Applied nutritional investigation

Common sources and composition of phytosterols and their estimated intake by the population in the city of São Paulo, Brazil

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ABSTRACT

Objective: Phytosterols have been used alone, or combined with lipid-altering drugs, to reduce cholesterol levels and the burden of cardiovascular disease. Considerable variation in the composition of phytosterols exists and its consumption, in a regular diet, by the Brazilian population is still unknown. Thus, the aim of the present study was to determine the phytosterols content of the most consumed plant foods and to estimate the phytosterols intake by this population.
Methods: Intake of plant foods of a representative population of the city of São Paulo (n = 1609), randomly selected on the basis of the Brazilian Institute for Geography and Statistics census data (2010), was obtained by a food frequency questionnaire (FFQ). Foods were chosen on the basis of the Consume Expenditure Survey (2002–2003) and from answers to the FFQ. Phytosterols composition of most consumed greens, legumes, cereals, and seeds, fruits, and vegetable oils was determined by gas chromatography (flame ionization detection). Daily phytosterols intake was estimated in terms of mg per 100 g (mg/100 g^{-1}) of edible portion. Underreporters and overreporters were excluded.
Results: Mean (SE) daily phytosterols intake in the diet of the study population was 100.6 (1.2) mg, with β-sitosterol as the largest sterol component (65.4%), followed by campesterol (23.2%), and stigmasterol (10%). No significant changes in daily phytosterols intake were observed after exclusion of underreporters and overreporters. Considerable variation was observed in phytosterols content among the most consumed plant foods.
Conclusions: Analysis of phytosterols composition in most consumed plant foods has shown that phytosterols content varied among food groups. Dietary intake of phytosterols in a large population of the city of São Paulo is in the same range of some countries.

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Introduction

Changes in diet are a most effective strategy to reduce the growing incidence of cardiovascular disease. Reduction in the intake of saturated fat and cholesterol combined with increase in fiber consumption are universally recommended and associated with a moderate reduction in the levels of low-density lipoprotein (LDL)-cholesterol. Furthermore, additional reduction in LDL-cholesterol can be achieved when these recommendations are combined with ingestion of adequate amounts of phytosterols, soy protein, and nuts [1].

Phytosterols are plant sterols with biologic functions similar to those of mammalian cholesterol. However, due to small differences in the chemical structure of phytosterols, they are much less absorbed (2–5%) [2,3] than cholesterol (56%) [4]. Moreover, phytosterols decrease the intestinal absorption of cholesterol and, therefore, they have been proposed as lipid-lowering agents. Recently, a synergistic effect of phytosterols and ezetimibe was demonstrated, providing additional inhibition of cholesterol absorption when compared with each therapy alone [5].

Although a daily consumption of 2 g to 3 g of phytosterol is associated with a 10% to 15% decrease in the levels of LDL-cholesterol [6], such phytosterol intake is not usually observed in a regular diet even among vegetarians.

Legumes, cereals, and seeds, oils, nuts, fruits, and greens are the main sources of phytosterols. Many differences in type and content of plant sterols have been reported for a variety of products in each group of food [7–9].

Whereas few reports were found regarding the mean daily consumption of plant sterols in developed countries [7,9], in developing nations, where food intake habits are markedly different, the available information is still lower.

Considerable variation in the composition of plant food sterols exist and their consumption in a regular diet by the Brazilian population is still unknown. Thus, the aim of the present study was to determine the phytosterol content of the most consumed plant foods and to estimate the phytosterol intake by the population.

Materials and methods

Population study

This cross-sectional study included 1609 individuals of both sexes. Sample-size calculation was based on the Brazilian Institute for Geography and Statistics census data (2010) and included adult individuals living in the city of São Paulo [10]. The study sample was selected using a non-stratified multistage cluster sampling (districts, census tracts, and households). In the first, second, and third stages, 25% of the districts were arbitrarily selected; four census tracts were randomly chosen for each district; and one of two households was selected for interview, respectively. Sixteen interviews per census tracts and 64 per district were performed.

The sample size was based on a population of 7,553,035 adult individuals (population in 2007, SEADE) [11] living in the city of São Paulo. In our calculations, the following equation (OpenEpi 2.0 statistical software) was used: $n = [DEFF*N*p(1-p)]/ [(d^2/Z^2_{1-\alpha/2}*(N-1)+p*(1-p)]$, where N is the population size, p is the expected proportion, $Z^2_{1-\alpha/2}$ is a constant related to the confidence interval (1.96), d^2 is the adopted error (0.05), DEFF is the effect of study design for complex methods, and n is the sample size. The calculated sample size for DEFF = 1 was 384 households; for DEFF = 2, was 768 households. The sample size has been duplicated to increase its representativeness.

The Terraview Social Policies software was used to map the selected households. Maps of the city of São Paulo were built containing information on selected districts with the respective census tracts.

Collection of population data

Interviews were performed by a group of trained dietitians and all procedures were explained to the adult living in the household.

The study protocol was conducted in accordance with the ethical guidelines for human experimentation and was approved by the Ethics Committee of the Federal University of São Paulo (#1328/09). Participants were included after they had signed a written informed consent form.

Self-reported data on socioeconomic characteristics (level of education and incomes), lifestyle (physical activity), body weight and height, and risk factor profile (hypertension, diabetes, hypercholesterolemia, and smoking) were obtained. This procedure was validated for population studies [12–16].

Dietary assessment

A 24-h recall of dietary intake was obtained to estimate the content and type of macronutrients (fat, protein, carbohydrate, cholesterol, fibers) [17]. Although 24-h recalls are easily and rapidly obtained, a single 24-h recall is not a good estimator of the usual diet because the intraindividual diet variability is not taken into account.

To assess the usual consumption of plant-derived foods we used a food frequency questionnaire that has been validated for population studies [18–21]. Common sources of plant sterols were identified and represented as portions for calculation of the daily intake.

To address adequacy of the information obtained in 24-h recalls, the reported energy intake (EI), estimated basal metabolic rate (BMR), and the EI:BMR ratios were obtained. BMR was calculated for gender, weight, height, and age according to the World Health Organization (1995) [22]. The Goldberg cut-off was used to verify underreporters (EI:BMR < 0.76), acceptable reporters (EI:BMR ≥ 0.76 and ≤1.24) and overreporters (EI:BMR > 1.24) [23,24]. Additionally, the Black criterion [25] also was used to better detect underreporters (EI:BMR < 0.87) for a single day's record.

The Avanutri software (5.0, for Windows; Rio de Janeiro, RJ, Brazil) was used to calculate the diet composition from 24-h recalls.

Common sources of plant sterols also were chosen on the basis of the Consume Expenditure Survey (CES, 2002–2003) for the city of São Paulo [12], which is a survey of food purchases covering all the economic and geographic sections of the city. For the final estimation of total phytosterol daily intake, we added the phytosterol content of soybean oil (20 mL), the type and amount of vegetable oil most consumed in Brazil according to the CES. The most consumed food plants and oils were then chosen for chemical analyses.

Analyses of plant sterols composition in plant foods

Plant materials

Food samples were obtained from different sources and were submitted to distinct preparations before analysis.

Legumes, cereals, and seeds

Two samples of cereals (brown and polished rice), legumes and seeds (green pea, brown and black bean, chickpea, lentil, soybean, black soybean, linseed) were purchased from a supermarket, and one sample was purchased from the largest warehouse of the city (Company of General Warehouse in São Paulo, CEAGESP, São Paulo, SP, Brazil). Legumes, cereals, and seeds were examined in raw. Food samples (150 g each) were homogenized to form a composed sample, powdered, and packed (100-g bags).

Fruits and vegetables

Fruits (açaí, avocado, pineapple, banana, coconut, guava, orange, apple, papaya, mango, and strawberry) and vegetables (zucchini, eggplant, broccoli, carrot, cauliflower, endive, spinach, white cabbage, tomato, and green bean) were purchased from CEAGESP (Sorocaba, SP, Brazil). Vegetables (800–4000 g) and fruits (650–1400 g) were washed with demineralized water and freeze-dried. Only the edible portion was used after husks and seeds were removed. Samples were crushed and, in some cases (zucchini, eggplant, broccoli, carrot, cauliflower, spinach, white cabbage, and green bean), submitted to a bleaching process, frozen (−30 to −40°C), and freeze-dried in a vacuum camera (759.6 mm Hg) for 20 to 25 h. The samples were then powdered and packed in 100-g bags.

Oils

Canola, coconut, sunflower, corn, soybean, olive, and composed oils were purchased from a supermarket and were examined in natura.

Quantification of phytosterols in plant foods

For lipid extraction, dry samples (5 g) or oils (5 mL) were used. Lipids were cold-extracted, as described by Bligh-Dyer [26]. Content analysis of phytosterols was initiated by a saponification step. In a capped test tube, ethanol (95%, 30 mL), KOH (50% in water, 5 mL), and dihydrocholesterol (0.2% in isopropanol, 0.5 mL; as internal standard) were added to the oil extracted from the sample (1.00 ± 0.03 g), and this solution remained in a water bath (100°C, 1 h). After cooling, the tube content was transferred to a separatory funnel, washed with petroleum ether (50 mL), and then shaken vigorously to separate the unsaponified fraction. After separation between the organic and aqueous phases, the petroleum ether fraction was removed and kept in another separatory funnel. This procedure was repeated with petroleum ether (50 mL, three times), by washing and vigorously shaking at each step. The four combined petroleum ether fractions were washed with distilled water until the KOH excess was removed, dried by filtration through anhydrous sodium sulfate and collected into a 250-mL flat-bottom flask. This flask was taken to a rotary evaporator and its content was concentrated to a volume of about 5 mL. This preconcentrated extract was transferred to a previously dried and weighted beaker and was heated in an oven (70°C) for solvent evaporation until dryness.

The unsaponified matter was dissolved in hexane (1 mL) and then applied onto activated (105°C, 30 min) thin-layer chromatography plates (20 x 20 cm, 0.5-mm thickness silica gel as stationary phase; Analtech, Newark, DE, USA)