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Examining Phytosterols In Nuts and Seeds For The USDA National Nutrient Database for Standard Reference

Robin Thomas, Susan Gebhardt

USDA-ARS Beltsville Human Nutrition Research Center, Nutrient Data Laboratory

Katherine Phillips

Virginia Polytechnic Institute and State University

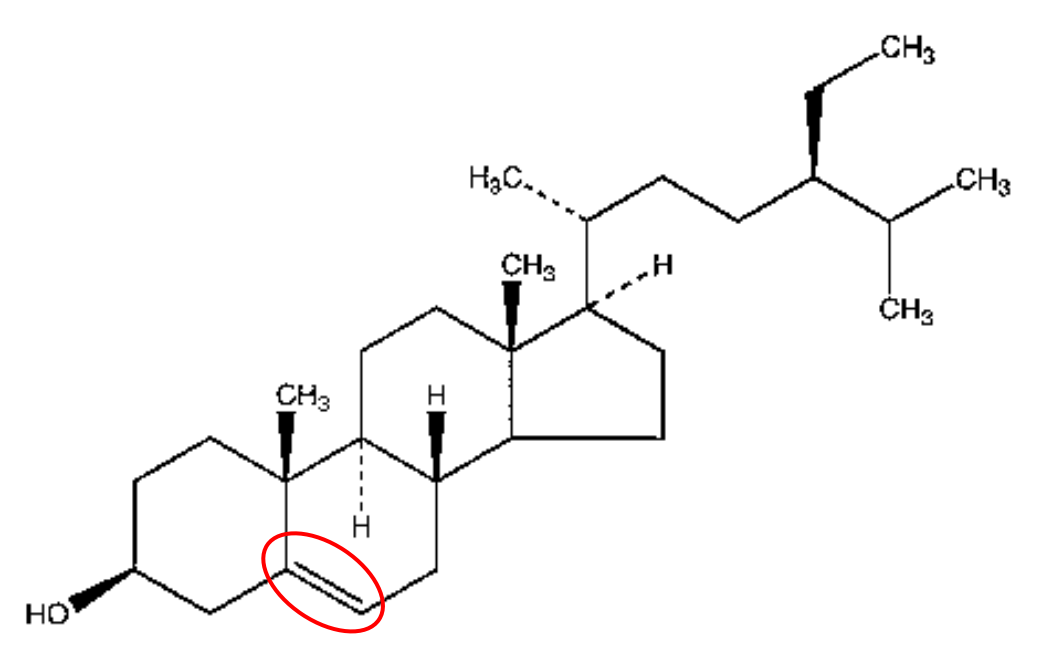
Introduction

- Phytosterols have been in the USDA National Nutrient Database for Standard Reference (SR) since early 1980's
- Research has been evolving
  - reduction in serum cholesterol levels
  - improvements in analytical methods
- Nuts and seeds are a significant dietary source of phytosterols

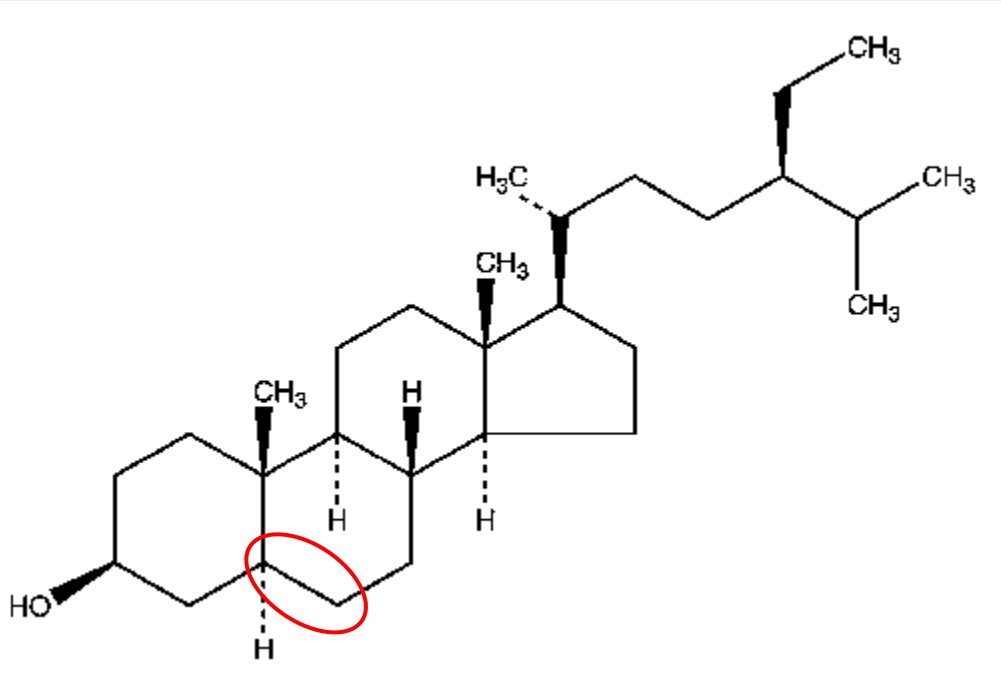
Occurrence of Phytosterols

- > 200 types
  - Most common = ß-sitosterol, campesterol, stigmasterol, Δ5-avenasterol
  - Stanols are subgroup of sterols
- 4 different forms
  - Free sterols, steryl esters, steryl glycosides, and acylated steryl glycosides

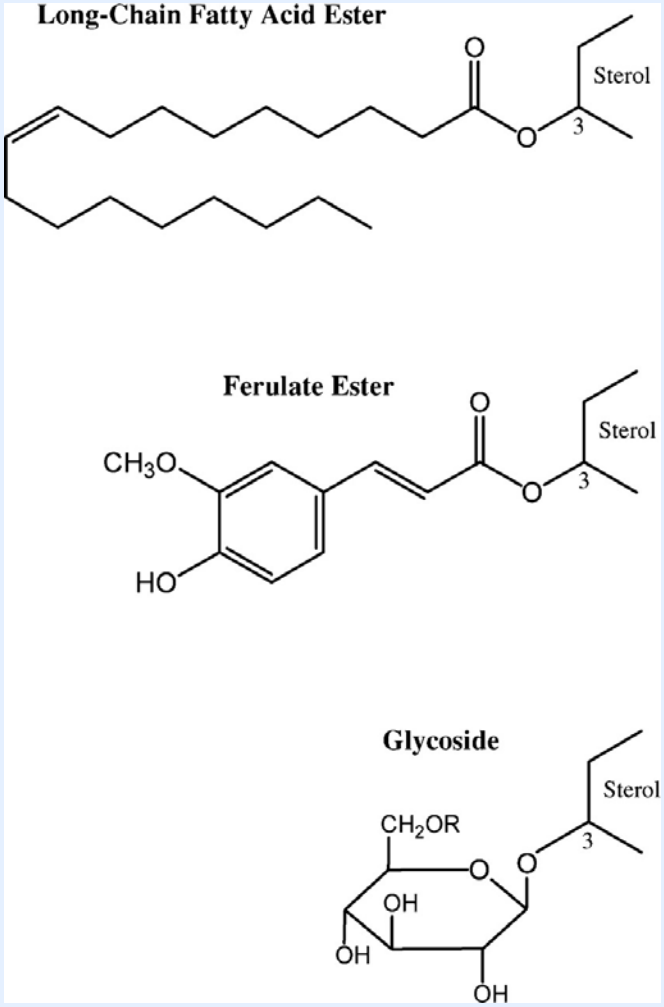
ß-Sitosterol



ß-Sitostanol



Sterol Conjugates



Steryl Esters

Steryl Glycoside

Health Effects

- Lower cholesterol
  - FDA health claim: ≥ 1.3 g/day plant sterol esters or ≥ 3.4 g/day of plant stanol esters may reduce risk of heart disease
  - ATP III: 2-3 g/day plant stanol/sterol esters reduce LDL cholesterol by 6-15 percent
- Reduced risk of certain cancers?

Addition of Phytosterols to Food

Foods or beverages containing at least 0.4 g plant sterols, when consumed twice a day for a total intake of 0.8 g/day as part of a diet low in saturated fat and cholesterol, may reduce the risk of coronary heart disease. (FDA health claim)

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Sources and Intake

- Primary natural sources:
  - vegetable oils (esp. corn oil), legumes, nuts, seeds, whole grains
- Typical intake
  - 150-400 mg/day naturally occurring
  - vegetarians nearly 1 g/day

Analytical Methods

- Separation of bound sterols
  - Acid hydrolysis to cleave free sterol from steryl glycoside
  - Alkaline hydrolysis (saponification) to cleave free sterol from steryl ester
- Gas chromatography to separate and quantify free and unbound sterols

Total Phytosterols[1] With and Without Steryl Glycosides, mg/100g

Food	F + E[2]	F + E + G[3]	Difference
Almonds	137	176	22% (p<0.007)
Flaxseed	134	169	21% (p<0.015)
Pine nuts	166	187	11% (p<0.025)
Sunflower kernels[4]	170	176	3%

1 Sum of ß-sitosterol, campesterol, stigmasterol, and Δ5-avenasterol
2 mg/100g free sterol + esterifed sterol
3 mg/100g free sterol + esterifed sterol + glycosylated sterol

4 Sunflower seed data from Toivo, J. et al. J Food Comp Anal 2001; remaining data from Phillips, K. et al. J Food Lipids 2005

Phytosterols in SR19

Currently report

- ß-sitosterol
- Campesterol
- Stigmasterol
- Phytosterols -- sum of these 3 sterols

Sources of Phytosterols in Nuts and Seeds in SR19

- 1978 Weihrauch paper (literature review)
  - Nut data calculated based on sterols in nut oils
  - Source of seed data not revealed
  - Spice seed data from 1974 Japanese paper
  - SR includes total phytosterol data from paper for:
    - cashew, European chestnut, coconut, pine nut, sesame seed, sunflower seed, and 9 spice seeds
  - Suggests GC method used

Sources of Phytosterols in Nuts and Seeds in SR19

- 1999 study
  - Collaboration between USDA and nut industry
  - Measured ß-sitosterol, campesterol, and stigmasterol in:
    - almonds, hazelnuts, macadamia nuts, pecans, pistachio nuts, and English walnuts
  - Mostly n=1; walnuts and pecans n=3
  - Various commercial labs
  - Gas chromatography

Recent Literature Sources of Nut and Seed Sterols

- 9 papers plus unpublished data, 2001-2007
- Grown in U.S. and other countries
- Some nuts fresh-harvested; others retail
- 2 papers used HPLC, remaining used GC
- Majority quantified more than 3 sterols
- 4 of 13 sources used acid hydrolysis

Phytosterols reported for almonds, mg/100g

Source	Number samples	ß-Sito-sterol	Campe-sterol	Stigma-sterol	Brassica-sterol	Δ^5-avena-sterol	Sito-stanol	Campe-stanol	Other	Total
Almond Board 06a	19	114	5	3					30*	**154**
Almond Board 06b	49	129	5	4						**138**
Maguire 04	1	85	2	2						**89**
Piironen 03	1	118	3	1	0	6†	2‡			**130**
Phillips 05	4	143	5	5		20	3	3	20	**199**
Normen 07	1	165	7	7	4	21	4	0		**208**
SR19	1	111	5	4						**120**

* total minor sterols (brassicasterol, campestanol, Δ7-campesterol, clerosterol, beta-sitostanol, Δ5-avenasterol, Δ7-avenasterol, Δ7-stigmasterol, and Δ7,25-stigmadienol)

† reported as Δ5- + Δ7-avenasterols

‡ reported as “stanols”

Red indicates use of acid hydrolysis

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Phytosterols reported for hazelnuts, mg/100g

Source	Number samples	ß-Sito-sterol	Campe-sterol	Stigma-sterol	Δ^5-avena-sterol	Δ^7-avena-sterol	Sito-stanol	Campe-stanol	Other	Total
Alasalvar 06	6	82	6	1	9*	1			2†	**101**
Amaral 06‡	19	94	7	1	7*	1			2†	**112**
Maguire 04	1	49	3	2						**54**
Phillips 05	4	102	7	0	3		4	3	3	**121**
Normen 07	1	122	8	2			7	0		**139**
SR19	1	89	6	1						**96**

* reported as Δ5-avenasterols and sitostanol

† clerosterol and Δ7-stigmastenol reported separately in paper, but combined here

‡ nuts were roasted

Red indicates use of acid hydrolysis

Phytosterols reported for English walnuts, mg/100g

Source	Number Samples	ß-Sito-sterol	Campe-sterol	Stigma-sterol	Brassica-sterol	Δ^5-avena-sterol	Δ^7-avena-sterol	Sito-stanol	Campe-stanol	Other	Total
Amaral 03	6	143	8	0		52	2			2	**206**
Maguire 04	1	57	3	3							**63**
Phillips 05	4	89	5	0		7		0	2	9	**113**
Normen 07	1	121	6	0	0	1		0	0		**128**
SR19	3	64	7	1							**72**

Red indicates use of acid hydrolysis

Phytosterols reported for pumpkin seed kernels*, mg/100g

Source	Number samples	ß-Sito-sterol	Campe-sterol	Stigma-sterol	Brassica-sterol	Δ^5-avena-sterol	Sito-stanol	Campe-stanol	Other	Total
Phillips 05	1	13	3	0		4	4	1	241	**265**
Normen 07	1	48	0	4	0	3	39			**94**

*Phillips specifies kernels but Normen does not specify if seeds are hulled

Red indicates use of acid hydrolysis

Phytosterols reported for sesame seeds, mg/100g

Source	Number Samples	ß-Sito-sterol	Campe-sterol	Stigma-sterol	Brassica-sterol	Δ^5-avena-sterol	Sito-stanol	Campe-stanol	Other	Total
Phillips 05*	1	232	53	22		40	0	6	47	**400**
Normen 07†	1	263	70	28	0	43	0	0		**404**
SR19‡	1									**714**

* Decorticated

† Not specified if decorticated or whole

‡ Whole

Red indicates use of acid hydrolysis

Summary of Results in Comparison to SR19

Type of Nut/Seed	Total Phytosterols, mg/100g	
	From Literature	In SR19
Almonds	89 - 208	120
Brazil nuts	97 - 131	*
Cashew	80 - 158	158
Flaxseed	197 - 214	*
Hazelnuts	54 - 121	96
Macadamia	96 - 187	115
Pecan	114 - 157	97
Pine nut	161 - 236	141
Pistachio	279 - 297	213
Pumpkin seed	94 - 265	*
Sesame seed	400 - 404	714
Sunflower seed	176 - 322	534
Walnuts, black†	177	108
Walnuts, English	63 - 206	72

* No data

† Same composite was assayed for paper and SR19

Variation in Ways Phytosterol Data Reported in Literature

- Number and types of different sterols quantified
- Sterol peaks not identified
- Minor sterols may be quantified individually or grouped together
- Some sterol values include glycosidic portion and others do not
- Some values are reported as mg/100g oil
- Lack of method validation/quality control in some studies

Additional Factors Affecting Differences Among Phytosterol Results in Nuts and Seeds

- Natural variability in sterol content between cultivars
- Lack of detailed sample description
- Sampling protocol
- Data reported for nuts grown in countries other than U.S.
- Number of samples

Conclusion

- Different analytical methods currently used to measure phytosterols
- Use of acid hydrolysis can have significant impact on results for nuts and seeds
- Limited data available
- Additional data on nuts and seeds needed
- Research on other food sources needed