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Analysis of Phenolic Compounds in White Rice, Brown Rice, and Germinated Brown Rice

SU TIAN,† KOZO NAKAMURA,*,‡ AND HIROSHI KAYAHARA*,‡

Department of Science of Biological Resources, United Graduate School of Agricultural Sciences, Gifu University, Gifu 501-1193, Japan, and Division of Food Functional Analysis, Science of Functional Foods, Graduate School of Agriculture, Shinshu University, Nagano 399-4598, Japan

Two hydroxycinnamate sucrose esters, 6′-*O*-(*E*)-feruloylsucrose and 6′-*O*-(*E*)-sinapoylsucrose, were isolated from methanol extracts of rice bran. Soluble and insoluble phenolic compounds as well as 6′-*O*-(*E*)-feruloylsucrose and 6′-*O*-(*E*)-sinapoylsucrose from white rice, brown rice, and germinated brown rice were analyzed using HPLC. The results demonstrated that the content of insoluble phenolic compounds was significantly higher than that of soluble phenolics in rice, whereas almost all compounds identified in germinated brown rice and brown rice were more abundant than those in white rice. 6′-*O*-(*E*)-Feruloylsucrose (1.09 mg/100 g of flour) and 6′-*O*-(*E*)-sinapoylsucrose (0.41 mg/100 g of flour) were found to be the major soluble phenolic compounds in brown rice. During germination, an ∼70% decrease was observed in the content of the two hydroxycinnamate sucrose esters, whereas free phenolic acid content increased significantly; the ferulic acid content of brown rice (0.32 mg/100 g of flour) increased to 0.48 mg/100 g of flour and became the most abundant phenolic compound in germinated brown rice. The content of sinapinic acid increased to 0.21 mg/100 g of flour, which is nearly 10 times as much as that in brown rice (0.02 mg/100 g of flour). In addition, the total content of insoluble phenolic compounds increased from 18.47 mg/100 g of flour in brown rice to 24.78 mg/100 g of flour in germinated brown rice. These data suggest that appropriate germination of brown rice may be a method to improve health-related benefits.

KEYWORDS: HPLC; *Oryza sativa*; white rice; brown rice; germinated brown rice; phenolic; 6′-*O*-(*E*)-feruloylsucrose; 6′-*O*-(*E*)-sinapoylsucrose

INTRODUCTION

Phenolic compounds are universally distributed in the plant kingdom as secondary metabolic products (*1*). Evidence indicates that phenolic compounds have potent antioxidant properties and free radical scavenging capabilities (*2*). Phenolic compounds are known to exert various physiological effects in humans, such as preventing oxidative damage of lipid and low-density lipoproteins (*3*), inhibiting platelet aggregation (*4*), and reducing the risk of coronary heart disease and cancer (*1*, *5*). Fruits and vegetables are known to be major dietary sources of phenolic compounds, whereas substantial research has demonstrated that cereal consumption is also an excellent way to increase phenolic compound intake (*6*). Cereal grains contain unique free phenolic compounds and their glycosides, which exist in solution, and a significant amount of insoluble phenolic compounds, most of which are bound to polysaccharides in the cell wall (*7*). Both types are important sources of phenolic compounds; however, consumption of these phenolic compounds is currently neglected. The major reason for this is that these compounds are concentrated in the bran layers and are lost with the separation of seed coat during processing. By the same token, most phenolic compounds in rice, which is a major staple cereal all over the world, particularly in Asia, are also lost with rice bran.

Recently, "germinated brown rice", a new cereal diet product, has attracted public attention in Japan. This product is simply brown rice soaked in water until it begins to bud. Germinated brown rice utilizes the physiologically active substances that are present in bran, and the soaking process improves the texture of brown rice (*8*), whereas the nutrients in the seed become easier to digest and absorb during germination (*9*). In rice, ferulic acid and *p*-coumaric acid are the major phenolic compounds and exist in the free form, the soluble conjugate form, or the insoluble bound form, which is found in dietary fiber (*10*).

High-performance liquid chromatography (HPLC) is widely used for the analysis of phenolic compounds. Adom et al. (*11*) determined the free, soluble conjugate, bound ferulic acid content of rice, wheat, corn and oat using HPLC and compared the total content of free and bound phenolic acids using the Folin−Ciocalteu method. Nishizawa et al. (*12*) reported the bound ferulic acid content in well-milled rice, well-milled rice with embryo, and brown rice using alkaline hydrolysis and HPLC analysis. Ayumi et al. (*13*) analyzed and quantified free

* Corresponding author (telephone/fax +81-265-771638; e-mail knakamu@gipmc.shinshu-u.ac.jp).
† Gifu University.
‡ Shinshu University.

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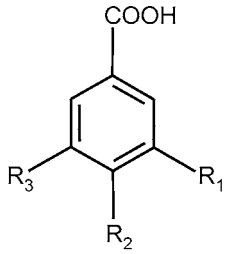
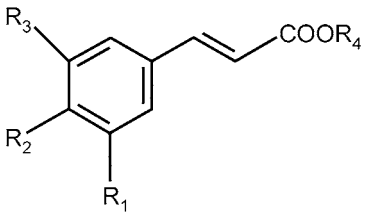


Protocatechuic acid: R_1=H, R_2=OH, R_3=OH
Hydroxybenzoic acid: R_1=H, R_2=OH, R_3=H
Vanillic acid: R_1=H, R_2=OH, R_3=OCH_3
Syringic acid: R_1=OCH_3, R_2=OH, R_3=OCH_3



Caffeic acid: R_1=OH, R_2=OH, R_3=OH, R_4=H
p-Coumaric acid: R_1=H, R_2=OH, R_3=H, R_4=H
Ferulic acid: R_1=OCH_3, R_2=OH, R_3=H, R_4=H
Sinapinic acid: R_1=OCH_3, R_2=OH, R_3=OCH_3, R_4=H
Chlorogenic acid: R_1=OH, R_2=OH, R_3=H, R_4=quinate

Figure 1. Chemical structures of phenolic compounds in rice.

ferulic and *p*-coumaric acid as well as bound ferulic, *p*-coumaric, and 5,5′-diferulic acid contents in 21 japonica cultivars (*Oryza sativa*) of rice bran and polished rice by HPLC with a photodiode array detector. Bunzel et al. (*14*) evaluated several bound hydroxycinnamic acid, hydroxybenzoic acid, and phenolic aldehyde contents in insoluble wild rice fiber. In this study, we investigated the changes in phenolic acid content in brown rice during germination. Soluble and insoluble protocatechuic, hydroxybenzoic, vanillic, syringic, chlorogenic, caffeic, *p*-coumaric, ferulic, and sinapinic acid (**Figure 1**) in white rice, brown rice, and germinated brown rice were analyzed and their contents estimated by HPLC analysis. (All hydroxycinnamates identified in this paper are trans configuration.)

MATERIALS AND METHODS

Chemicals. The phenolic acid standards protocatechuic, hydroxybenzoic, vanillic, syringic, ferulic, *p*-coumaric, caffeic, and sinapinic acid were purchased from Wako Pure Chemical Industries Co., Ltd. (Osaka, Japan). Chlorogenic acid was from Sigma Chemical Co. (St. Louis, MO). 6′-*O*-(*E*)-Feruloylsucrose (**1**) and 6′-*O*-(*E*)-sinapoylsucrose (**2**) were purified in our laboratory, and the method of isolation is described below. Sucrose, D-glucose and D-fructose were obtained from Nacalai Tesque Co., Ltd. (Kyoto, Japan). Acetonitrile and tetrahydrofuran (THF) used in HPLC analysis were of HPLC grade from Nacalai Tesque Co., Ltd. (Kyoto, Japan). Other chemicals and solvents were of analytical grade.

General Procedures. ^{1}H (500 MHz), ^{13}C (125 MHz), and two-dimensional (2D) NMR spectroscopic data were recorded in D_2O using a Bruker DRX500 spectrometer (Bruker BioSpin Crop., Billerica, MA) with the sodium salt of 3-(trimethylsilyl)propionic-*2,2,3,3-d*$_6$ acid as an internal standard. Electrospray ionization mass spectra (ESI-MS) were obtained using an Agilent 1100 series LC/MSD trap system (Agilent Technologies, Palo Alto, CA). IR spectra were measured on an FT/IR-480 plus Fourier transform infrared spectrometer (Jasco Co., Tokyo, Japan) in KBr pellet. UV spectra were determined in methanol using a V-530 UV−vis spectrophotometer (Jasco Co.). HPLC was performed on a Shimadzu HPLC system (Kyoto, Japan) consisting of an SCL-10Avp system controller, two LC-6AD solvent delivery units, a CTO-10Avp column oven, an SPD-M10Avp UV−vis photodiode array detector, and a Multi-PDA class-VP workstation.

Plant Material. Brown rice (*Oryza sativa* L., koshihikari) was obtained from Domer Inc. (Nagano, Japan), and white rice was obtained by polishing the brown rice using a household rice-cleaning mill (Zojirushi Corp., Osaka, Japan). Marketed germinated brown rice (koshihikari), which is produced by Domer Inc., was purchased from a local supermarket in Ina, Japan. Rice bran was obtained from a milling company in Ina, Japan. Germination was carried out in an HP-100 Hatsuga Bijin microcomputer electric germination appliance (Takekoshi Co., Niigata, Japan) according to the following procedure: 400 g of brown rice was cleaned and soaked in 2000 mL of water at 32 °C for 21 h, until a 0.5−1 mm bud developed. The germinated brown rice was lyophilized by using a VD-400F lyophilizer (Taitec Co., Kosigaya, Japan) and was ground with an ultracentrifugal mill. The powder was sieved (355 μm/m; 42 mesh) and then stored at −20 °C until analysis. Brown rice and white rice were also cleaned, lyophilized, powdered, sieved, and stored at −20 °C.

Extraction of Soluble Phenolic Compounds. Soluble phenolic compounds in white rice, brown rice, and germinated brown rice flour (5 g each) were extracted with 70% ethanol (4 × 50 mL, 10 min each) according to the method of Ayumi et al. (*13*). Each extract was pooled and evaporated to ∼10 mL at 30 °C under reduced pressure and was then lyophilized to dryness. Dry samples were dissolved in 2 mL of 15% methanol and subjected to HPLC analysis. All analyses were performed in triplicate.

Extraction of Insoluble Phenolic Compounds. Two grams of white rice, brown rice, and germinated brown rice flour was extracted with hexane (4 × 50 mL) and 70% ethanol (4 × 50 mL), and each extract was discarded to remove fat and soluble phenolic acids, respectively. The residue was hydrolyzed with 1 M sodium hydroxide (NaOH) (2 × 100 mL, 2 h each) at room temperature with stirring under nitrogen gas (*15*). The clear supernatants were pooled and acidified with 4 N HCl to pH 1 and were then extracted four times with ethyl acetate (200 mL each). The ethyl acetate fractions were evaporated to dryness, and the phenolic acids were dissolved with 5 mL of 15% methanol and analyzed by HPLC. All analyses were performed in triplicate.

Isolation of Compounds 1 and 2. Rice bran (820 g) was extracted three times with 16 L of methanol under reflux. The methanol extract was evaporated under reduced pressure at 30 °C. The viscous concentrate (146.1 g) was then successively defatted with *n*-hexane (2.9 L) and diethyl ether (1.5 L). The defatted residue (80.1 g) was suspended in 800 mL of water and partitioned with 2.4 L of ethyl acetate to remove the free phenolic acids. The remaining water solution was dried (65.8 g), and 10 g was subjected to chromatography on a 300 mm × 45 mm i.d. lipophilic Sephadex LH-20 (Sigma Chemical Co., St. Louis, MO) column with either purified water or 5, 10, 20, or 100% methanol (400 mL each). The 5 and 10% methanol fractions (2.1 g) were further subjected to preparative HPLC on a 250 mm × 20 mm i.d., 5 μm, Cosmosil 5C_{18}-AR-300, C_{18}-ODS column (Waters, Milford, MA) with 10% tetrahydrofuran (THF) at a flow rate of 7 mL/min, giving crude substances **1** and **2**, which were then repeatedly purified by semipreparative HPLC on a 250 mm × 10 mm i.d., 5 μm, Cosmosil 5C_{18}-AR-300, C_{18}-ODS column with 12% acetonitrile (t_R **1** = 14 min, t_R **2** = 16 min) and 6% THF (t_R **1** = 24 min, t_R **2** = 16 min) at a flow rate of 3.0 mL/min to afford **1** (16.3 mg) and **2** (5.4 mg), respectively.

6′-O-(E)-Feruloylsucrose (1): amorphous powder; UV (MeOH) λ_{max} (log ϵ) 325 (4.43), λ_{min} (log ϵ) 264 (3.96); IR (KBr) 3377, 1695, 1632, 1596, 1517 cm^{-1}; HRFAB-MS, [M]$^-$ 517.1557 for $C_{22}H_{29}O_{14}$, calcd, 517.1548; ESI-MS, *m/z* 541, 177; ^{1}H NMR, ^{13}C NMR, and HMBC, shown in **Table 1**.

6′-O-(E)-Sinapoylsucrose (2): amorphous powder; UV (MeOH) λ_{max} (log ϵ) 327 (4.22), λ_{min} (log ϵ) 266 (3.64); IR (KBr) 3377, 1699, 1633, 1556, 1517 cm^{-1}; HRFAB-MS, [M]$^-$ 547.1663 for $C_{23}H_{31}O_{15}$, calcd, 547.1653; ESI-MS, *m/z* 571, 369, 207; ^{1}H NMR, ^{13}C NMR, and HMBC, shown in **Table 1**.

Chemical Analysis of 1 and 2. Acid and alkaline hydrolyses of compounds **1** and **2** were performed according to the method described by Miyase (*16*). Compounds **1** and **2** were hydrolyzed with 2 N HCl in boiling water for 45 min, and the sugar moiety was identified by comparison with authentic samples using TLC (silica gel 60 F_{254}, E.

Table 1. ^{1}H, ^{13}C, and HMBC NMR Spectroscopic Data for Compounds **1** and **2** (in D_2O)

	compound **1**		compound **2**		HMBC (C–H) of compounds **1** and **2**
	δ_H[a] [*J* (Hz)]	δ_C[a]	δ_H[a] [*J* (Hz)]	δ_C[a]	
	feruloyl moiety		sinapoyl moiety		
1		129.6		128.6	H-5 (**1**); H-6 (**2**), H-8
2	7.20 s	114.2	6.96 s	109.0	H-7, H-6
3		150.6		150.6	H-2, H–OMe
4		151.0		139.9	H-5 (**1**); H-2/H-6 (**2**)
5	6.92 d (8.2)	118.5		150.6	H-2/H-6, H–OMe (**2**)
6	7.14 d (8.2)	126.3	6.96 s	109.0	H-7, H-2
7	7.61 d (15.9)	149.3	7.62 d (15.9)	149.4	H-6 (**1**); H-2/H-6 (**2**)
8	6.38 d (15.9)	116.9	6.44 d (15.9)	117.4	H-7
C=O		172.0		171.9	H-8, H-7, Glu-6′a, Glu-6′b
OMe	3.89 s	58.7	3.88 s	59.1	OMe
	sucrose moiety				
Glu-1′	5.43 d (3.5)	94.8	5.44 d (3.6)	94.8	
2′	3.62 dd (3.6, 6.3)	73.8	3.62 dd (3.8, 6.2)	73.8	
3′	3.81[b]	75.3	3.81[b]	75.3	Glu-1′, Glu-2′, Glu-4′
4′	3.49 t (9.6)	72.7	3.50 t (9.6)	72.8	
5′	4.15 m	73.4	4.16 m	73.4	Glu-1′, Glu-3′, Glu-4′
6′a	4.38 dd (5.8, 6.4)	66.5	4.39 dd (5.9, 6.3)	66.6	Glu-4′
6′b	4.54 d (12.0)		4.55 d (12.0)		Fru-3″
Fru-1″	3.68 d (2.4)	64.4	3.67 d (2.3)	64.4	
2″		106.5		106.5	Glu-1′, Fru-1″
3″	4.23 d (8.7)	79.3	4.22 d (8.7)	79.3	Fru-1″, Fru-4″
4″	4.06 t (8.5)	77.2	4.06 t (8.6)	77.2	Fru-3″
5″	3.91[b]	84.2	3.90[b]	84.2	Fru-3″
6″a	3.80[b]	65.7	3.80[b]	65.7	Fru-4″
6″b	3.82[b]		3.82[b]		

[a] δ_H and δ_C, ^{1}H and ^{13}C NMR chemical shifts, respectively. [b] Overlapped.

Merck, Darmstadt, Germany) in ethyl acetate/2-propanol/acetone/purified water (20:10:7:6) (*17*). Alkaline hydrolysis was performed in 2 M NaOH for 2 h at room temperature under nitrogen gas. The reaction mixture was acidified with HCl and extracted with ethyl acetate. Phenolic acids were analyzed by HPLC.

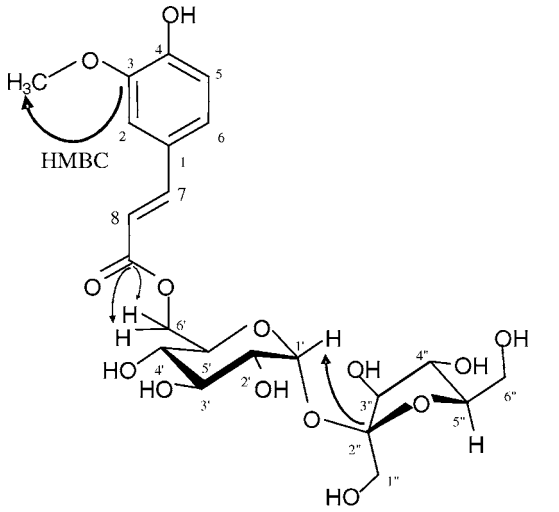
HPLC Analysis. Phenolic compounds in white rice, brown rice, and germinated brown rice were estimated by reversed-phase HPLC. All samples were filtered though a 0.45-μm pore size syringe-driven filter before injection. A 20-μL aliquot of sample solution was separated using a Shimadzu HPLC system equipped with a diode array detector on a 150 mm × 4.6 mm i.d., 5 μm, Cosmosil 5C_{18}-MS-II, C_{18}-ODS analytical column (Waters). The mobile phase consisted of acetonitrile (B) and purified water with 0.1% trifluoroacetic acid (TFA) at a flow rate of 0.8 mL/min. Gradient elution was performed as follows: from 0 to 5 min, linear gradient from 5 to 9% solvent B; from 5 to 15 min, 9% solvent B; from 15 to 22 min, linear gradient from 9 to 11% solvent B; and from 22 to 35 min, linear gradient from 11 to 18% solvent B. Column temperature was set at 40 °C. Hydroxybenzoic acid compounds were detected at a wavelength of 280 nm and hydroxycinnamic acid compounds at 325 nm. Phenolic compounds in the samples were identified by comparing their relative retention times and UV spectra with authentic compounds and were detected using an external standard method.

RESULTS AND DISCUSSION

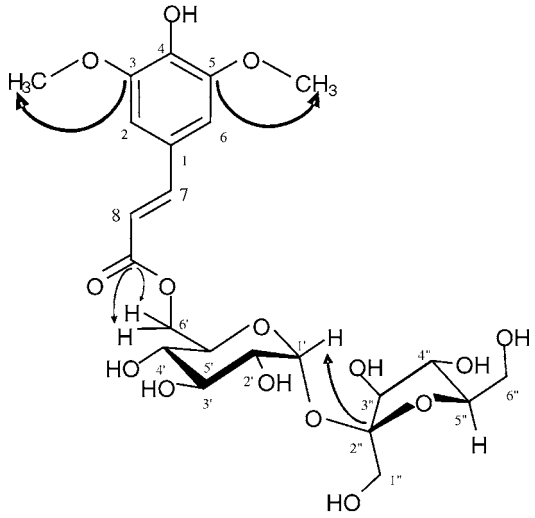
Identification of 6′-*O*-(*E*)-Feruloylsucrose (1) and 6′-*O*-(*E*)-Sinapoylsucrose (2). The hydroxycinnamate sucrose esters **1** and **2** were isolated from the methanol extract of rice (*O. sativa* L.) bran. On alkaline hydrolysis, **1** gave ferulic acid on the basis of HPLC analysis, whereas on acid hydrolysis, it gave D-glucose (R_f = 0.29) and D-fructose (R_f = 0.86) on the basis of TLC analysis. The cationic ESI-MS spectrum showed an $[M + Na]^+$ peak at *m*/*z* 541 and fragment ions at *m*/*z* 177 $[M - 2\text{hexose} + H]^+$. The UV absorption of **1** correlated closely with ferulic acid. The IR (KBr) spectrum revealed hydroxyl groups (3377 cm^{-1}), unsaturated carbonyl (C=O, 1695 cm^{-1}; C=C, 1632 cm^{-1}), and aromatic absorptions (1516 cm^{-1}). All of the above data suggested that **1** was composed of ferulic acid and D-glucose and D-fructose in a 1:1 ratio. ^{1}H, ^{13}C, and 2D NMR spectral data, summarized in **Table 1**, also displayed characteristic signals for the feruloyl group and sucrose moiety, which were identified by comparison with reported data (*18*) and experimental data for authentic sucrose. All proton and carbon signals of **1** were assigned from its ^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY spectra. On the ^{1}H NMR spectrum, the feruloyl moiety was determined to be in the *E* configuration on the basis of the large coupled doublets (J = 15.96 Hz) of H-7 (δ_H 7.61) and H-8 (δ_H 6.38). The deshielding shifts of H-6′ (δ_H 4.54 and 4.38) and H-5′ (δ_H 4.15) of the glucosyl residue confirmed that the feruloyl group was adjacent to the glucosyl-6′-OH of sucrose. The attachment position was determined from the correlation between the carbonyl carbon signal (δ_C 172.0) and glucosyl H-6′ (δ_H 4.38, 4.54) of sucrose in the HMBC spectrum (**Figure 2**). On the basis of these data, **1** was confirmed to be 6′-*O*-(*E*)-feruloylsucrose. This chemical structure is primarily consistent with the literature (*19*).

Alkaline and acid hydrolysis of **2** gave sinapinic acid, D-glucose, D-fructose. The cationic ESI-MS spectrum showed three major peaks at *m*/*z* 571 $[M + Na]^+$, *m*/*z* 369 $[M - \text{hexose} + H]^+$, and *m*/*z* 207 $[M - 2\text{hexose} + H]^+$, 30 mass units higher than that of **1**. The UV spectrum exhibited absorptions at λ_{max} = 327 nm (log ϵ = 4.22) and λ_{min} = 266 nm (log ϵ = 3.64). The spectroscopic data from ^{1}H, ^{13}C, and 2D NMR are shown in **Table 1**. The ^{1}H NMR spectrum was similar to that of **1** but showed the presence of an (*E*)-sinapoyl residue. HMBC revealed a correlation between the glucosyl H-6′ of sucrose and an ester carbonyl carbon at δ_C 172.0 (**Figure 2**). Thus, **2** was confirmed to be 6′-*O*-(*E*)-sinapoylsucrose.

Soluble Phenolic Content of White Rice, Brown Rice, and Germinated Brown Rice. Typical HPLC chromatogram traces of standards and soluble phenolic compounds in white rice, brown rice, and germinated brown rice are shown in **Figure 3**. The contents of soluble phenolic compounds in white rice,



Compound **1**



Compound **2**

Figure 2. Structures of compounds **1** and **2**, isolated from rice bran.

brown rice, and germinated brown rice are shown in **Table 2**. Our results showed that soluble phenolic compounds contained free phenolic acids and hydroxycinnamate sucrose esters. Feruloylsucrose and sinapoylsucrose were found to be the major soluble phenolic compounds in brown rice, along with ferulic acid (**Figure 3C**). During germination, an ~70% decrease was observed in feruloylsucrose (from 1.09 to 0.27 mg/100 g of flour) and sinapoylsucrose (from 0.41 to 0.13 mg/100 g of flour) contents, whereas free ferulic acid content increased, becoming the most abundant phenolic compound (0.48 mg/100 g of flour) (**Figure 3B**). Moreover, the content of sinapinic acid in germinated brown rice increased to 0.21 mg/100 g of flour, which is nearly 10 times as much as that in brown rice (0.02 mg/100 g of flour). Levels of other soluble phenolic compounds identified were generally low. With regard to total content of soluble phenolic compounds, brown rice (2.17 mg/100 g of flour) and germinated brown rice (1.45 mg/100 g of flour) were found to have significantly higher levels than white rice (0.28 mg/100 g of flour), and this result was consistent with previous studies (*20*). However, germinated brown rice contained slightly lower amounts of soluble phenolic compounds than brown rice.

Recent studies have indicated that hydroxycinnamates had strong antioxidant activity, but the antioxidant effects of

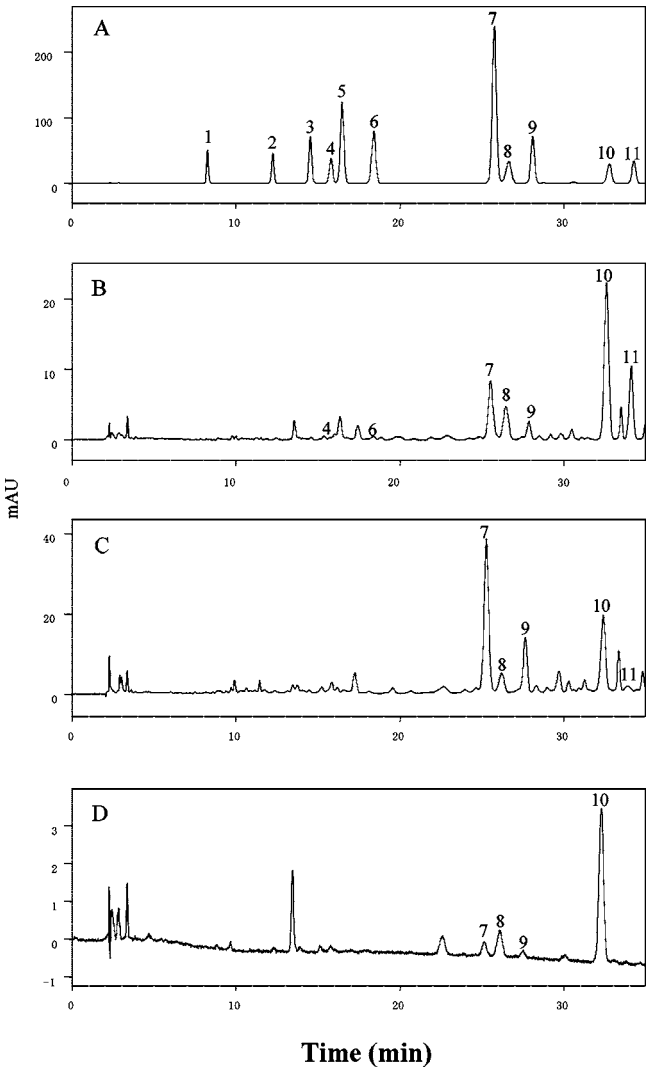


Figure 3. Typical HPLC chromatograms of (**A**) soluble and insoluble phenolic compounds in (**B**) germinated brown rice, (**C**) brown rice, and (**D**) white rice. Peaks: 1, protocatechuic acid; 2, hydroxybenzoic acid; 3, chlorogenic acid; 4, vanillic acid; 5, caffeic acid; 6, syringic acid; 7, 6′-*O*-feruloylsucrose; 8, *p*-coumaric acid; 9, 6′-*O*-sinapoylsucrose; 10, ferulic acid; 11, sinapinic acid. Detection is at 325 nm.

Table 2. Soluble Phenolic Acid Content[a] of Germinated Brown Rice, Brown Rice, and White Rice (Milligrams per 100 g of Flour)

	white rice	brown rice	germinated brown rice
protocatechuic acid	0.01 ± 0.00	0.04 ± 0.00	0.05 ± 0.00
hydroxybenzoic acid	0.02 ± 0.00	0.04 ± 0.00	0.01 ± 0.00
vanillic acid	0.03 ± 0.00	0.07 ± 0.00	0.06 ± 0.00
syringic acid	0.01 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
chlorogenic acid	0.03 ± 0.00	0.03 ± 0.00	0.04 ± 0.00
caffeic acid	0.02 ± 0.00	0.02 ± 0.00	0.05 ± 0.00
p-coumaric acid	0.02 ± 0.00	0.10 ± 0.00	0.12 ± 0.00
ferulic acid	0.07 ± 0.01	0.32 ± 0.01	0.48 ± 0.01
sinapinic acid	0.01 ± 0.00	0.02 ± 0.00	0.21 ± 0.01
feruloylsucrose	0.03 ± 0.00	1.09 ± 0.01	0.27 ± 0.01
sinapoylsucrose	0.03 ± 0.00	0.41 ± 0.01	0.13 ± 0.00
total	0.28	2.17	1.45

[a] Mean value ± SD ($n = 3$).

hydroxycinnamates depend on their available absorption in the gut (*21*). Zhao et al. (*22*) reported that the form of hydroxycinnamates in the diet has an effect on their absorption. Free

Table 3. Insoluble Phenolic Acid Content[a] of Germinated Brown Rice, Brown Rice, and White Rice (Milligrams per 100 g of Flour)

	white rice	brown rice	germinated brown rice
protocatechuic acid	0.17 ± 0.00	0.17 ± 0.00	0.19 ± 0.01
hydroxybenzoic acid	nd[b]	0.16 ± 0.01	0.28 ± 0.02
vanillic acid	nd[b]	0.17 ± 0.01	0.20 ± 0.01
syringic acid	nd[b]	0.14 ± 0.00	0.16 ± 0.00
chlorogenic acid	nd[b]	nd[b]	nd[b]
caffeic acid	nd[b]	0.22 ± 0.00	0.22 ± 0.00
p-coumaric acid	0.34 ± 0.01	2.10 ± 0.08	3.05 ± 0.02
ferulic acid	5.26 ± 0.14	15.19 ± 0.52	20.04 ± 0.77
sinapinic acid	nd[b]	0.32 ± 0.01	0.64 ± 0.01
feruloylsucrose	nd[b]	nd[b]	nd[b]
sinapoylsucrose	nd[b]	nd[b]	nd[b]
total	5.77	18.47	24.78

[a] Mean value ± SD ($n = 3$). [b] Not detectable.

Table 4. Soluble Phenolic Acid and Insoluble Phenolic Acid Content[a] of Marketed Germinated Brown Rice (Milligrams per 100 g of Flour)

	free	bound
protocatechuic acid	0.03 ± 0.00	nd[b]
hydroxybenzoic acid	0.12 ± 0.00	0.26 ± 0.01
vanillic acid	0.06 ± 0.00	0.18 ± 0.01
syringic acid	0.05 ± 0.00	0.15 ± 0.00
chlorogenic acid	0.03 ± 0.00	nd[b]
caffeic acid	0.05 ± 0.00	0.20 ± 0.00
p-coumaric acid	0.34 ± 0.01	3.00 ± 0.00
ferulic acid	0.28 ± 0.01	16.39 ± 0.65
sinapinic acid	0.11 ± 0.00	0.77 ± 0.04
feruloylsucrose	0.22 ± 0.01	nd[b]
sinapoylsucrose	0.10 ± 0.00	nd[b]
total	1.39	20.95

[a] Mean value ± SD ($n = 3$). [b] Not detectable.

ferulic acid was absorbed almost completely, whereas one of the conjugated phenolic compounds with a relatively simple structure present in cereal grains, feruloylarabinose, showed lower bioavailability in rat than free ferulic acid. The increase in free phenolic compound content in germinated brown rice suggests that it has a higher potential bioavailability and thus a higher antioxidant potential.

Insoluble Phenolic Content of White Rice, Brown Rice, and Germinated Brown Rice. The content of insoluble phenolic compounds, which are bound to polysaccharides in the cell wall, of white rice, brown rice, and germinated brown rice is shown in **Table 3**. The content of insoluble phenolic compounds in rice was found to be significantly higher than that of soluble phenolics, and that in germinated brown rice and brown rice was substantially higher than that in white rice. This could be because phenolic compounds are mostly located in the bran layer of rice grains (*13*). The most abundant insoluble phenolic compound in white rice, brown rice, and germinated brown rice was ferulic acid, followed by *p*-coumaric acid, and the content of other bound phenolic compounds was lower. This result is in agreement with the literature (*13*, *14*). In germinated brown rice, a 1−2-fold increase in all insoluble phenolic compounds was observed when compared with brown rice; the total content of insoluble phenolic compounds in germinated brown rice was 24.78 mg/100 g of flour, whereas that of brown rice was 18.47 mg/100 g of flour. Insoluble phenolic compounds in rice have been found to be cell wall components (*23*). In the cell wall, phenolic compounds, particularly hydroxycinnamates, are ester linked to insoluble fiber, polysaccharides, and lignin components (*24*). The increase in phenolic compounds in germinated brown rice could be explained as an increase in the free forms with alkaline hydrolysis, due to dismantling of the cell wall during germination. The increase in insoluble phenolic compounds may be intended to increase the availability of hydrolyzable insoluble phenolic compounds during the germination of brown rice. Ferguson et al. (*25*) reported that hydroxycinnamates from the cell wall had antimutagenic properties and that this physiological activity could have significance in the preventative activity of dietary fiber against cancer. Therefore, the increases in insoluble but hydrolyzable phenolic compounds during germination suggest that germinated brown rice can potentially reduce mutations and improve human health.

Soluble Phenolic and Insoluble Phenolic Compound Content of Marketed Germinated Brown Rice. The content of soluble phenolic and insoluble phenolic compounds of marketed germinated brown rice is shown in **Table 4**. Insoluble bound ferulic acid (16.39 mg/100 g of flour) was a major phenolic acid, along with *p*-coumaric acid (3.00 mg/100 g of flour) and sinapinic acid (0.77 mg/100 g of flour). With regard to soluble phenolic acids, the contents of *p*-coumaric acid (0.34 mg/100 g of flour), ferulic acid (0.28 mg/100 g of flour), feruloylsucrose, hydroxybenzoic acid, sinapinic acid, and sinapoylsucrose were relatively high when compared with the other phenolic acids. These results are consistent with the germinated brown rice prepared in our laboratory.

Some research has suggested that germination may bring about changes in nutrients and physiologically active substances. During the germination of wheat (*26*) and *Pangium edule* Reinw. (*27*), vitamin C, vitamin E, ferulic acid, and total phenolic acid contents increased. Upon malting of finger millet, changes in both free and bound phenolic acid contents were observed (*28*), and these reflected their antioxidant properties. During the germination of rice seeds, alanine and γ-aminobutyrate have been reported to increase significantly (*29*, *30*). However, none of these reports have investigated the changes in the existing forms and content of phenolic compounds during the germination. Phenolic compounds, particularly hydroxycinnamates, are found in significant quantities in their insoluble bound forms in rice grains. Ferulic acid in rice may be ester linked to sterol as part of γ-oryzanol in rice bran oil (*31*) but may also be bound to insoluble fiber forming the cell walls (*26*). However, there are few occurrences of hydroxycinnamate sucrose ester in rice. In this study, two hydroxycinnamate sucrose esters, 6′-*O*-(*E*)-feruloylsucrose and 6′-*O*-(*E*)-sinapoylsucrose, were isolated and identified from the methanol extract of rice bran, and their content was estimated in white rice, brown rice, and germinated brown rice. Our results clearly showed significant increases in free ferulic, *p*-coumaric, and sinapinic acid and insoluble but hydrolyzable phenolic compounds, together with decreases in the two hydroxycinnamate sucrose esters in germinated brown rice. On the basis of these results, it appears that the two hydroxycinnamate sucrose esters in brown rice are important intermediate products of phenolic metabolism. We speculate that, during germination, as seed moisture increases, the seed coat has the potential for injury by oxidation and/or microorganism infiltration. Induced saccharolytic enzymes to hydrolyze starch would produce free phenolic compounds having more effective antioxidant activity from hydroxycinnamate sucrose esters. As a result, the content of hydroxycinnamate sucrose esters decreases, whereas that of free phenolic compounds increases. This hypothesis requires verification through further experiments; however, the changes in content and form of phenolic compounds in germinated brown rice suggest that

appropriate germination of brown rice may be a method to improve health-related benefits.

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