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Nutraceutical Lipid Substances in Korean Rice Cultivars

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Abstract The lipid soluble substances, such as tocols (tocopherols and tocotrienols), phytosterols, squalene, and γ-oryzanol, levels in 22 cultivars of Korean brown rice were investigated. The phytosterol levels in 22 cultivars varied from 255.0 mg/kg rice in *Daerip* to 492.0 mg/kg rice in *Kuennun* and the major phytosterol was β-sitosterol in all cultivars. In all the cultivars, there was a high correlation coefficient (R^2 = 0.7996) between the lipid level and the phytosterol level. The major tocols homologues in the 19 *Japonica* type cultivars were α-tocopherol and α-tocotrienol, and the major homologue in the *Indica* type cultivars namely *Segyuejinmi*, *Hanareum*, and *Dasan No.1* was γ-tocotrienol. Among the γ-oryzanol species, the proportions of cycloartenylferulate in *Japonica* type cultivars were significantly higher than those of 24-methylene cycloartanylferulate ($p < 0.05$). This study provided information on lipid soluble substances level, such as tocols, phytosterol and γ-oryzanol, in different rice cultivars.

Keywords: *brown rice cultivars, tocols, γ-Oryzanol, phytosterols, squalene*

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1. Introduction

Rice (*Oryza sativa* L.) has long been a staple in Asian countries and is considered an excellent energy source. Rice is commonly consumed in milled or white form, which is palatable to Asian consumers. However the parts removed during the milling process, namely the germ and bran, reportedly contain plentiful nutraceutical substances such as dietary fiber, tocopherols, phytosterols and γ-oryzanol [1,2,3]. Because tocols (tocopherols and tocotrienols), phytosterols and γ-oryzanol are lipid soluble, these substances are present in larger quantities in rice germ, bran, or brown rice, of which the lipid contents are higher than milled rice [4]. Therefore, as the degree of rice milling increased, the rice lipid soluble substances contents significantly decreased [5]. Lipid soluble substances such as tocols, phytosterols and γ-oryzanol were reported to have beneficial effects in lowering blood cholesterol, protection against oxidative damage and inhibition of tumor induced by chemicals in animals [3,6,7,8]. Therefore, in addition to rice palatability, the nutraceutical contents of brown rice cultivars are considered to be one factors used by consumers when choosing a rice cultivar.

Hence, the aim of this study was to provide comprehensive information on the lipid content, tocols (tocopherols and tocotrienols), phytosterols, squalene and γ-oryzanol in 22 different rice cultivars grown in South Korea.

2. Materials and methods

2.1. Materials

Twenty two rice cultivars harvested during 2010 in Korea were supplied by the Korean Rural Developmental Administration (KRDA) (Suwon, Korea). Tocopherols and tocotrienols standards were purchased from Merck (Darmstadt, Germany) and their purity was 95%. Squanlene, 5α-cholestane, campesterol, β-sitosterol, and stigmasterol were purchased from Sigma-Aldrch Co. (St.Louis, MO, USA) with a purity of 95%. γ-Oryzanol standards were obtained from Oryza Oil & Fat Chemical Co. Ltd (Ichinomiya, Japan) at a purity of 98%. *n*-Hexane, isopropanol, chloroform, methanol, acetonitrile and acetic acid were purchased from Fisher Scientific Korea (Seoul, Korea) and of HPLC grade. Other chemicals used in this study were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Methods

2.2.1. Seed Plantation Environmental Conditions and Sample Preparation

All cultivars from this study were grown in an experimental plot at the NICS (National Institute of Crop Science), Suwon, South Korea. Seeds were sown on April 25, 2010, and the seedlings were transplanted on May 25, 2010. All plants were transplanted to 15 x 30 cm^2 plot with 3 replications. Cultivation and management were

performed according to the rice cultivation standards of the KRDA (Korean Rural Development Administration) experimental farm in South Korea. All seeds were threshed, and then air dried in a shaded greenhouse. Fully mature grains were used for chemical property evaluation. All sample grains were dehulled and then stored -70°C to use in the experiments. The samples were harvested in August, 2010 and this study was performed 2011.

2.2.2. Extraction and Determination of Brown Rice Oil

Dehulled brown rice (1kg) from KRDA was ground using a cyclomill, Cyclotech (Foss, Hillerød, Denmark). The oil was extracted in a 3 L flask by stirring it into 2.5 L of *n*-hexane for 3 h at ambient temperature. The extract was filtered through a filter paper (Whatman No. 2) over a Buchner funnel. The extraction was repeated, and the two extracts were combined. *n*-Hexane was evaporated by a rotary evaporator (Eyela, Japan) at 30°C and the residual *n*-hexane was removed completely at a high vacuum of 0.1 kPa. The lipid content of each brown rice was determined by the percentage of the resulting oil weight to used brown rice weight. The oil was stored at –70°C under nitrogen until analysis.

2.2.3. Saponification of Brown Rice Oil

Brown rice oil was saponified by the Ko et. al's method [4]. One gram of sample oil, 4 mL of 5% pyrogallol solution in ethanol, 30 mL ethanol, and a few boiling chips were placed in a 120 mL flat-bottomed flask fitted with a reflux condenser and heated on a hot plate. When the mixture started boiling, 1 mL of 50% aqueous potassium hydroxide solution was added. The sample was saponified for 5 min. After saponification, the flask was placed in an ice bath, and 20 mL of water and 50 mL of diethyl ether were added. The mixture was transferred to a 250 mL separatory funnel. Sample was repeated twice with 50 mL of diethyl ether. The pooled diethyl ether layer was washed three times with 20 mL of distilled water, filtered through anhydrous sodium sulfate and then evaporated at 30°C. Finally, the extracts were dissolved in appropriate solvents (*n*-hexane or chloroform) and filtered through a Millipore 0.45□ m membrane. This filtrate was used to analyze of tocols, phytosterols, andsqualene by HPLC or GC.

2.2.4. Tocols Analysis

Tocols contents were quantified by HPLC using the Ko et. al's method [4], in which the chromatographic separation was performed with a normal phase Lichrospher Si-60 column (250 mm×4.6mm i.d Merck Co., Germany) and each peak was detected at an excitation wavelength of 298 nm and an emission wavelength of 325nm using a JASCO FP-1520 fluorescent detector (JASCO Co., Japan). The mobile phase was a solvent mixture of isopropanol, and *n*-hexane (1:99, by volume) in isocratic mode, and the flow rate was 1 mL/min. Tocols peaks were identified and quantified by comparing their retention times and peak areas to those of standards.

2.2.5. γ-Oryzanol Analysis

The γ-oryzanol content was determined by the HPLC method [9], chromatographic separation was performed by the Optimapark C_{18} column (250mm × 4.6mm, RS Tech. Co., Korea). Each peak was detected at 330nm by a JASCO UV-2075 UV/VIS detector (JASCO Co., Japan). A solvent mixture of methanol, acetonitrile, and acetic acid (53:44:3, by volume) was used as a mobile phase in isocratic mode, and the flow rate was 1.4 mL/min. γ-Oryzanol peaks were identified and quantified by comparing their retention times and peak areas to those of standards.

2.2.6. Squalene and Phytosterols Analysis

The squalene and phytosterols contents were determined by gas chromatography using 5α-cholestane as the internal standard following Ha's method [5]. The squalene, campesterol, β–sitosterol and stigmasterol were identified using standard materials and cycloartenol and 24-methyl cycloartenol were identified by GC-MS analysis according to the previous method [5]. The GC (Varian CP-3800, Varian Inc., CA, USA) was equipped with a SAC^{TM}-5 capillary column (30m × 0.32mm i.d., Supelco, PA, USA) and squalene and phytosterols were detected using a flame ionization detector. The column was maintained at 280°C for 1min and programmed to rise to 300°C at a rate of 2°C/min and was then held stable for 20min. Helium was used as the carrier gas and the flow rate was 1.0 mL/min. The injector and the detector temperature were 310°C and 320°C, respectively and the split ratio was 50:1.

2.2.7. Recovery and Precision

The amount of each tocols, squalene, phytosterol and γ-oryzanol was added to the samples corresponded to 50~150% of those expected levels and recovery was calculated by the following equation

$$R\ (\%) = \left[(Cs - Cp)/Ca\right] \times 100$$

Where R (%) is the percent recovery of added standard; Cs the each tocols, squalene, phytosterol and γ-oryzanol content in the spiked sample; Cp the each each tocols, squalene, phytosterol and γ-oryzanol content; and Ca the each tocols, squalene, phytosterol and γ-oryzanol standard added.

Repeatability and reproducibility of the tocols, squalene, phytosterol and γ-oryzanol were determined by carrying out on a sample by analyzing five replicates of the sample on the same day and on the different days, respectively.

2.2.8. Statistical Analysis

Each reported value is the mean of triplicate samples from each cultivar. The significance of differences in the sample group was determined by Student's *t*-test and ANOVA with Duncan's multiple range tests and the data was analyzed with a statistical software package (SPSS version 12.0, SPSS Institute). Statistical significance was accepted at a level of $p < 0.05$.

3. Results and Discussion

3.1. Assay Repeatability and Reproducibility

The repeatability and reproducibility for tocols, phytosterol, squalene and γ-oryzanol analyses were assessed by rice cultivars (Table 1). The coefficient of

variations (CV) for repeatability and reproducibility were less than 5%. The accuracy was evaluated by measuring recovery. The recoveries of the tocols, phytosterol, squalene and γ-oryzanol were from 92% to 101%.

Table 1. Precisions and recoveries of tocols, squalene, phytosterol and g-oryzanol assay for rice cultivars

	Repeatability [1]		Reproducibility [2]		Recovery (%) [3]	
	Mean ± std	CV (%) [4]	Mean ± std	CV (%)	Mean ± std	CV (%)
α-tocopherol	11.98 ± 0.06	0.50	12.02 ± 0.09	0.76	97.82 ± 3.62	3.70
β-tocopherol	0.45 ± 0.01	1.30	0.46 ± 0.01	2.92	97.78 ± 2.22	2.27
γ-tocopherol	0.36 ± 0.00	0.68	0.37 ± 0.01	3.30	100.98 ± 3.17	3.14
δ-tocopherol	0.43 ± 0.01	1.80	0.43 ± 0.01	2.59	98.36 ± 2.65	2.70
α-tocotrienol	10.97 ± 0.06	0.53	11.01 ± 0.08	0.74	98.12 ± 3.80	3.87
γ-tocotrienol	4.73 ± 0.19	4.01	4.82 ± 0.22	4.57	98.77 ± 5.05	5.11
δ-tocotrienol	3.09 ± 0.04	1.18	3.13 ± 0.05	1.65	99.40 ± 3.54	3.56
Squalene	44.89 ± 1.18	2.62	45.43 ± 0.99	2.18	92.55 ± 1.44	1.56
Phytosterols	496.54 ± 6.14	1.24	496.36 ± 9.75	1.96	101.66 ± 4.70	4.63
γ-Oryzanol	310.04 ± 5.83	1.88	312.79 ± 6.36	2.03	95.83 ± 3.29	3.43

[1] Repeatability refers to the results of independent determinations carried out on a sample by analyzing five replicates of the sample on the same day. The unit is mg/kg rice.
[2] Reproducibility refers to the results of independent determinations carried out on a sample by analyzing five replicates of the sample at different times. The unit is mg/kg rice.
[3] Recovery is a measure of the closeness of the analytical result to the value evaluated by analyzing spiked samples.
[4] Coefficient of variation calculated as the SD divided by the mean.

3.2. Phytosterol and Lipid Levels

Phytosterols are important bioactive constituents as well as the most abundant lipid soluble substances in rice. Thetotal phytosterol and lipid levels in 22 types of rice cultivars are given in Figure 1. As reported by Ha et al. [5], β-sitosterol was the predominant sterol (> 40%) followed by campesterol, stigmasterol, and others, namely cycloartenol and 24-methylene cycloartanol in this study (data not shown). There was a significant difference in the total phytosterol levels between the highest and lowest levels. For example, the levels of total phytosterols ranged from 492 mg/kg of rice in the *Keunnun* cultivar to 255 mg/kg of rice in the *Daerip* cultivar. These significant differences may be attributed to different genotypes of a crop in same location and same year [10]. It is reported that the factors to effect on phytosteol level are climate and growth location as well as genotype [11,12]. Lipid level of each rice cultivars ranged from 2.78% in *Goami* 3 cultivar to 1.40% in *Daerip* cultivar. Meanwhile, there was a high correlation coefficient (R^2 = 0.7996) between the lipid and phytosterol levels. A positive correlation was reported between the phytosterol and the oil levels when the effects of genetic variation and genotype x environmental interactions for rapeseed phytosterol levels was investigated [13]. In the case of spring wheat, there was a positive correlation between phytosterols and total lipid contents [10]. Similar results were obtained in our rice study. The study of Nurmi et al. [10] suggested that larger wheat bran, which was known to be rich in sterol, caused higher lipid content and higher phytosterol contents of spring wheat.

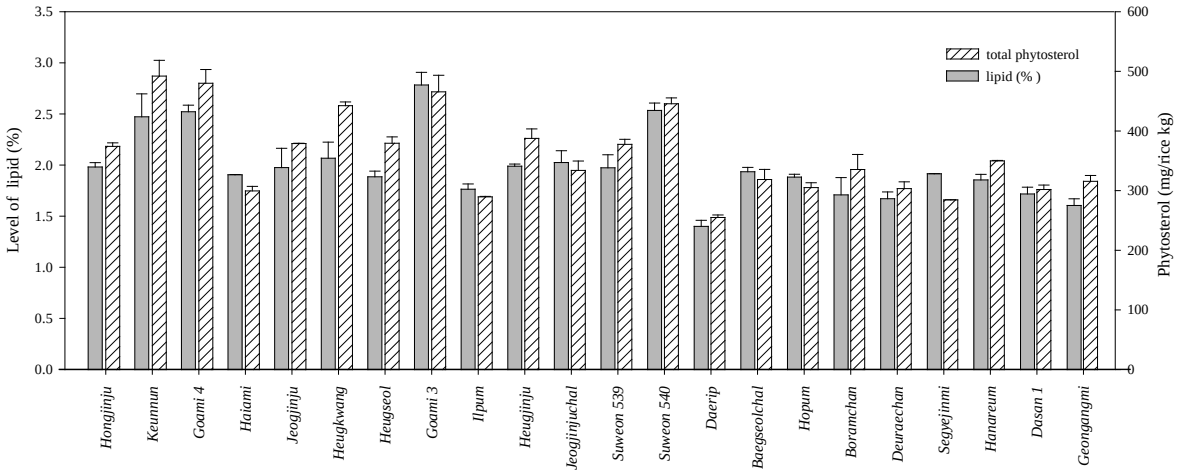

Figure 1. The levels of total phytosterols and lipids in various brown rice cultivars

3.3. Tocols Levels

The tocols levels in 22 brown rice cultivars were given in Figure 2. Four tocopherol homologues, *i.e.*,α-, β-, γ-, and δ-tocopherol, and three tocotrienol homologues, *i.e.*,α-, γ- and δ-tocotrienol were identified. β-Tocotrienol was not detected in this study. The total tocols levels of various brown rice cultivars were detected from14.86 to 38.19 mg/kg rice. *Heugkwang* had the highest tocols level as 38.19mg/kg rice, whereas *Geongangmi* had the lowest

total tocols level as 14.86mg/kg rice. The predominant tocols homologues in all tested rice cultivars were α-tocopherol, α-tocotrienol, and γ-tocotrienol even though there were differences in the three tocols homologues levels for individual cultivar. The relative percentages of seven tocols homologues in 22 cultivars are shown in Table 2. For four *Japonica* type cultivars, namely *Hongjinju*, *Keunnun*, *Suweon 539* and *Suweon 540*, the proportions of α-tocopherol were higher than 50%, whereas those of γ-tocotrienol were lower than 15%. Meanwhile, the proportions of γ-tocotrienol in all cultivars of *Indica* type, namely, *Segyejinmi*, *Hanareum* and *Dasan 1*, ranged from 56 to 71%, whereas those of α-tocopherol were lower than 19%. The HPLC chromatograms of the tocols in *Hongjinju* as the cultivar with a high percentage of α-tocopherol and in *Hanareum* as the cultivar with a high percentage of γ-tocotrienol are shown in Figure 3. These results are consistent with those of other studies [14,15]. The levels of tocopherol and tocotrienol in plant were reported to relate with homogentisic acid geranylgeranyl transferase (HGGT, known as α-tocotrienolsynthetase) and Homogentistic acid phytyltransferase (HPT, known as α tocopherolsynthetase) activities [16,17]. Cahoon et al. [17] was reported that the increases in HGGT activity in transgenic *Arabidopsis thaliana* and corn seed resulted in the increases of tocotrienol. Although there is little study of the two transferases activity in rice cultivars, according to the study of Cahoon et al. [17], the varieties of tocopherol and tocotrienol levels in rice cultivars may be resulted from inherent differences of HGGT or HPT activities in each rice cultivar.

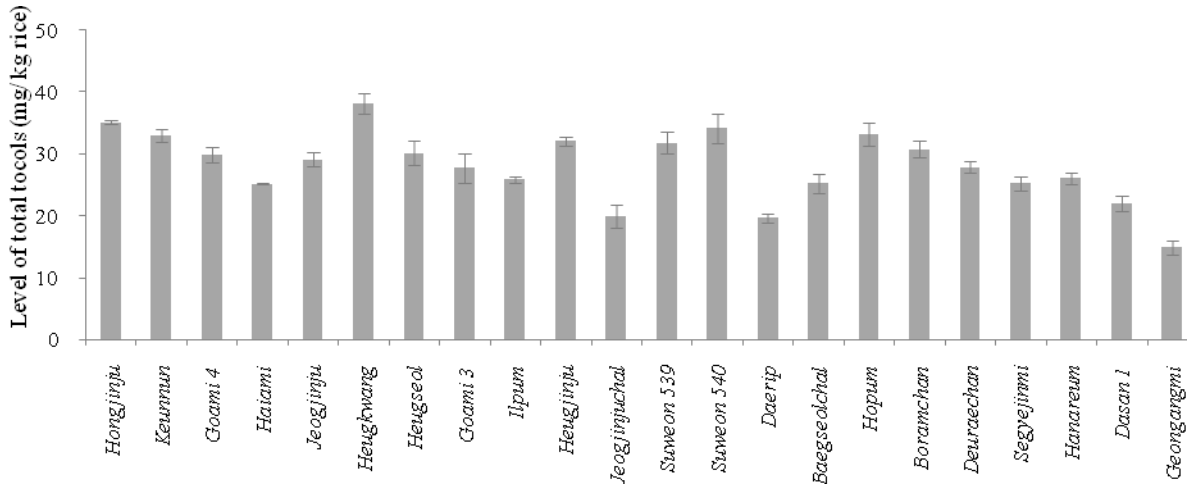

Figure 2. The levels of total tocols for various brown rice cultivars

Table 2. The compositions (w %) of tocols homologues in various brown rice cultivars[1)]

Cultivars	α-tocopherol	β-tocopherol	γ-tocopherol	δ-tocopherol	α-tocotrienol	γ-tocotrienol	δ-tocotrienol
Hongjinju	55.47 ^{lm}	2.95 ^{def}	1.10 ^{bc}	0.16 ^{abcd}	23.52 ^{cd}	15.61 ^{c}	1.18 ^{bcd}
Keunnun	57.11 ^{m}	2.99 ^{def}	0.36 ^{a}	0.04 ^{a}	26.61 ^{ef}	11.44 ^{a}	1.46 ^{cde}
Goami 4	39.48 ^{f}	7.21 ^{k}	0.56 ^{ab}	0.09 ^{ab}	33.25 ^{i}	14.50 ^{bc}	4.91 ^{n}
Haiami	45.77 ^{j}	2.62 ^{cdef}	3.99 ^{g}	0.22 ^{bcde}	26.17 ^{ef}	19.46 ^{d}	1.77 ^{efgh}
Jeogjinju	44.66 ^{ij}	2.72 ^{cdef}	2.74 ^{f}	0.29 ^{defg}	27.82 ^{fg}	19.92 ^{d}	1.85 ^{efgh}
Heugkwang	34.41 ^{d}	2.43 ^{bcde}	3.24 ^{f}	0.35 ^{fgh}	28.43 ^{g}	28.30 ^{g}	2.83 ^{jk}
Heugseol	40.68 ^{fgh}	2.20 ^{bc}	6.59 ^{j}	0.40 ^{ghi}	24.19 ^{cd}	24.46 ^{ef}	1.48 ^{cde}
Goami 3	38.60 ^{ef}	3.13 ^{ef}	1.40 ^{cd}	0.21 ^{bcde}	32.03 ^{hi}	20.12 ^{d}	4.50 ^{mn}
Ilpum	42.34 ^{ghi}	2.61 ^{cdef}	1.84 ^{de}	0.13 ^{abc}	30.72 ^{h}	20.76 ^{d}	1.60 ^{defg}
Heugjinju	35.78 ^{d}	2.44 ^{bcde}	4.60 ^{h}	0.43 ^{hi}	24.94 ^{de}	29.99 ^{g}	1.82 ^{efgh}
Jeogjinjuchal	43.03 ^{hi}	2.16 ^{bc}	2.10 ^{e}	0.21 ^{bcde}	32.04 ^{hi}	19.84 ^{d}	0.62 ^{a}
Suweon 539	54.56 ^{l}	2.13 ^{bc}	5.73 ^{i}	0.36 ^{fgh}	23.56 ^{cd}	12.80 ^{ab}	0.86 ^{ab}
Suweon 540	57.71 ^{m}	3.31 ^{fg}	0.22 ^{a}	0.09 ^{ab}	25.32 ^{de}	11.20 ^{a}	2.15 ^{hi}
Daerip	40.25 ^{fg}	1.80 ^{b}	3.31 ^{f}	0.18 ^{bcde}	27.07 ^{fg}	26.05 ^{f}	1.34 ^{bcde}
Baegseolchal	35.61 ^{d}	2.35 ^{bcd}	0.83 ^{abc}	0.11 ^{ab}	35.75 ^{j}	23.98 ^{e}	1.36 ^{bcde}
Hopum	41.15 ^{fgh}	2.71 ^{cdef}	1.76 ^{de}	0.20 ^{bcde}	23.65 ^{cd}	28.47 ^{g}	2.04 ^{fghi}
Boramchan	43.14 ^{hi}	2.64 ^{cdef}	1.96 ^{de}	0.14 ^{abc}	25.02 ^{de}	24.99 ^{ef}	2.11 ^{ghi}
Deuraechan	48.61 ^{k}	2.19 ^{bc}	6.39 ^{j}	0.30 ^{efg}	22.45 ^{c}	19.04 ^{d}	1.03 ^{abc}
Segyejinm[2)]	15.99 ^{a}	1.00 ^{a}	11.59 ^{n}	0.52 ^{ij}	5.25 ^{b}	62.61 ^{i}	3.04 ^{cdef}
Hanareum[2)]	11.55 ^{b}	0.50 ^{a}	16.57 ^{l}	0.53 ^{ij}	7.50 ^{a}	61.81 ^{i}	1.54 ^{k}
Dasan 1[2)]	10.77 ^{a}	0.49 ^{a}	9.88 ^{k}	0.26 ^{cdef}	4.78 ^{a}	71.30 ^{j}	2.51 ^{ij}
Geongangmi	36.74 ^{de}	3.88 ^{g}	2.81 ^{f}	0.45 ^{hij}	22.63^{c}	29.28 ^{g}	4.22 ^{l}

[1)] Each value represents the average of triplicates.
[2)] These subcultivars are *Indica* type and the others are *Japonica* type.
[a-n)] Means within a column with different superscript letters are significantly different ($p < 0.05$)

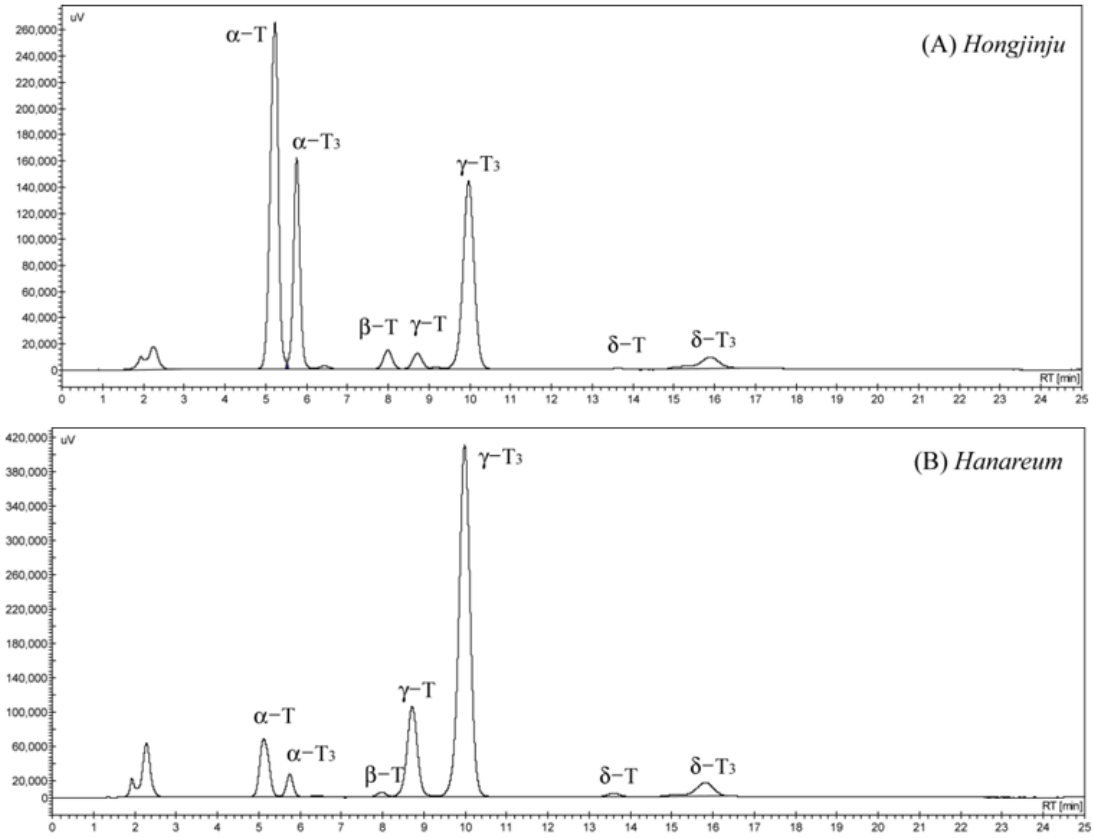

Figure 3. HPLC chromatograms of tocols for *Hongjinju* cultivar as a Japonica type (A) and *Hanareum* cultivar as an Indica type (B). T and T3 represent tocopherol and tocotrienol, respectively

3.4. Squalene Levels

Squalene is reported to be a quencher of singlet oxygen and a free radical scavenger [18]. The squalene levels of 22 brown rice cultivars are given in Figure 4 and were detected in a wide range of 10.6 to 59 mg/kg of rice. Three cultivars in particular, namely *Segyejinmi*, *Hanareum* and *Dasan 1*, possessed significantly lower squalene levels than the other cultivars ($p < 0.05$). These three cultivars were *Indica* type rice. There is little information available about rice squalene contents, especially in different rice cultivars. Rice squalene contents decreased significantly as the degree of milling increased [5] and the squalene contents of 14 day old rice were higher than those of 28 day old rice and varied with the rice cultivar [19]. The squalene level for olives reportedly varied up to 20% in relation to different cultivars [20]. Interestingly, three cultivars (*Segyejinmi*, *Hanareum* and *Dasan 1*) of higher level in γ-tocotrienol were much lower in squalene level than the others. Because squalene biosynthesis needs farnesyl pyrophosphate, which is precursor of geranyl pyrophosphate, and tocotrienol biosynthesis needs geranylgeralnyl pyrophosphate [17,21], tocotrienol biosynthesis and squalene biosynthesis may be in competition with the same substrates, farnesyl pyrophosphate. Therefore it is suggested that the levels of tocotrienol and squalene in rice be different in accordance to their synthesis process.

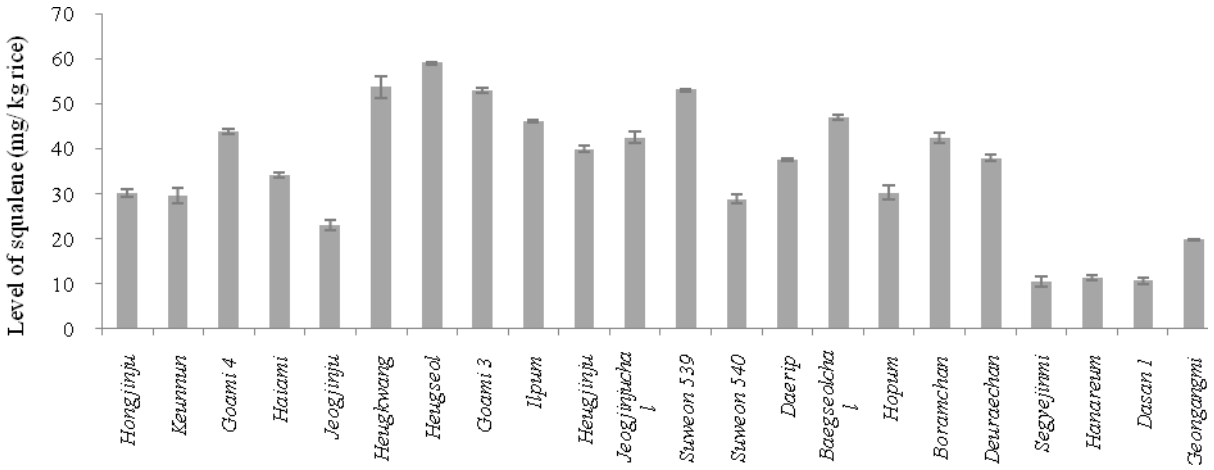

Figure 4. Squalene levels for various brown rice cultivars

3.5. γ-Oryzanol Levels

γ-Oryzanol is a naturally occurring component in rice, which consists of a mixture of ferulic acid esters from sterols and triterpene alcohols. Cycloartenylferulate, 24-methylene cycloartanylferulate, campesterylferulate, and β-sitosterylferulate are four major components and account for >95% of γ-oryzanol [22,23]. The γ-oryzanol levels are shown in Figure 5. The γ-oryzanol levels was the lowest (155.1mg/kg rice) in *Daerip*, while the highest level (372.7 mg/kg rice) was in *Keunnun*. Interestingly, there was a significant difference on the proportion of four γ-oryzanol species in subcultivars in this study (Table 3). For example, the proportion of 24-methylene cycloartanyl ferulate in *Segyejinmi*, *Hanareum* and *Dasan 1* were significantly higher than those of cycloartenylferulate ($p < 0.05$), whereas the proportion of cycloartenylferulate in the other cultivars was significantly higher than those of 24-methylene cycloartanylferulate ($p < 0.005$). Miller and Engel [22] reported no difference between long and short grain rice in terms of γ-oryzanol levels, composition, but the environmental condition such as location or season, may have influenced the of γ-oryzanol level.

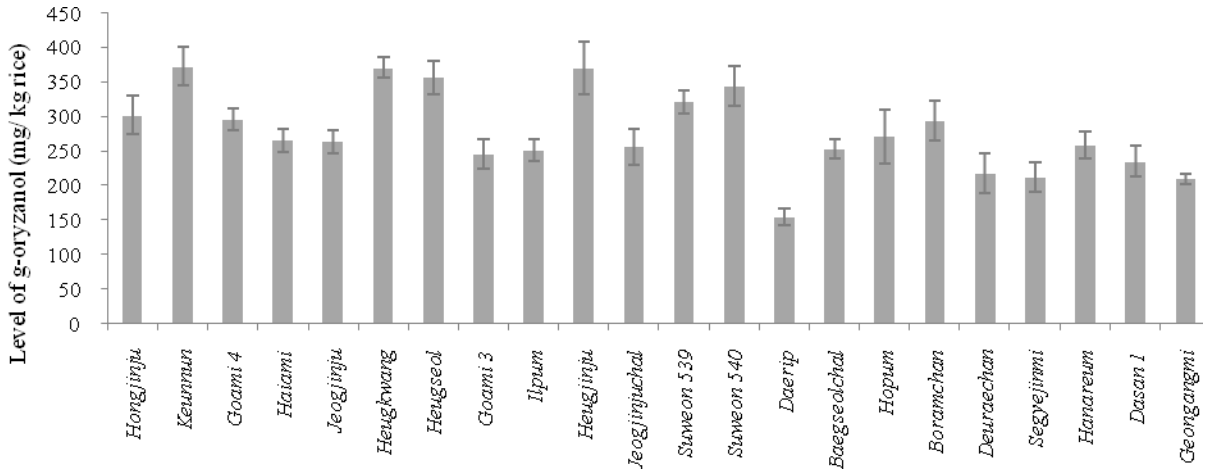

Figure 5. The levels of g-oryzanol in various brown rice cultivars

Table 3. The compositions (w %) of g-oryzanol species in various brown rice cultivars[1)]

Cultivars	γ-Oryzanol species			
	Cycloartenylferulate	24-methylene cycloartanylferulate	campestrylferulate	β-sitosterylferulate
Hongjinju	34.27 ^{ghij}	36.18 ^{abcd}	14.64 ^{abc}	14.91 ^{bc}
Keunnun	33.59 ^{fghij}	43.19 ^{cde}	10.29 ^{a}	12.93 ^{abc}
Goami 4	35.79 ^{i}	37.39 ^{abcd}	12.97 ^{abc}	13.84 ^{abc}
Haiami	30.37 ^{cdef}	42.68 ^{cde}	12.42 ^{abc}	14.53 ^{abc}
Jeogjinju	29.07 ^{cd}	41.60 ^{cd}	15.95 ^{abcd}	13.37 ^{abc}
Heugkwang	39.82 ^{k}	39.74 ^{abcd}	12.01 ^{ab}	8.42 ^{a}
Heugseol	44.15 ^{l}	31.74 ^{ab}	11.86 ^{ab}	12.25^{abc}
Goami 3	32.36 ^{defghi}	37.62 ^{abcd}	14.39 ^{abc}	15.63 ^{bc}
Ilpum	34.68 ^{hij}	35.18 ^{abc}	14.54 ^{abc}	15.59 ^{bc}
Heugjinju	42.05 ^{kl}	30.75 ^{a}	14.10 ^{abc}	13.10 ^{abc}
Jeogjinjuchal	31.31 ^{defg}	37.77 ^{abcd}	15.60 ^{abc}	15.32 ^{bc}
Suweon 539	43.92 ^{l}	30.69 ^{a}	13.32 ^{abc}	12.07 ^{abc}
Suweon 540	32.48 ^{efghi}	45.17 ^{de}	12.00 ^{ab}	10.35 ^{ab}
Daerip	31.81 ^{defgh}	31.78 ^{ab}	18.86 ^{bcd}	17.55 ^{c}
Baegseolchal	29.92 ^{cde}	37.37 ^{abcd}	15.85 ^{abcd}	16.86 ^{c}
Hopum	29.26 ^{cde}	40.16 ^{bcd}	17.19 ^{abcd}	13.38 ^{abc}
Boramchan	35.15 ^{ij}	37.58 ^{abcd}	13.34 ^{abc}	13.94 ^{abc}
Deuraechan	30.28 ^{cde}	39.52 ^{abcd}	13.46 ^{abc}	16.74 ^{c}
Segyejinmi[2)]	13.59 ^{a}	51.14 ^{e}	17.84 ^{bcd}	17.44 ^{c}
Hanareum[2)]	27.61 ^{c}	43.96 ^{cde}	15.06 ^{abc}	13.37 ^{abc}
Dasan 1[2)]	22.87 ^{b}	43.53 ^{cde}	19.54 ^{cd}	14.07 ^{abc}
Geongangmi	29.53 ^{cde}	37.35 ^{abcd}	22.78 ^{d}	10.34 ^{ab}

[1)] Each value represents the average of triplicates.
[2)]These subcultivars are *Indica* type and the others are *Japonica* type.
[a-l)] Means within a column with different superscript letters are significantly different (p<0.05).

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