food Items Selected in the Study

| food | variety/brand | origin | sample preparation[a] | taxonomy |
|---|---|---|---|---|
| vegetables | | | | |
| artichoke | Blanca Provenza | Almería | ELR, C, FD, M | *Cynara scolymus* L. |
| asparagus | green | Granada | ELR, C, FD, M | *Asparagus officinalis* L. |
| bean, green | Garrafal | Málaga | P, C, FD, M | *Phaseolus vulgaris* Savi |
| broccoli | Maratón | La Rioja | SR, C, FD, M | *Brassica oleracea* L. var. *botrytis* subvar. *cymosa* Duch. |
| cabbage | white | Castilla y León | C, FD, M | *Brassica rubla oleracea* L. var. *capitata* subvar. *alba* D.C. |
| carrot | Mokim | Cádiz | P, W, C, FD, M | *Dacus carota* L. var. *sativa* D.C. |
| cauliflower | Súper Siria | La Rioja | SR, C, FD, M | *Brassica oleracea* L. var. *botrytis* subvar. *cauliflora* Duch. |
| celery | Dulce Mill | Andalucía | ELR, FD, M | *Apium graveolens* L. |
| chard | Lyon | Castilla-La Mancha | W, C, FD, M | *Beta vulgaris* L. var. *cycla* L. |
| cucumber | Midi | Almería | W, P, C, FD, M | *Cucumis sativus* L. |
| eggplant | Belleza Negra | Málaga | SR, W, C, FD, M | *Solanum melongena* L. |
| endive | Latifolia | Andalucía | ELR, C, W, FD, M | *Cichorium endivia* L. |
| escarole | Crispum | Andalucía | ELR, C, W, FD, M | *Cichorium intybus* L. |
| garlic | Flor | Andalucía | P, C, FD, M | *Allium sativum* L. |
| leek | Margarita | Segovia | ELR, C, FD, M | *Allium porrum* L. |
| lettuce | Romana | Murcia | ELR, C, W, FD, M | *Lactuca sativa* L. |
| marrow | Verde Hortelano | Zaragoza | P, C, FD, M | *Cucurbita pepo* L. var. *medullusa* Alef. |
| onion | Recas | Castilla-La Mancha | P, C, FD, M | *Allium cepa* L. |
| parsley | undetermined | Castilla-La Mancha | C, FD, M | *Petroselinum sativum* Hofpm. |
| potato | Monalisa | Castilla y León | W, P, C, FD, M | *Solanum tuberosum* L. |
| pepper, green | Lamallo | Almería | SR, W, C, DS, FD, M | *Capsicum annuum* L. var. *grosum* Bailey |
| pepper, red | Genil | Almería | SR, W, C, DS, FD, M | *Capsicum annuum* L. var. *grosum* Bailey |
| spinach | Glabra | Andalucía | W, C, FD, M | *Spinacea oleracea* L. |
| tomato | Daniella | Murcia | W, C, FD, M | *Lycopersicon esculentum* Mill. |
| oils | | | | |
| virgin olive | Carbonell | Córdoba | N | *Olea europaea sativa* Hoffg. Link |
| refined olive | Carbonell | Córdoba | N | *Olea europaea sativa* Hoffg. Link |
| sunflower | Koipesol | Andalucía | N | *Helianthus annuus* L. |
| fruits | | | | |
| apple | Golden Delicious | Lleida | W, P, CR, C, FD, M | *Pyrus malus* L. |
| apricot | Bulida | Castilla-La Mancha | W, P, CR, C, FD, M | *Prunus armeniaca* L. |
| banana | Cavendish | Canarias | P, C, FD, M | *Musa paradisiaca* L. |
| cherry | Navalinda | Extremadura | W, DS, C, FD, M | *Prunus avium* L. |
| grape | white, Moscatel | Andalucía | W, C, FD, M | *Vitis vinifera* L. |
| kiwi fruit | Hayward | Asturias | W, P, C, FD, M | *Actinidia deliciosa* [A. Chev.] C.F. Liang et A.R. Ferguson |
| lemon | Fino | Andalucía | SQ, FD | *Citrus limonis* Osbeck. |
| mandarin | Clementine, Fortuna | Valencia | P, C, FD, M | *Citrus deliciosa* Ten. |
| melon | Piel de Sapo | Castilla-La Mancha | P, C, FD, DS, M | *Cucumis melo* L. |
| olive | Brown, Picual | Andalucía | P, C, DS, FD, M | *Olea europaea sativa* Hoffg. Link. |
| orange | Navel | Valencia | P, C, FD, M | *Citrus sinensis* L. |
| peach | Baby Gold | Tarragona | W, P, DS, C, FD, M | *Prunus persica* Sieb. et Zuce. |
| pear | Conferencia | Aragón | W, P, CR, C, FD, M | *Pyrus communis* L. |
| plum | Golden Japan | Aragón | W, DS, C, FD, M | *Prunus domestica* L. |
| strawberry | Camarrosa | Huelva | SR, W, C, FD, M | *Fragaria virginiana* Dutch. |
| watermelon | Crimson Sweet | Almería | P, C, FD, DS, M | *Citrullus vulgaricus* Schered. |
| legumes | | | | |
| chickpea | Koifer | León | M | *Cicer arietinum* L. |
| lentil | Koifer | León | M | *Lens sculenta* Moench. |
| white bean | Koifer | León | M | *Phaseolus vulgaris* L. Savi. |
| nuts | | | M | |
| almond, roasted | Marcona | Alicante | M | *Prunus amygdalus* Stokes. |
| hazelnut | Negret | Alicante | M | *Juglans regia* L. |
| peanut | undetermined | Brasil | P, M | *Arachis hypogaea* L. |
| pistachio, roasted | Kerman | Iran | P, M | *Pistacia vera* L. |
| walnut, roasted | Howard | USA | P, M | *Corylus avellana* L. |
| sunflower seed | Arpón | Andalucía | P, M | *Helianthus annuus* L. |
| cereals | | | | |
| rice | S.O.S. | Spain | M | *Oryza sativa* L. |
| white bread | wheat | Spain | C, FD, M | *Triticum* spp. L. |

[a] C, cut; CR, cored; DS, deseeded; ELR, external leaves removed; FD, freeze-dried; M, milled; N, none; P, peeled; SQ, squeezed; SR, stalk removed; W, washed.

by adding 100 $\mu$L of the silylation reagent (BSTFA/TMCS, 99:1) and 100 $\mu$L of anhydrous pyridine. Then the samples were heated at 60 °C for 30 min or placed overnight at room temperature for silylation. The excess silylating reagent was removed under nitrogen at 50 °C, and the residue was dissolved in hexane (200 $\mu$L) and transferred to a gas chromatographic vial.

**Gas Chromatography (GC) Analysis of PS.** The procedure followed for the GC analysis of PS was a modification of the method reported earlier by Toivo et al. (*19*, *20*). The TMS ether solutions (0.5 $\mu$L) were injected into a biphenyl polysiloxane column (Restek, 60 m × 0,32 m i.d., film thickness = 0.10 $\mu$m) fitted in a Hewlett-Packard

chromatograph with a flame ionization detector (300 °C). The column temperature program was 245 °C (1 min hold), 3 °C/min ramp to 275 °C, and 28.5 min hold; the nitrogen carrier flow was 3 mL/min.

Peaks (**Figure 1**) were identified by comparison of their retention times with those of TMS ethers of available standards. PS contents were calculated as milligrams per 100 g on a fresh weight basis of the edible portion. Campesterol, $\beta$-sitosterol, stigmastanol, and stigmasterol standards were used to identify and quantify PS. Cholesterol was used as an internal standard. This fraction is called the identified PS fraction throughout the paper. In addition, certain peaks that eluted after the cholesterol peak were tentatively identified as PS by applying the criteria
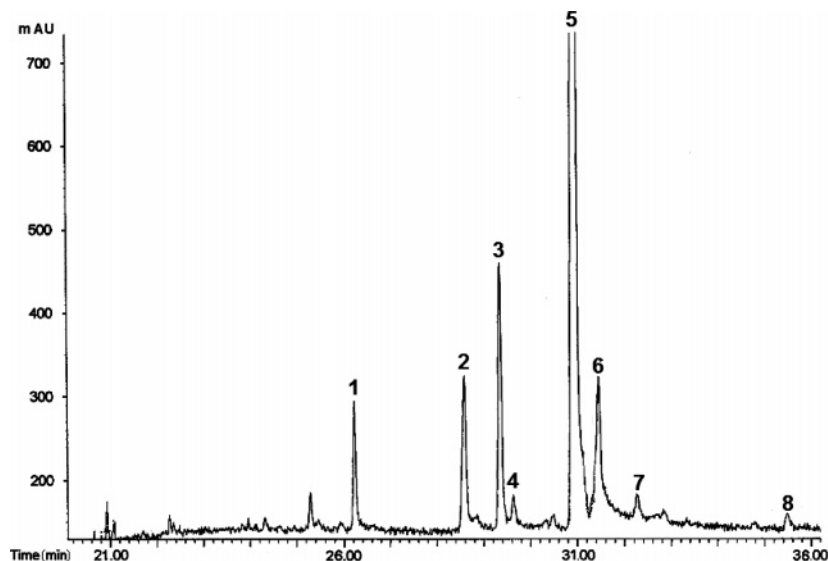
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**Figure 1.** Gas chromatogram of the plant sterols in escarole. Peaks: 1, internal standard; 2, campesterol; 3, stigmasterol; 4, unknown plant sterol; 5, $\beta$-sitosterol; 6, stigmastanol; 7, unknown plant sterol; 8, unknown plant sterol.

used by De Vries et al. (*11*); this fraction constitutes the unknown PS fraction. All PS extractions and analyses were carried out at least in triplicate. If triplicate samples differed by >10%, the samples were prepared and analyzed again.

The GC separation and quantification was checked by a mixture of PS standards: campesterol, $\beta$-sitosterol, and stigmasterol. Routine analysis was checked by the area of the internal standard. The detection limit of the GC analysis calculated per 0.5 $\mu$L was 1.03 ng. The lowest PS concentration given was 0.05 mg/100 g of dry weight.

**Estimation of PS Intake in Spain.** The estimation of PS intake in the Spanish diet was obtained by applying plant plant food composition analyses of the identified fraction of PS to the amount of plant food recorded on 2000 in the SNFC study implemented by the Department of Agriculture (*33*). In addition, the tentatively identified PS fraction was applied to obtain a potential estimate of PS intake. This fraction will need further confirmation.

SNFC is a continuous survey of direct food purchases in Spain that covers all economical and geographical sections of the Spanish population. The purpose of SNFC is to ascertain trends in food consumption and their economic implications. Data are collected from an effective sample and a total universe (value in parentheses) of 6 000 (15 066 810) households, 840 (263 375) hospitality and restaurant businesses, and 230 (25 179) institutional centers. Twenty-one groups of similar foods, which include 130 food items, are specified. The main diary keeper records the quantities of all food items brought into the home, restaurant, or institution. The data are registered day by day during a year and collected each month. SFNC gives purchase (edible portion) of each food item.

### RESULTS AND DISCUSSION

**PS Composition. Tables 2** and **3** show the content and composition of PS in the foods from plant origin commonly consumed in the Spanish diet. According to the SNFC study (*33*), these foods reflect 96% of the total plant-based food intake in the Spanish diet. In each group, food items were analyzed to reflect intakes of >96% of the total plant-based food intake except in the case of cereals, which reflect nearly 80%. A number of foods of low consumption in each food group were reported as "others". PS content of certain items of low consumption was estimated as the mean value of the corresponding group to estimate the 100% of PS intake from plant origin (**Table 4**.)

The analysis showed a considerable variation of PS content in plant-based foods. In the case of vegetables, the present results

(identified fraction) are similar to those found in the study by Normén et al. (*24*), reporting the PS composition (fresh weight) in fruits and vegetables commonly consumed in Sweden. Certain data from this study are used to estimate the PS intake in the Dutch diet (*26*). This work is performed using a methodology similar to that of the present study. A GC method after acid hydrolysis, alkaline hydrolysis, and silylation with TMS ether is applied. A comparison of 10 specific vegetable items analyzed in both studies showed the following values for campesterol, $\beta$-sitosterol, stigmasterol, and stigmastanol, respectively: carrot, 2.7, 11, 2.8, and 0.05 mg/100 g; cauliflower, 9.5, 26, 3.7, and 0.06 mg/100 g; green pepper, 2.0, 2.7, 0.4 mg/100 g and not detected; potato, 0.2, 2.7, 0.4, and 0.56 mg/100 g; and tomato 0.3, 2.4, 1.7, and 0.23 mg/100 g. In the previous items similar values were obtained in the present study. Broccoli (39 mg/100 g), celery (17 mg/100 g), olive (35 mg/100 g), and onion (8.4 mg/100 g) show higher values, whereas white cabbage (13 mg/100 g) shows a lower value than those in the present study. We have made a similar comparison with eight vegetables analyzed in the Finnish diet (*22*). The following items show values for campesterol, $\beta$-sitosterol, and stigmasterol, respectively, similar to those found in the present study: carrot, 2.0, 10.4, and 2.7 mg/100 g; cauliflower, 7.2, 21.6, and 1.6 mg/100 g; onion, 0.6, 7.0, and 1.2 mg/100 g; potato, 0.2, 3.2, and 0.3 mg/100 g; and tomato, 0.5, 3.5, and 1.6 mg/100 g. Broccoli (43.2 mg/100 g) and parsley (37.1 mg/100 g) show in the Finnish study higher values, whereas cucumber (0.2 mg/100 g) shows a lower value than that found in the present study. Swedish and Finnish studies are performed using a methodology similar to that of the present study; thus, the differences found in a few food items could be due to different variety. Other causes of variation in PS content could be maturity level, stress during the growing period, prestorage treatment, and storage (*22*).

The total PS contents—campesterol, $\beta$-sitosterol, and stigmasterol—were compared with those (values in parentheses) found in the revised data reported by Weihrauch and Gardner (*14*) for 17 plant-based foods measured in both studies. These revised data are from different sources in which different methodologies have been applied and probably are underestimated, because the recovery of PS is higher if an acid hydrolysis step that includes a glycosidic sterol fraction is used (*21, 23*),

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**Table 2.** Plant Sterols (PS) in Cereals, Oils, and Vegetables (Milligrams per 100 g of Fresh Weight, Edible Portion)[a]

|  | campesterol | stigmasterol | $\beta$-sitosterol | stigmastanol | unknown PS[b] | total PS |
|---|---|---|---|---|---|---|
| cereals |  |  |  |  |  |  |
| rice | 4.6 ± 0.2 | 3.0 ± 0.1 | 19.8 ± 0.4 | nd[c] | 1.3 ± 0.1 | 29.0 ± 0.8 |
| white wheat bread | 7.7 ± 0.2 | 1.1 ± 0.1 | 26.1 ± 1.0 | 3.9 ± 0.1 | 3.3 ± 0.9 | 41.9 ± 2.1 |
| oils |  |  |  |  |  |  |
| refined olive | 6.2 ± 0.4 | 5.2 ± 0.5 | 121.0 ± 9.6 | 4.2 ± 0.1 | 99.5 ± 5.9 | 235.9 ± 11 |
| sunflower | 36.4 ± 1.0 | 8.2 ± 1.4 | 219.3 ± 5.4 | 10.6 ± 0.3 | 217.9 ± 6.4 | 492.5 ± 12 |
| virgin olive | 6.3 ± 0.5 | 2.4 ± 0.0 | 158.5 ± 6.7 | 3.1 ± 0.3 | 89.6 ± 0.6 | 259.7 ± 8.3 |
| vegetables |  |  |  |  |  |  |
| artichoke | 1.9 ± 0 | 7.4 ± 0.3 | 16.9 ± 0.7 | 3.8 ± 0.1 | 18.5 ± 1.5 | 48.5 ± 1.7 |
| asparagus, green | 2.2 ± 0.2 | 1.0 ± 0.1 | 5.4 ± 0.4 | nd | 1.9 ± 0.2 | 10.6 ± 0.7 |
| bean, green | 1.2 ± 0 | 4.7 ± 0.2 | 9.3 ± 0.4 | 1.5 ± 0.1 | 2.2 ± 0.2 | 18.8 ± 0.8 |
| broccoli | 3.7 ± 0 | 0.7 ± 0 | 13.9 ± 0.3 | nd | nd | 18.3 ± 1.3 |
| cabbage | 4.7 ± 0.4 | 1.6 ± 0.2 | 17.6 ± 1.4 | 0.5 ± 0.1 | 2.9 ± 0.1 | 27.4 ± 1.9 |
| carrot | 2.7 ± 0.3 | 3.1 ± 0.9 | 12.6 ± 0.9 | 0.2 ± 0 | nd | 18.6 ± 1.5 |
| cauliflower | 11.1 ± 0.3 | 4.3 ± 0.1 | 27.8 ± 0.4 | nd | 1.1 ± 0.1 | 44.3 ± 1.2 |
| celery | 0.7 ± 0 | 4.1 ± 0.3 | 2.9 ± 0.2 | nd | nd | 7.8 ± 0.6 |
| chard | 0.9 ± 0.1 | 5.3 ± 0.4 | 6.9 ± 0.6 | 0.2 ± 0 | 3.4 ± 0.3 | 16.6 ± 1.2 |
| cucumber | 0.1 ± 0 | 1.5 ± 0.1 | 3.0 ± 0.1 | nd | 2.3 ± 0.1 | 7.0 ± 0.2 |
| eggplant | 0.4 ± 0 | 1.6 ± 0.1 | 3.2 ± 0.2 | nd | 0.6 ± 0 | 5.9 ± 0.6 |
| endive | 1.6 ± 0.1 | 6.1 ± 0.1 | 7.7 ± 0.2 | 1.5 ± 0 | 7.7 ± 0.2 | 16.9 ± 0.6 |
| escarole | 1.0 ± 0 | 2.8 ± 0 | 13.1 ± 0.3 | 1.6 ± 0.2 | 1.2 ± 0.1 | 18.1 ± 0.6 |
| garlic | 2.7 ± 0.1 | nd | 2.6 ± 0.2 | 12.9 ± 0.3 | nd | 18.2 ± 0.6 |
| leek | 0.9 ± 0 | 5.9 ± 0.2 | 4.5 ± 0.2 | nd | 0.4 ± 0 | 11.7 ± 0.4 |
| lettuce | 0.9 ± 0 | 3.4 ± 0.1 | 7.3 ± 0.3 | 0.6 ± 0 | 1.2 ± 0 | 13.5 ± 0.4 |
| marrow | 0.1 ± 0 | 0.6 ± 0 | 1.2 ± 0.1 | nd | 0.5 ± 0 | 2.4 ± 0.2 |
| onion | 0.3 ± 0 | 1.3 ± 0 | 2.6 ± 0.3 | nd | 2.8 ± 0.3 | 7.2 ± 0.4 |
| parsley | 0.2 ± 0.1 | 2.3 ± 0.1 | 3.1 ± 0.2 | nd | 1.8 ± 0.1 | 7.4 ± 0.4 |
| potato | 0.1 ± 0 | 0.6 ± 0 | 3.4 ± 0.1 | nd | nd | 4.3 ± 0.2 |
| pepper, green | 1.9 ± 0 | 0.4 ± 0 | 5.6 ± 0.1 | 0.3 ± 0 | 1.3 ± 0.1 | 9.4 ± 0.2 |
| pepper, red | 1.3 ± 0 | 0.2 ± 0 | 3.9 ± 0.1 | 0.2 ± 0 | 1.7 ± 0.1 | 7.4 ± 0.2 |
| spinach | 0.5 ± 0 | 8.1 ± 0.8 | 4.2 ± 0.4 | 0.7 ± 0 | 2.9 ± 0.4 | 16.3 ± 1.2 |
| tomato | 0.8 ± 0 | 2.1 ± 0.2 | 3.5 ± 0.8 | 0.7 ± 0 | 2.8 ± 0.1 | 9.9 ± 0.7 |

[a] $n = 9$ analytical replicates. [b] Refers to certain unknown peaks that eluted after the cholesterol peak on gas chromatography tentatively identified as PS. [c] nd, not detected.

**Table 3.** Plant Sterols (PS) in Fruits, Legumes, and Nuts (Milligrams per 100 g of Fresh Weight, Edible Portion)[a]

|  | campesterol | stigmasterol | $\beta$-sitosterol | stigmastanol | unknown PS[b] | total PS |
|---|---|---|---|---|---|---|
| fruits |  |  |  |  |  |  |
| apple | 0.8 ± 0 | 0.3 ± 0 | 13.8 ± 0.4 | nd[c] | 1.1 ± 0 | 16.0 ± 0.9 |
| apricot | 0.8 ± 0 | 1.9 ± 0.1 | 5.9 ± 0.2 | 0.4 ± 0 | 6.1 ± 0.4 | 15.2 ± 0.7 |
| banana | 1.8 ± 0.1 | 2.3 ± 0.2 | 13.3 ± 0.1 | 0.1 ± 0 | 2.7 ± 0.2 | 20.1 ± 1.2 |
| cherry | 1.1 ± 0.3 | 0.5 ± 0.1 | 16.2 ± 1.2 | 0.1 ± 0 | 2.2 ± 0 | 20.1 ± 1.4 |
| grape, white | 2.1 ± 0.2 | 0.3 ± 0 | 22.8 ± 0.5 | nd | 7.4 ± 0.2 | 32.6 ± 1.0 |
| kiwi fruit | 0.2 ± 0 | 0.7 ± 0 | 4.9 ± 0.2 | nd | 1.2 ± 0.2 | 7.1 ± 0.4 |
| lemon | 0.6 ± 0 | 0.2 ± 0 | 2.5 ± 0.1 | nd | 0.2 ± 0 | 3.3 ± 0.7 |
| mandarin | 3.4 ± 0.2 | 1.3 ± 0 | 15.3 ± 0.2 | nd | 2.6 ± 0.1 | 22.1 ± 0.6 |
| melon | 0.3 ± 0 | 0.8 ± 0.1 | 2.3 ± 0.2 | 0.2 ± 0 | 1.1 ± 0 | 4.6 ± 0.3 |
| olive | 1.3 ± 0.1 | 0.4 ± 0 | 27.2 ± 0.8 | 2.5 ± 0 | 6.5 ± 0.9 | 37.7 ± 1.7 |
| orange | 3.8 ± 0.1 | 1.2 ± 0.1 | 22.0 ± 1.4 | 0.4 ± 0.1 | 3.2 ± 0.3 | 30.4 ± 2.1 |
| peach | 0.4 ± 0 | 2.5 ± 0.1 | 8.8 ± 0.4 | nd | 2.9 ± 0.2 | 14.6 ± 0.8 |
| pear | 0.4 ± 0 | nd | 10.6 ± 0.6 | nd | nd | 11.0 ± 0.6 |
| plum | 1.6 ± 0 | 0.7 ± 0 | 15.0 ± 0.4 | nd | 1.7 ± 0.2 | 18.9 ± 0.6 |
| strawberry | 0.7 ± 0 | 0.2 ± 0.1 | 8.2 ± 0.7 | 0.1 ± 0 | 2.1 ± 0.1 | 11.3 ± 0.9 |
| watermelon | 0.6 ± 0.1 | 0.4 ± 0 | 2.4 ± 0.6 | 0.2 ± 0.1 | 1.0 ± 0.4 | 4.5 ± 0.9 |
| legumes |  |  |  |  |  |  |
| chickpea | 12.4 ± 0.6 | 8.3 ± 0.1 | 84.1 ± 1.3 | 10.5 ± 0.1 | 5.4 ± 0.5 | 121.2 ± 4.7 |
| lentil | 10.0 ± 0.4 | 10.8 ± 0.4 | 80.8 ± 2.2 | 8.4 ± 0.4 | 7.2 ± 0.6 | 117.3 ± 5.6 |
| white bean | 4.0 ± 0.1 | 35.2 ± 0.9 | 56.3 ± 2.3 | 1.0 ± 0.3 | 11.5 ± 0.5 | 108.1 ± 5.8 |
| nuts |  |  |  |  |  |  |
| almond | 4.4 ± 0.2 | nd | 125.5 ± 3.1 | 1.4 ± 0 | 16.3 ± 0.5 | 148.6 ± 4.5 |
| hazelnut | 6.9 ± 0.1 | 1.8 ± 0 | 110.9 ± 3.4 | 4.0 ± 0.1 | 5.4 ± 0.1 | 128.1 ± 3.9 |
| peanut | 19.0 ± 0.6 | 14.5 ± 0.2 | 99.4 ± 3.1 | 6.9 ± 0.1 | 2.8 ± 0 | 143.6 ± 4.1 |
| pistachio | 12.2 ± 0.6 | 2.3 ± 0 | 201.9 ± 5.0 | 5.9 ± 0.2 | 19.4 ± 0.6 | 242.7 ± 5.8 |
| sunflower seed | 22.7 ± 0.9 | 15.1 ± 0.3 | 146.4 ± 4.2 | 2.3 ± 0 | 39.4 ± 1.5 | 226.9 ± 6.9 |
| walnut | 5.3 ± 0.1 | nd | 100.2 ± 2.1 | 0.7 ± 0 | 25.1 ± 0.6 | 131.3 ± 3.1 |

[a] $n = 9$ analytical replicates. [b] Refers to certain unknown peaks that eluted after the cholesterol peak on gas chromatography tentatively identified as PS. [c] nd, not detected.

and this sample preparation step appears not to have been use in the previous miscellaneous methodologies. In this sense, acid hydrolysis leads to 20% higher values in vegetable and fruit samples (*22*). Artichoke (23 mg/100 g), cabbage (25 mg/100

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**Table 4.** Intakes[a] of Plant Foods (PF) and Plant Sterols (PS) in the Spanish Diet

| PF group | PF | PF intake[b] | | PS intake, mg | |
|---|---|---|---|---|---|
| | | g | % of total intake in each PF group | identified[c] | identified[c] plus unknown[d] |
| cereals | rice | 16.7 | 7.5 | 4.7 | 4.9 |
| | white wheat bread | 160.0 | 72.1 | 61.7 | 67.0 |
| | others[e] | 45.2 | 20.4 | 15.0 | 16.0 |
| | subtotal cereals | 221.9 | 100 | 81.4 | 87.9 |
| fruits | apple | 29.7 | 12.3 | 4.4 | 4.7 |
| | apricot | 3.1 | 1.1 | 0.3 | 0.5 |
| | banana | 17.6 | 8.9 | 3.0 | 3.5 |
| | cherry | 2.9 | 0.9 | 0.5 | 0.6 |
| | grape | 6.2 | 2.1 | 1.6 | 2.1 |
| | kiwi fruit | 6.2 | 1.5 | 0.3 | 0.4 |
| | lemon | 3.4 | 4.1 | 0.1 | 0.1 |
| | mandarin | 11.7 | 5.5 | 2.3 | 2.6 |
| | melon | 13.2 | 9.2 | 0.5 | 0.6 |
| | olive | 5.7 | 2.3 | 1.8 | 2.2 |
| | orange | 46.6 | 24.5 | 12.7 | 14.2 |
| | peach | 11.8 | 5.0 | 1.4 | 1.7 |
| | pear | 18.4 | 6.6 | 2.0 | 2.0 |
| | plum | 3.3 | 1.2 | 0.5 | 0.6 |
| | strawberry | 6.0 | 2.1 | 0.6 | 0.7 |
| | watermelon | 8.3 | 9.7 | 0.3 | 0.4 |
| | others[f] | 8.7 | 7.3 | 1.3 | 1.5 |
| | subtotal fruits | 202.6 | 100 | 33.6 | 38.4 |
| legumes | bean | 6.6 | 27.5 | 7 | 7.3 |
| | chickpea | 7.4 | 34.9 | 8.9 | 9.5 |
| | lentil | 7.1 | 32.7 | 8.0 | 8.9 |
| | others[g] | 1.1 | 4.8 | 1.3 | 1.4 |
| | subtotal legumes | 22.2 | 100 | 25.1 | 27.1 |
| nuts | almond | 0.8 | 13.6 | 1.1 | 1.2 |
| | hazelnut | 1.9 | 32.2 | 2.4 | 2.5 |
| | peanut | 1.1 | 18.6 | 1.6 | 1.6 |
| | walnut | 1.1 | 18.6 | 1.3 | 1.4 |
| | others[h] | 1.0 | 16.9 | 1.5 | 1.6 |
| | subtotal nuts | 5.9 | 100 | 6.7 | 8.3 |
| oils and fats | refined olive oil | 23.4 | 42.4 | 35.2 | 60.9 |
| | sunflower oil | 19.2 | 41.8 | 52.7 | 94.6 |
| | virgin olive oil | 5.2 | 8.1 | 8.1 | 12.3 |
| | others[i] | 6.3 | 7.7 | 12.4 | 20.7 |
| | subtotal oils | 54.1 | 100 | 108.4 | 188.5 |
| vegetables | asparagus, green | 1.1 | 0.3 | 0.1 | 0.1 |
| | bean, green | 7.4 | 2.7 | 1.2 | 1.4 |
| | cabbages[j] | 3.8 | 1.4 | 1.0 | 1.0 |
| | chard | 2.4 | 1.2 | 0.3 | 0.4 |
| | cucumber | 5.2 | 1.7 | 0.4 | 0.4 |
| | escarole | 0.7 | 2.3 | 0.1 | 0.1 |
| | garlic | 3.5 | 1.3 | 0.6 | 0.6 |
| | lettuce | 12.6 | 2.3 | 1.6 | 1.7 |
| | onion | 20.0 | 6.7 | 0.9 | 1.4 |
| | pepper, green | 5.7 | 1.9 | 0.4 | 0.5 |
| | pepper, red | 5.7 | 1.9 | 0.3 | 0.4 |
| | potato | 119.5 | 45.2 | 5.0 | 5.0 |
| | spinach | 2.8 | 1.2 | 0.4 | 0.5 |
| | tomato | 60.1 | 12.9 | 4.2 | 5.9 |
| | others[k] | 28.7 | 16.8 | 3.9 | 4.6 |
| | subtotal vegetables | 279.3 | 100 | 20.2 | 24.0 |
| total foods | | 786.0 | 100 | 275.5 | 374.2 |

[a] Per person daily (edible portion). [b] SNFC study, confidence level, 95.45%; error range, 3% in amount of food (*33*). [c] Estimated on the basis of campesterol, β-sitosterol, stigmasterol, and stigmastanol concentrations. [d] Estimated on the basis of the concentrations of certain unknown compounds tentatively identified as PS. [e] Cookies, bunes, paste, other cereals. [f] Avocado, cherimoya, fig, grapefruit, mango, pineapple, other fruits. [g] Peas, other legumes. [h] Cashew, pistachio, sunflower seed, corn seed, other seeds. [i] Vegetable-fat spreads, other seed oils. [j] Refers to cabbage, cauliflower, and broccoli. [k] Artichoke, carrot, celery, eggplant, leek, mushroom, marrow, other vegetables.

g), carrot (11 mg/100 g), cauliflower (17 mg/100 mg), celery (5 mg/100 g), garlic (1 mg/100 g), and spinach (not detected) showed lower values than those found in the present study. Six food items, bean (14 mg/100 g), eggplant (5 mg/100 g), lettuce (10 mg/100 g), parsley (4 mg/100 g), potato (4 mg/100 g), and tomato (7 mg/100 g), showed similar values in both reports.

Asparagus (22 mg/100 g), cucumber (14 mg/100 g), onion (13 mg/100 g), and red pepper (12 mg/100 g) showed higher values than those in the Spanish study.

In the case of fruits, the identified results (campesterol, β-sitosterol, and stigmasterol) were similar to those found in the study by Normén et al. (*24*), reporting the PS available in

fruits commonly consumed in Sweden. A comparison of six specific foods analyzed in both studies show values in the same range as in the present study for campesterol, $\beta$-sitosterol, and stigmasterol, respectively. Apple gives 0.4, 13, and 0.1 mg/100 g; banana, 1.5, 11, and 1.8 mg/100 g; clementine, 4.0, 12, and 0.78 mg/100 g; orange, 3.0, 20, and 1.0 mg/100 g; peach, 0.6, 13, and 1.8 mg/100 g; pear, 0.3 and 12 mg/100 g and not detected. Two items—kiwi fruit (9 mg/100 g) and lemon (18 mg/100 g)—showed higher values, whereas one item—watermelon (1.3 mg/100 g)—showed lower value than those in the present study. In the case of lemon the difference could be due to the sample preparation.

We have made a similar comparison with six fruits analyzed in the Finnish study (*22*). The following fruit items show slightly lower values for campesterol, $\beta$-sitosterol, and stigmasterol, respectively, than those found in the present study: banana, 1.3, 8.4, and 1.3 mg/100 g; grape, 1.4, 14.3, and 0.2 mg/100 g; orange, 3.4, 17.0, and 0.9 mg/100 g, and plum, 1.1, 10.6, and 0.7 mg/100 g. Similar values were found in both studies for apple (0.9, 15.7, and 0.2 mg/100 g), whereas kiwi fruit (0.5, 13.7, and 2.3 mg/100 g) showed higher value than those in the present study.

The identified sterol contents—campesterol, $\beta$-sitosterol, and stigmasterol—were compared with those found in the revised data reported by Weihrauch and Gardner (*14*) (values in parentheses) for 12 fruits measured in both studies: apple (12 mg/100 g), apricot (17 mg/100 g), banana (16 mg/100 g), orange (24 mg/100 g), peach (10 mg/100 g), strawberry (10 mg/100 g), and watermelon (2.5 mg/100 g) have shown values slightly lower than or similar to those found in the present study, whereas cherry (12 mg/100 g), grape (23 mg/100 g), pear (7 mg/100 g), and plum (7 mg/100 g) have shown lower values. As in the Finnish study, lemon (11 mg/100 g) showed higher values than in the present study.

The total PS value (identified fraction) in legumes was within the range reported in the review by Weihrauch and Gardner (*14*), which gives a range from 23 (mung beans) to 161 mg/100 g (soybeans), with a median value of 95 ± 46 mg/100 g. As far as we know, literature reports on the contents of different sterols in legume seeds are scarce and expressed as relative percentages (*34*).

In the case of oils in which glycosidic PS are not present (*19, 25*), the total values obtained for the identified fraction—campesterol, $\beta$-sitosterol, stigmastanol, and stigmasterol—in virgin and refined varieties were in the range of values reported by Piironen et al. (*4*) (144−150 mg/100 g) and Weihrauch and Gardner (*14*) (212 and 161 mg/100 g for virgin and refined oils, respectively). Oil refining decreased the sterol content either 10 to 70% (*4*) or to 16 to 50% (*14*). In sunflower oil the PS value was slightly higher than the values given by Trautwein et al. (*35*) (190−203 mg /100 g) and lower than the values reported by Weihrauch and Gardner (*14*) (610 mg/100 g) and Piironen et al. (*4*) (374−725 mg/100 g).

Among the different plant-based groups analyzed, the oil group showed the major content in identified PS (136−275 mg/100 g). Nuts showed relatively high values (123−223 mg/100 g), which were similar to those found by Phillips et al. (*9*), followed by legumes (97−116 mg/100 g). The contents in vegetables and fruits were under 43 mg/100 g. Regarding vegetables, the higher concentrations were found in artichoke (30.0 mg/100 g), a vegetable consumed mainly in the Mediterranean area (*36*), cauliflower (43.2 mg/100 g), and cabbage (24.5 mg/100 g). The higher values of these two last items are explained on the basis of their relatively high meristematic tissue

content, with relatively high membrane-rich content in comparison with vascular or starch tissues (*22*). Olive and orange, which are within the more representative fruits in the Mediterranean diet (*31*), showed the highest contents within the fruit group (31.2 and 27.2 mg/100 g, respectively). These values could be related, in part, to their relative high oil content among fruits. Consistently, avocado shows a relatively high oil (*37*) and PS (*22*) content within fruits.

In all of the items analyzed $\beta$-sitosterol was the main PS, except in celery, garlic, leek, and spinach, in which it was stigmasterol. Piironen et al. (*22*) found the same exception in the case of spinach and cucumber, whereas Weihrauch and Gardner (*14*) and Normén (*24*) found it in cerely. No literature data for garlic were found. In contrast, Normén (*24*) did not found this feature in leek.

With regard to the hydrolysis step in the preparation of nonoil samples, it is reported (*9, 28*) that minor components $\Delta^5$- and $\Delta^7$-avenasterol are destroyed or suffer isomerization under the conditions of strong acid and high temperatures used for hydrolysis. Certain items in which these compounds should be in relatively medium content—nuts, seeds, and legumes—may be affected. It seems that cucumber, which contains a high proportion of $\Delta^7$ sterols (*22*), may be affected in the present study, because the analyzed value was lower than those found in the literature data (*14, 22*).

**PS Intake.** From the PS content calculated and on the basis of the intake of foods from plant origin from the SNFC study (*33*), the daily intake per person of PS from plant-based foods in the Spanish diet was estimated (**Table 4**). The dietary intake of the identified PS in the Spanish diet was found to be 276 mg. If the estimated data for the unknown PS fraction were included, an additional 99 mg could be considered.

The main contributor groups to the total plant-food based intake of PS (identified fraction) were oils (39.3%), cereals (29.6%), and fruits (12.2%), followed by legumes (9.1%), vegetables (7.3%), and nuts (2.4%). Of special importance is the intake from oils, because the higher bioavailability of these compounds is through the oil matrix (*38*). The main individual item contributors to the PS intake were sunflower oil [52.7 mg per person daily (pd)], refined olive oil (35.2 mg per pd), orange (12.7 mg per pd), chickpeas (8.9 mg per pd), virgin olive oil (8.1 mg per pd), lentils (8.0 mg per pd), beans (7.0 mg per pd), potato (5.0 mg per pd), rice (4.7 mg per pd), apple (4.4 mg per pd), and tomato (4.2 mg per pd).

Among the identified PS compounds $\beta$-sitosterol was the major contributor to the total intake (79.7%), followed by campesterol (9.5%), stigmasterol (6.8%), and stigmastanol (4.1%). If the intake from the unknown PS fraction was taken into account, its contribution to the total estimated intake was considerable (26%).

Intakes of PS have previously been reported in Finland (*22*), The Netherlands (*26*), and the United Kingdom (*29*). Normén et al. (*26*) state that variations in food patterns in different countries influence total PS intakes. Thus, we have made a comparison between the British, Finnish, and Dutch diets and the Spanish diet in order to search for qualitative/quantitative differences in the PS intake in the northern and southern European regions.

Comparison of these intakes with the intake detailed in the present study, on the basis of identified fraction of PS—campesterol, $\beta$-sitosterol, stigmasterol, and stigmastanol—shows that Spanish PS intake is much higher than the PS intake in the British diet and slightly higher (Finnish) or equal (Dutch) in the case of the other two populations.
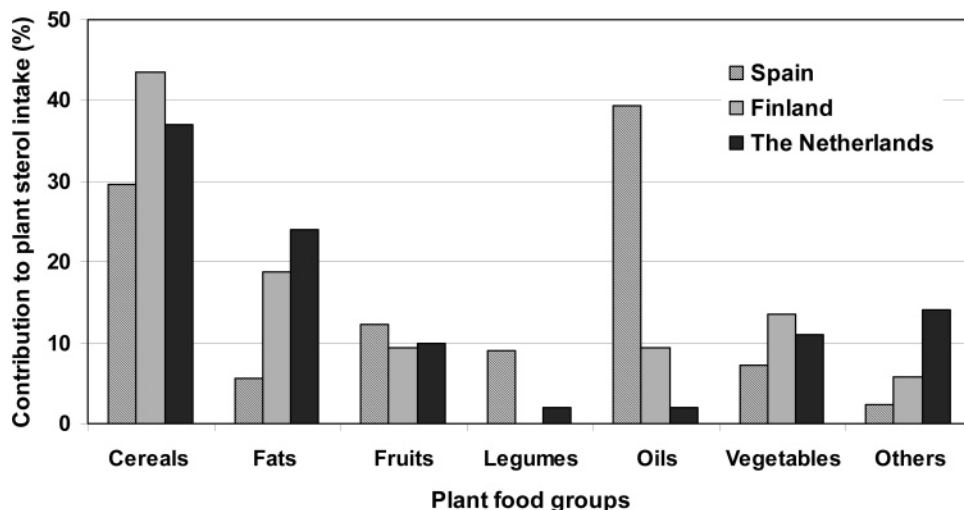
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**Figure 2.** Contribution of plant-based food groups to the estimated daily intake per person of plant sterols in the Spanish diet, in comparison to Finnish (*13*) and Dutch (*26*) diets. Legume data in Finnish diet are not available.

The estimated PS intake in the Spanish population was 39% higher than those found for the British population (163 mg). The comparison was made without stigmastanol data because this stanol is not available in the British study. The main contributor groups to the total dietary intake of PS in the British diet were oils (49%), cereals (32%), vegetables (8%), and nuts (5%). Thus, oils and cereals represent the two most important sources in both diets. Within the oils, in the British diet rapeseed oil represents 41% of the total vegetable oil intake (*39*). Rapeseed oil is the main oil source of brassicasterol (*14*) and a very rich source of campesterol (248 mg/100 g) (*4*). Thus, in contrast to the Spanish diet, rapeseed oil could be considered a good source of campesterol and brassicasterol in the British diet.

The Spanish estimated intake was ∼16% higher than those found for the Finnish population (238 mg) by Piironen et al. (*13*). The contributions of PS in the Finnish diet to the total PS intake are (approximate rates of plant-based food groups to total PS) as follows: cereal products (43.5%), fats (18.7%), vegetables (13.5%), fruits (9.3%), oils (9.3%), and other plant sources (5.7%). Cereals represent a good source of PS in Finnish and Spanish diets (**Figure 2**). Specifically rye accounts for most of the intake of PS (23%) in the Finnish diet, whereas wheat represents a similar percentage in the Spanish diet. It is worth mentioning the relatively high contribution of vegetables and fruits (29%) in the Finnish diet. This feature can be interpreted as a reflection in the Finnish diet of the recent trend of consumers toward diets that are rich in vegetables (*13*). The daily intake per person of PS from vegetables and fruits is estimated as 60 mg in the Finnish diet (*22*), which is quite similar to the corresponding value estimated in the Spanish diet (54 mg). In both diets, the great amount of PS is estimated to be supplied by potato in the vegetable group, although its PS content is low because potato, as a rich starchy food (*40*), gives low PS values (*22*). The great difference between the diets is the ranking of oil as a source of PS, whereas in the Spanish diet oil represented the first PS source, in the case of the Finnish diet it represents only 9.3% of the total PS intake. This feature could be interpreted by the predominant use of margarines and vegetable-fat spreads in the northern European diets (*41*) and of olive and sunflower oils in the Spanish diet (*33*).

The estimated daily intake of PS per person in the present study was equal to that found for the Dutch population (274 mg). The vegetable sources of PS in this diet are bread (37%), fats (24%), fruits (10%), vegetables (11%), cakes and chocolate

(5%), oil (2%), and other food items (9%) (**Figure 2**). As in the case of all of the diets compared in the present study, cereals represent an important food source of PS, being in the Finnish and Dutch diets the highest source. The common Dutch (*26*) and Finnish (*42*) habit of eating brown bread (from wheat and rye, respectively) for breakfast and lunch might cause this feature. It is worth mentioning the low source of PS from plant oils in the Dutch diet as compared with the rest of the diets; this intake is "compensated" with the high source of PS through other plant fats such as margarines. It is also worth mentioning the relatively high contribution of the estimated intake due to vegetables in the Dutch diet in comparison with the contribution in the Spanish diet. The daily intake of vegetables—without potato, which is quite similar in both diets—is 194 g in the Dutch diet (*26*) and 160 g in the Spanish diet. Because this difference is not quite high, it could be speculated that Dutch vegetable intake is especially rich in vegetables with high contents of PS, such as cruciferous plants. Consistently, the daily intake per person of PS through cabbage is 9.4 mg in the Dutch diet (*26*), whereas in the Spanish diet the intake of cabbage, broccoli, and cauliflower was 1.0 mg. The intake of PS through the consumption of legumes is relatively low in comparison with the Spanish diet. This feature does not correspond to a relatively low intake of legumes in the Dutch diet (25 ± 19 mg) (*26*) with respect to the Spanish diet. Thus, it seems that the legumes commonly consumed in The Netherlands present an important relatively lower content than the legumes analyzed in the present study.

SFNC is a continuous survey of direct food purchases; thus, the data of food waste and gifts are not recorded. Another limitation of the present study is that a detailed analysis validation of PS composition by mass spectroscopy in the plant foods was beyond the aim of the present study. Finally, we consider only the plant-based foods as sources of PS, because the analysis of other sources—such as animal foods or beverages—will complicate the study in terms of time, effort, and cost and their contribution to total estimated intake would likely be <3.5% (*22*). Consistently, in the Dutch study PS content from these groups of products is set at zero in the estimation of total PS in the diet (*26*).

The estimated PS intakes in different diets may clarify the role in health of other bioactive components of plant-based foods. Normén et al. (*26*) indicate that because of the high correlation between PS and dietary fiber in the Dutch diet it is difficult to determine which substances are responsible for the

health benefits inflected by epidemiological studies. This aspect may be worthy in those diets in which the source of PS through brown bread is relatively high. In this sense, Phillips et al. (*43*) determine the relationship between PS and fatty acid composition in experimental diets designed to have particularly fatty acid profiles, concluding an inverse relationship between PS and saturated fatty acid concentrations. Also, they evidence that plant foods, which are higher sources of polyunsaturated fatty acids in the diets, are rich in PS.

In summary, the results of the present study provide a quantitative estimation of the total PS content that comprises all forms in which these compounds exist in plant foods in the Spanish diet. The intake of PS in the Spanish diet was in the same range as that of other European countries such as Finland or The Netherlands. However, some qualitative differences in the PS sources were detected. This was the predominant use as PS sources of brown bread and vegetable fat in the northern diets versus the use of white bread and vegetable oil in the Spanish diet.

## ABBREVIATIONS AND NOMENCLATURE USED

BSTA, *N,O*-bis(trimethylsilyl)trifluoroacetamide; campesterol, 24-methylcholest-5-en-3$\beta$-ol; CAS, Chemical Abstracts Service; cholesterol, cholest-5-en-3$\beta$-ol; EU, European Union; GC, gas−liquid chromatography; i.d., internal diameter; LDL, low-density lipoproteins; PD, person day; M, molar concentration; nd, not detected; PS, plant sterols; $\beta$-sitosterol, 24-ethyl-5-cholesten-3$\beta$-ol; SNFC, Spanish National Food Consumption; stigmastanol, 24$\alpha$-ethyl-5$\alpha$-cholestan-3$\beta$-ol; stigmasterol, 24-ethylcholesta-5,22-dien-3$\beta$-ol; TMCS, trimethylchlorosilane; TMS, trimethylsilyl.

## ACKNOWLEDGMENT

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