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Rice Bran Phytosterols of Three Widespread Iranian Cultivars

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ABSTRACT

Rice bran sterols have antioxidant activity as well as physiological and biological effects. Because of these benefits, the aim of this study is evaluating the quantity and quality of Iranian rice bran sterols. Three widespread Iranian rice cultivars (Khazar, Hashemi, and Alikazemi) were used for determination of their sterol contents. Two methods of sample preparation were compared and the better one was used in this work. Rice bran samples were saponified directly after acid hydrolysis. Unsaponified materials were extracted, purified by solid phase extraction, silylated, and their sterol fractions determined by GC-MS. The sterol composition (in mg kg^{-1} bran) of three cultivars (Khazar, Alikazemi, and Hashemi) were 1,330.69, 1,279.95, 1,313.17 *β*-sitosterol; 747.52, 696.05, 756.8 campesterol; 112.8, 115.36, 114.24 Δ-5-avenasterol, 38.912, 33.08, 38.24 Δ-7-avenasterol; 8.05, 7.07, 7.56c holesterol; 4.20, 3.99, 4.23 brassicasterol; and 2,722.016, 2,706.176, 2,717.68 total sterols, respectively. The results showed that there were no significant differences between the individual and total sterol contents of these three cultivars (P> 0.05), except campesterol, β-sitosterol, and Δ-7-avenasterol. The highest and lowest sterols were *β*-sitosterol and campesterol.

Keywords: Phytosterols, Plant sterols, Rice bran, Sterol content determination.

INTRODUCTION

Phytostrols (Ps) are biosynthetically derived from squalen and belong to the group of triterpenes that includes more than 4,000 different compounds. Phytosterols are made up of a tetracyclic cyclopenta (α) phenanthrene ring and a flexible side chain at C17. The most common Ps -sitosterol, campestrol and stigmasterol- are structurally closely related to cholesterol. In plants, phytosterols exist as free and conjugated (Loharson, 2004).

Stero in rice-bran oil contain considerable quantities of Δ-5-avenasterol and related sterols. As with common vegetable oils, *β*-sitosterol is the major sterol in rice oil. More than 75% of the sterols of rice bran-oil are esterified and are collectively called orizanol. These sterol esters of ferulic acid show antioxidant activity as well as physiological and biological effects (Gunstone, 2002). Vegetables and fruits as well as cereals are suitable sources of plant sterols in diet. However, the quantity of phytosterols in a regular diet is not sufficient for these health effects (Gunstone, 2002). Therefore, they are extracted and added to some foodstuffs for enrichment.

Rice bran is a byproduct in Iran which is used primarily as domestic animal foodstuff. So, in this work we used the bran of three widespread cultivars as a cheap source for the evaluation of their sterol content. We used the solid phase extraction (SPE) method which is more rapid and convenient than TLC (Thin Layer Chromatography) for cleaning up unsaponifiable matters

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(Azadmard-Damirchi, 2006, Pironen and Toivo, 1999; Toivo, 2000)

MATERIALS AND METHODS

The bran of three widespread cultivars of Iranian rice (*Oryza sativa*) named Khazar, Hashemi, and Alikazemi were taken from the Rice Research Center in Rasht, Iran. The samples after milling (Cyclotec 1093 sample mill, Tekator, Sweden) and sieving (20 meshe) were packaged in nylon bags and kept frozen until analysis. The average oil content of the bran was 16%.

Saponification

Two methods were used for saponification of plant materials for phytosterol extraction which had been developed by Toivo (1999) and Pironen (2001). Table 1 shows the fatty acid profile of extracted unsaponifiable matters by two methods. So, we used the Piironen method which showed lower residual fatty acids in unsaponifiable fraction. Acid hydrolysis can hydrolyze glycoside sterol esters in cereals (Toivo, 2000), so we used it as a pretreatment of saponification in cereals.

One gram of each sample was weighed in a 50 ml flask and betulin (Sigma-Aldrich) was added as an internal standard. For acid hydrolysis, 5 ml of 6M HCl was added to the flask and the contents were mixed for 10 seconds then refluxed for 60 minutes on a water bath. The content of the flask was mixed manually every 10 minutes, then the flask was cooled to the room temperature, and its contents were transferred to a screw capped test tube. Lipids were extracted with 20 ml of hexane and diethyl ether (1:1) solvent mixture by shaking for 10 minutes. The organic layer was separated by letting the tube stand for 15 minutes (and centrifuging if necessary), and was transferred to a round-bottom flask and evaporated to dryness in a rotary evaporator at 50°C. For saponification, 8 ml of absolute ethanol and 0.5 ml of saturated aqueous KOH solution were added. The flask contents were mixed (10 seconds) and refluxed at 80-85°C for 30 minutes, then cooled to room temperature, and transferred into a screw capped test tube; 12 ml of water and 20 ml of cyclohexane were added, followed by shaking for 10 minutes. An aliquot of 15 ml of the organic layer was transferred to a round-bottom flask and evaporated to dryness in a rotary evaporator at 50°C. The residue was dissolved in 1 ml of hexane (Pironen and Toivo, 1999).

A SiOH cartridge (chromo bond SiOH, 6 ml, 1 gr) was activated by 5 ml of hexane. The sample solution was first filtered through a nylon acrodisc (13 Pall Perman Laboratory, Ann Arbot, and MI). The solution was eluted by gravity flow for several minutes and then by vacuum (5-10 Hg) for 5 minutes. The sterol fraction was eluted with 5ml of hexane and diethyl ether (90:10) solvent and evaporated to dryness at 50°C. The sterol fraction residue was dissolved in 500 µl of dichloromethane.

Table 1. Fatty acid residual of two methods of saponification.

Fatty acid residue	Toivo Method (%)	Piironen Method (%)
Lauric acid	0.15	1.89
Myristic acid	1.97	1.97
Palmitic acid	16.8	2.8
Margaric acid	0.6	1.6
Oleic acid	18.17	4.87
Linoleic acid	24.59	7.99
Stearic acid	6.53	5.53
Total fatty acid	44.22	23.76

Identification and Quantification

Sterols were analyzed on GC by their trimethylsillyl (TMS) ether derivatives. In a presanitized sample vial, the solvent was evaporated under nitrogen (50°C) and then 100 µl of anhydrous pyridine (E. Merck, Darmstadt, Germany) and 100 µl of derivatization reagent containing 99% of BSTFA (E. Merck, Darmstadt, Germany) and 1% of TMCS (Flucka Chemie AG, Switzerland) were added. To complete the silylation, solutions were allowed to stand overnight at room temperature before GC analysis (Pironen and Toivo 1999).

GC-MS analysis was used to identify the major sterols in sample matrix. MS analysis was also used to evaluate peak purities of sterols.

A Hewlett-Packard (HP, Palo Alta, USA) HP 6890 series GC equipped with a split/splitless injector and a HP 5973 mass-selective detector system were used. The MS was operated in the EI mode (70 eV). Helium (99.999%) was employed as carrier gas and its flow rate was adjusted to 1 ml min^{-1}. The separation was performed on a 60 m×0.25 mm I.D. fused silica capillary column of HP-1. The GC column temperature was programmed at 100°C and raised to 270°C at 30°C min^{-1}, 90 minutes hold at this temperature. The injector temperature was set at 260°C, and all the injection was carried out on the splitless mode. The ion source and quadrupole temperature were set at 230°C and 150°C, respectively.

The area of each peak was measured relative to the internal standard peak area and, then, the concentration of each sterols was calculated.

Analysis of Results

All the analysis was performed in triplicate. SPSS was used for calculation of the statistical analysis.

RESULTS AND DISCUSSION

The sterol contents of the three cultivars are given in Table 2. Table 3 shows there is not significant difference ($P > 0.05$) between their sterol and individuals except campesterol, Δ-7 avenasterol and β-sitosterol. The decreasing order of sterols was sitosterol, campesterol, Δ-5 avenasterol, Δ-7 avenasterol, cholesterol, and brassicasterol. Because the rice bran sterols reported by others (19, 20) were in $mgkg^{-1}$ oil, for comparison we calculated our results in $mgkg^{-1}$ oil. Table 4 demonstrates that the total contents of Iranian cultivars are higher that the sterols reported by Firestone (1999) and lower than those of Rossel, (2001).

Table 2. Sterol content of rice bran and calculated rice bran oil.

Sterol	Khazar		ALIKAZEMI		HASHEMI	
	mg/kg oil*	Sterol Fraction Percentage	mg/kg oil*	Sterol Fraction Percentage	mg/kg oil*	Sterol Fraction Percentage
Cholesterol	50.3±2.1	0.3	44.16±3.1	0.26	47.23±0.5	0.28
Campesterol	4672±11.0	27.8	4350.3±47.5	25.72	4730±19.9	27.84
Sitosterol	8129.3±706.3	47.8	7999.7±108.1	47.30	8207.33±7.4	48.3
Brassicasterol	26.3±1.0	0.15	24.93±0.9	0.15	26.45±0.7	0.16
Δ-5-Avenasterol	705±6.0	4.14	720.97±5.9	4.26	714±2.0	4.20
Δ-7-Avenasterol	243.2±2.1	1.43	206.77±1.7	1.22	239±5.3	1.41
Others	3185±1.9	18.72	3566.77±101.1	21.11	3021.43±2.1	17.88
TOTAL	17012.6±11.3		16913.6±118.0		16985.5±30.2	

Table 3. Statistics assessment of sterols content.

Source*		SS	d f	MS	F
1	SS_M	57.0422	2	28.5211	6.21
	SS_P	27.9600	6	4.6600	
2	SS_M	251,322.6667	2	125,666.3333	135.7251**
	SS_P	5,555.3333	6	925.8889	
3	SS_M	66022.89	2	33011.44	7.96**
	SS_P	24882.00	6	4,147.000	
4	SS_M	4.2654	2	2.1327	2.9925
	SS_P	4.2760	6	0.7127	
5	SS_M	384.4689	2	192.2344	7.6159
	SS_P	151.4467	6	25.2411	
6	SS_M	12,386.7267	2	1,193.3633	101.0564**
	SS_P	70.8533	6	11.8089	
7	SS_M	15,681.7267	2	7,840.8633	1.5715
	SS_P	29,935.7533	6	4,989.2922	

* 1=Cholesterol, 2= campesterol, 3= β-Sitosterol, 4=Brassicasterol, 5=Δ-5-avenasterol, 6= Δ-7-avenasterol, and 7=Total sterol.
** Significant difference (P<0.05).

Table 4. Rice bran oil Sterol composition % reported by others.

	Rossel(2001)	Firestone(1999)
Cholesterol	2.2	_
Brassicasterol	0.3	_
Campesterol	20.4	24
Stigmasterol	10.8	11.5
Sitosterol	34.8	51.5
Δ-5-avenasterol	3.2	8
Δ-7-Stigmasterol	11.6	1.5
Δ-7-avenasterol	2.4	3
Total(mg/kg)	36758	10550

CONCLUSIONS

The average of total sterol contents of Khazar, Alikazemi and Hashemi cultivars were in the range of 2,706.176-2,722.016 mg kg^{-1} bran or 16,913-1,712.06 mg kg^{-1} oil, β- sitosterol was in the highest and brassica in the lowest proportions.

The result showed that there was not any significant differences (P> 0.05) between the total sterol content of the three cultivars, and between individual sterols except campesterol, brassicaesterol, and Δ-7 avenasterol.

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تعیین محتوی فیتوسترول سه واریته برنج ایرانی

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چکیده

محتوی استرول سه واریته پرمصرف برنج ایرانی (خزر، هاشمی و علی کاظمی) اندازه گیری گردید. سبوس ها بعد از هیدرولیز اسیدی و قلیایی، صابونی شدند. مواد غیر قابل صابونی استخراج گردیدند ، به روش SPE خالص سازی شدند، سیلیله شدند و قسمت استرولی آنها توسط دستگاه GC-MS اندازه گیری شد. محتوی استرولی سه واریته به ترتیب نزولی عبارت بودند از : بتا سیوسترول (۱۳۳۰/۶۹، ۱۲۷۹/۹۵، ۱۳۱۳/۱۷ به ترتیب در مورد واریته های خزر، علی کاظمی، و هاشمی) برحسب میلی گرم در کیلوگرم سبوس، کمپسترول (۷۴۷/۵۲، ۶۹۶/۰۵، ۷۵۶/۸۰ به ترتیب در مورد واریته های خزر، علی کاظمی، و هاشمی) برحسب میلی گرم در کیلوگرم سبوس، Δ- ۵- آوناسترول (۱۱۲/۸، ۱۱۵/۳۶، ۱۱۴/۲۴ به ترتیب در مورد واریته های خزر، علی کاظمی، و هاشمی) برحسب میلی گرم در کیلوگرم سبوس، Δ-۷- آوناسترول (۳۸/۹۱۲، ۳۳/۰۸، ۳۸/۲۴ به ترتیب در مورد واریته های خزر، علی کاظمی، و هاشمی) برحسب میلی گرم در کیلوگرم سبوس، کلسترول (۸/۰۵، ۷/۰۷، ۷/۵۶ به ترتیب در مورد واریته های خزر، علی کاظمی، و هاشمی) برحسب میلی گرم در کیلوگرم سبوس، براسیکاسترول (۴/۲۰، ۳/۹۹، ۴/۲۳ به ترتیب در مورد واریته های خزر، علی کاظمی، و هاشمی) برحسب میلی گرم در کیلوگرم سبوس، و استرول تام (۲۷۲۲/۰۱۶، ۲۷۰۶/۱۷۶، ۲۷۱۷/۶۸ به ترتیب در مورد واریته های خزر، علی کاظمی، و هاشمی) برحسب میلی گرم در کیلوگرم سبوس. نتایج نشان داد که تفاوت قابل ملاحظه آماری بین مقادیر استرول تام در سه واریته وجود نداشت و دربین اجزاء استرولی به جز کمپسترول وΔ-۷- آوناسترول تفاوت قابل ملاحظه آماری وجود نداشت.