SUPPLEMENTARY MATERIAL

Phytosterols in supplements containing *Serenoa repens*: an example of variability of active principles in commercial plant based products.

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Abstract

Phytosterols are one of the bioactive components responsible for the beneficial effects of *Serenoa repens* in Benign Prostate Hyperplasia. The aim of this study was to verify the actual variability of the phytosterols content in supplements containing serenoa, in order to provide useful elements to check the effectiveness of these preparations. The amount of campesterol, stigmasterol and β-sitosterol were determined by gas-chromatography in commercial raw materials and supplements containing serenoa in association or not with other botanicals. The experimental data were used to calculate amounts of phytosterols for recommended daily dose. The overall results of this study show an extreme variability in the content and also in the amounts per daily dose of phytosterols of the examined supplements (both mono/multi-components). These data confirm that the characterization of serenoa based supplements is insufficient to ensure comparable effects between different products and therefore can explain the conflicting results of clinical trials.

Key words: *Serenoa repens,* food supplements, phytosterols, characterization.

1. Experimental

1.1 Samples

Different products containing *Serenoa repens* were collected to take into account the different forms of marketing of these products: three raw materials (a powdered dried fruit and two liquid extracts); three mono-component food supplements, containing only dry extract or powder of the *Serenoa repens* fruit; and four multi-components, containing other herbal powders or extracts in addition to *Serenoa repens*. An aliquot of the powdered fruit and one of an extract were provided by PlantLIBRA project (see Funding section), the purified alcoholic extract by Indena (Batch no. 30293/B1/4), and all the supplements were obtained from the local market. The supplement producers are Aboca, Arkopharma, Body Spring, Erba Vita, Long Life, and Solgar (2 products).

1.2 Materials

Standard Reference Material (SRM) 3251, a *Serenoa repens* extract, was purchased from the National Institute of Standards and Technology (NIST; Gaithersurg, MD, USA) to verify the reliability of the phytosterol analysis.

β-sitosterol, stigmasterol and 5α-cholestane were obtained from Sigma-Aldrich, campesterol from Larodan Fine Chemicals and the silylation reagents (hexamethyl-disilazane and trymetilchlorosilane) from Thermo Scientific.

1.3 Experimental procedure

The determination of phytosterols (campesterol, stigmasterol and β-sitosterol) was performed for all samples (raw materials and supplements) according to the AOAC Official Method 2007.03 First Action (Sorenson and Sullivan 2006, 2007)**.** Briefly the samples were saponified at high temperature with ethanolic KOH solution, the unsaponifiable fraction was extracted with toluene, the phytosterols derivatized to trimethylsilyl (TMS) ethers and then quantified by GC-FID, using 5α-cholestane as internal standard. The gascromatographic analysis was performed on a Perkin Elmer Clarus 500 GC, equipped with a Supelco Equity-5 capillary column (30 m x 0.25 mm ID, 0.25 μm d_f), with the following operating conditions: carrier gas helium 0.8 ml/min; injector 250°C; FID 300°C; oven 220°C (isotherm 2 min), ramp 40°C/min to 275°C (isotherm 30 min). The data were processed with the Perkin Elmer TotalChrom Workstation.

Some of the validation data presented in the single-laboratory validation (Sorenson and Sullivan 2006) of the AOAC method were verified. In particular the repeatability was evaluated on *Serenoa repens* extract and supplements, and the trueness from the analysis of the NIST Standard Reference Material (SRM 3251). The repeatability for all phytosterols obtained was <6% and trueness between 99 and 120%.

References

Sorenson WR, Sullivan D. 2006. Determination of campesterol, stigmasterol, and beta-sitosterol in saw palmetto raw materials and dietary supplements by gas chromatography: single-laboratory validation. J AOAC Int. 89:22-34.

Sorenson WR, Sullivan D. 2007. Determination of campesterol, stigmasterol, and beta-sitosterol in saw palmetto raw materials and dietary supplements by gas chromatography: collaborative study. J AOAC Int. 90:670-678.

Table S1 - Labelled herbal ingredients of examined food supplements.

Supplements	Ingredients
Mono components	
Sample 1	*Serenoa repens* fruit dry extract (45% fatty acids) (Batch no. P25188G)
Sample 2	*Serenoa repens* Bart. fruit dry extract (Batch no. BCP02682A)
Sample 3	*Serenoa repens* Bart. fruit dry extract; *Serenoa repens* fruit powder (Batch no. 498606-01)
Multi components	
Sample 1	*Serenoa repens* fruit fat-soluble extract (90% free fatty acids and 0,2% beta-sitosterol); *Cucurbita maxima* Duchesne seeds oil (Batch no. LR002A?)
Sample 2	*Serenoa repens* fruit powder (lipido-sterolic fraction 14%); *Urtica dioica* root dry extract (0,01% scopoletin); *Arctostaphylos uva-ursi* leaves dry extract; *Malaleuca viridifolia* essential oil; *Juniperus communis* essential oil (Batch no. 12F1048)
Sample 3	*Serenoa repens* Bart. fruit dry extract (40% fatty acids); Graminex pollen extract; *Arctostaphylos uva-ursi* (L.) Spreng leaves dry extract (20% arbutin); *Cucurbita pepo var. oleifera* Pietsch seeds dry extract (40% fatty acids and 0,075% phytosterols) (Batch no. 32613)
Sample 4	*Urtica dioica L.* leaves standardized extract (1% silicic acid); *Serenoa repens* Bart. fruit standardized extract (45% free fatty acids); *Cucurbita* seeds lyophilized; *Soja ispida* Moench. seeds powder (2% isoflavones) (Batch no. 428843-01)

Table S2 - Amount (mg) of *Serenoa repens* per daily doses indicated on the label of the examined food supplements. .

Supplements	**Recommended daily dose**	**Amount of *Serenoa repens***
Mono components		
Sample 1	4 capsules	1200 mg dry extract (180 mg fatty acids)
Sample 2	2-4 capsules	320-640 mg dry extract
Sample 3	1-3 capsules	300-900 mg dry extract; 220-660 mg fruit powder
Multi components		
Sample 1	2 capsules	320 mg extract (288 mg free fatty acids, 0.64 mg β-sitosterol)
Sample 2	4 capsules	1500 mg fruit powder (210 mg lipido-sterolic fraction)
Sample 3	2 capsules	330 mg fruit dry extract (132 mg fatty acids)
Sample 4	2 capsules	150 mg fruit standardized extract (68 mg free fatty acids)

Table S3 - Content of phytosterols (mg/100g) and relative percentages in the examined products containing *Serenoa repens*

Samples	**Campesterol**	**Stigmasterol**	**β-Sitosterol**	**Total Sterols**
Powdered dried fruit	**8.8** (22%)	**3.6** (9%)	**27.7** (69%)	**40.1**
NIST SRM 3251 *Serenoa repens* extract	**63.7 (24%)**	**24.5 (9%)**	**182.0 (67%)**	**270.2**
Extract	**72.0** (23%)	**28.5** (9%)	**210.0** (68%)	**310.5**
Purified alcoholic extract	**63.7** (22%)	**25.8** (9%)	**196.0** (69%)	**285.5**
Mono component 1	**12.6** (23%)	**4.9** (9%)	**38.0** (68%)	**55.5**
Mono component 2	**19.1** (23%)	**7.8** (9%)	**57.1** (68%)	**84.0**
Mono component 3	**14.6** (20%)	**5.6** (8%)	**52.0** (72%)	**72.2**
Multi component 1	**62.0 (**20%)	**44.0** (14%)	**202.0** (66%)	**308.0**
Multi component 2	**19.7** (15%)	**5.0** (4%)	**106.4** (81%)	**131.1**
Multi component 3	**9.8** (16%)	**10.4** (17%)	**42.0** (68%)	**62.2**
Multi component 4	**8.9** (15%)	**4.6** (8%)	**46.1** (77%)	**59.6**

Values represent the mean of two determinations.

Table S4

Amounts (mg) of phytosterols of examined food supplements per daily doses. *

Supplements	Recommended daily dose	Campesterol		Stigmasterol		β-Sitosterol		Total phytosterols	
		Min	Max	Min	Max	Min	Max	Min	Max
Mono components									
Sample 1	4 capsules	-	208	-	81	-	626	-	915
Sample 2	2-4 capsules	121	242	49	98	362	724	532	1064
Sample 3	1-3 capsules	83	249	32	96	298	882	413	1227
Multi components									
Sample 1	2 capsules	-	574	-	407	-	1871	-	2825
Sample 2	4 capsules	-	411	-	105	-	2230	-	2746
Sample 3	2 capsules	-	81	-	87	-	353	-	521
Sample 4	2 capsules	-	114	-	59	-	591	-	764

*The reported values were calculated on the basis of the analytical data for phytosterols (mg/100g), the content of the individual capsules (mg) and the recommended daily doses showed on the label.