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Quantification of Tocopherols, Tocotrienols, and γ-Oryzanol Contents and Their Distribution in Some Commercial Rice Varieties in Taiwan

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ABSTRACT: The eight vitamin E isomers [α-, β-, γ-, and δ-tocopherols (T) and α-, β-, γ-, and δ-tocotrienols (T3)] and γ-oryzanol are known to possess diverse biological activities. This study examined the contents of these compounds and their distribution in 16 commercial rice varieties in Taiwan. Results showed that the order of vitamin E, total T, total T3, and γ-oryzanol contents was rice bran > brown rice > rice husk > polished rice. γ-T3 was the highest vitamin E isomer present in all rice samples, while β-T, β-T3, δ-T, and δ-T3 were present in trace amounts. The *Japonica* varieties contained a higher total T, total T3, and γ-oryzanol than the *Indica* varieties. They also have a higher level of α-T and α-T3 but a lower level of γ-T and γ-T3 than the *Indica* varieties. However, no obvious difference in total T, total T3, and γ-oryzanol content was noted between black- and red-colored rice varieties.

KEYWORDS: *Oryza sativa*, vitamin E, tocopherols, tocotrienols, γ-oryzanol

INTRODUCTION

Rice (*Oryza sativa* L.) is an important agricultural commodity and the main staple food in many Asian countries. Although brown rice has long been considered as an excellent source of energy and nutrients, rice is consumed mostly in its polished form. Studies have shown that rice bran contains a complex mixture of unique biologically active phytochemicals such as tocopherols (T), tocotrienols (T3), γ-oryzanol, and others.[1] These phytochemicals are believed to exert important roles in protecting against degenerative diseases.[2] Among the many phytochemicals in rice, γ-oryzanol and vitamin E including the four analogues (α, β, γ, and δ) of T and T3 have received the most research attention.

γ-Oryzanol is present abundantly in rice and exists mainly in rice bran layers. It has been shown to reduce serum cholesterol[3] and possess potent antioxidant properties.[1,4] Both T and T3 are regarded as the most important natural antioxidants; however, T3 was shown to have a greater biopotency and certain bioactivities that are not found in T.[5] With growing interests in the functional products worldwide, some specialty rice cultivars, either pigmented or nonpigmented rice, have been commercially cultivated in Taiwan. Studies have reported that consumption of brown rice and pigmented rice provided many health benefits, such as preventing heart diseases, diabetes, atherosclerosis, etc.[6,7] However, detailed information on the contents of these bioactive phytochemicals in the commercial rice varieties remains limited. In this study, our aim was to examine the contents and distribution of T, T3, and γ-oryzanol in different grain parts of 16 commercially available rice varieties in Taiwan.

MATERIALS AND METHODS

Materials. Tocopherols (α-, β-, γ-, and δ-T), γ-oryzanol, 2,2,5,7,8-pentamethyl-6-chromanol (PMC; used as an internal standard), and butylated hydroxytoluene (BHT) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Hexane and isopropanol of HPLC grade were purchased from Merck (Darmstad, Germany). Tocotrienols (α-, β-, γ-, and δ-T3) were obtained from Davos Life Science Pte. Ltd. (Helios, Singapore). All other chemicals used were of analytical grade.

Rice Samples. Sixteen different nonpigmented (n = 12) and pigmented (n = 4) genotypes (*Oryza sativa* L.) belonging to either *Indica* or *Japonica* subspecies were collected from various Agricultural Research Stations in Taiwan (i.e., Hualian, Taoyuan, and Tainan) during the same period of the year. They were chosen to represent the pigmented and nonpigmented commercially available rice varieties in Taiwan.

Standard Stock Solution. All vitamin E analogs (α-, β-, γ-, and δ-T and α-, β-, γ-, and δ-T3) and γ-oryzanol were prepared to a concentration of approximately 500 mg/mL in methanol. The stock solutions were stored at −20 °C until use. The combined working solution was prepared by pooling suitable amounts of each standard compound (T, T3, and γ-oryzanol) and diluting with hexane to obtain the required concentrations ranging from 1 to 100 ng per injection.

Preparation of Rice Sample Extract. Grain of each of the rice varieties was individually separated into rice husk, rice bran, brown rice, and polished rice. It was threshed to separate the rice husk and brown rice by an automatic rice husker (Kett TR200, Tokyo, Japan). The brown rice samples were further polished by a grain polisher (Kett PEARLEST, Tokyo, Japan). The different grain parts were then ground into fine powder by a pulverizing machine (RT-02B, Rong Tsong Iron Co., Taichung, Taiwan).

All samples were subjected to extraction procedures as described by Jang and Xu.[8] Briefly, 0.5 g of each rice sample was taken and transferred into a test tube (25 × 150 mm) to which 3 mL of hexane and 0.1 mL of BHT (10 mg/mL) were added, and the mixture was vortexed for 30 s. The test tubes were capped and then placed in a 60 °C water bath undergoing extraction for 20 min with shaking. The hexane layer in each test tube was separated by centrifugation at 2000*g* for 15 min. The supernatant was collected, while the residue was further subjected to similar procedures of extraction twice. The supernatants collected from three separate extractions were combined, filtered, and diluted to a volume of 10 mL. Twenty microliters of the filtrate was taken for HPLC analysis.

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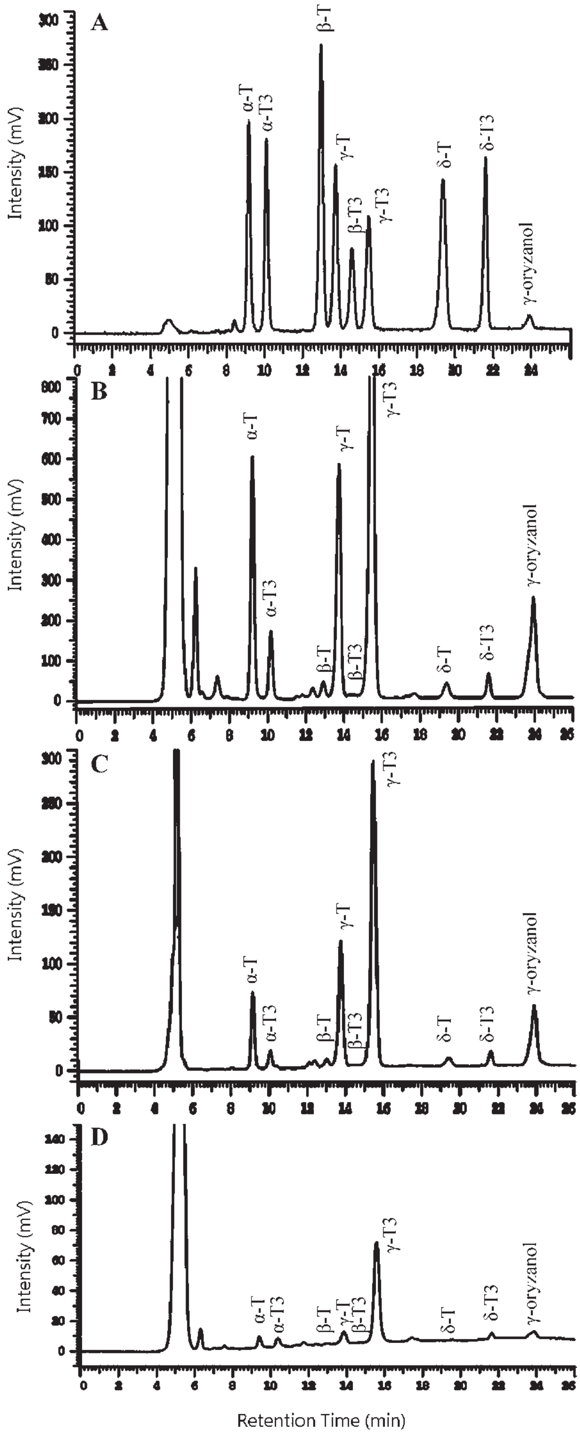

Figure 1. Chromatograms of a standard mixture and different grain parts. (A) Reference standards; (B) rice bran; (C) brown rice; and (D) polished rice. α-T, α-tocopherol; β-T, β-tocopherol; γ-T, γ- tocopherol; δ-T, δ-tocopherol; α-T3, α-tocotrienol; β-T3, β-tocotrienol; γ-T3, γ- tocotrienol; δ-T3, δ-tocotrienol; and γ-oryzanol.

Analysis of Tocopherols, Tocotrienols, and γ-Oryzanol. The analysis was carried out by a method described previously.[9] Briefly, the HPLC system consisted of a Hitachi L-2130 pump and a Hitachi L-2485 fluorescence detector (Hitachi, Japan) set at an excitation wavelength of 290 nm and an emission wavelength of 330 nm. Chromatographic separation was performed by a normal phase Inertsil SIL 100A (5 μM, 4.6 × 250 mm) column coupled with an Inertsil SIL 100A (5 μM, 4 × 10 mm) guard column (GL Sciences Inc., Tokyo, Japan) and a mobile phase composed of hexane/isopropanol/ethylacetae/acetic acid (97.6:0.8:0.8:0.8, v/v/v/v) operating at room temperature. The flow rate of isocratic elution varied from 0.7 to 1.5 mL/min. All compounds were confirmed by chromatographic comparisons with their respective authentic standards. T, T3, and γ-oryzanol in rice samples were identified by retention time and quantified by the calibration curve of external standards.

RESULTS

Contents of Tocopherols, Tocotrienols, and γ-Oryzanol. A typical chromatogram of eight vitamin E analogs (α-, β-, γ-, and δ-T and α-, β-, γ-, and δ-T3) and γ-oryzanol in standard solution and rice samples is shown in Figure 1. The contents of different isomers of T and T3 in the selected 16 rice varieties are individually presented in Tables 1−4, and their contents of total T, total T3, and γ-oryzanol in different grain parts are reported in Tables 5−8.

Results showed that the total vitamin E (T + T3) content varied from 0.41 mg/kg (in the rice husk of Tainung-Shien 22) to 314.96 mg/kg (in the rice bran of Tainung 71). The total T content varied from 0.18 mg/kg (in the rice husk of Taichung-Shien 10) to 126.00 mg/kg (in the rice bran of Kaohsiung 139), while the total T3 content ranged from 0.15 mg/kg (in the rice husk of Tainung-Shien 22) to 191.44 mg/kg (in the rice bran of Tainung 71). All rice varieties contained a significant amount of γ-oryzanol whose values varied from 32.64 mg/kg (in the polished rice of Kaohsiung 139) to 3133.14 mg/kg (in the rice bran of Tainan 11). The major forms of vitamin E present in rice samples were γ-T3 and α-T, while the contents of δ-T and β-T3 were the lowest.

Variation between Cultivars. The cultivars with the highest total vitamin E content in rice bran were Tainung 71 (314.96 mg/kg), followed by Kaohsiung 139 (289.27 mg/kg) and Taoyuan 3 (286.28 mg/kg), while the cultivar Taichung-Shien 10 (181.23 mg/kg) had the lowest (Table 5). For total T3 content, the rice bran of Tainung 71 (191.44 mg/kg) showed the highest content, followed by Taoyuan 3 (178.13 mg/kg) and Long Glutinous (171.38 mg/kg). The cultivar with the highest total T content was in the rice bran of Kaoshiung 139 (126.00 mg/kg), followed by Tainung 71 (123.52 mg/kg) and Taoyuan 3 (108.15 mg/kg). The highest γ-oryzanol content was in the rice bran of Tainan 11 (3133.14 mg/kg), followed by Kaohsiung 139 (2723.15 mg/kg) and Taoyuan 3 (2631.84 mg/kg).

Among the rice varieties analyzed, the order of T3 isomer content was γ-T3 > α-T3 > δ-T3 > β-T3, and the order of T isomer content was α-T > γ-T > β-T > δ-T (Tables 1−4). The cultivar having the highest γ-T3 content was in the rice bran of Long Glutinous (151.09 mg/kg) and the lowest was Kaohsiung 139 polished rice (0.52 mg/kg). The rice bran of Tainung 71 (67.01 mg/kg) showed the highest α-T3 content, while Kaohsiung 139 polished rice had the lowest amount (0.13 mg/kg). The cultivars with the highest α-T and γ-T were in the rice bran of Tainung 71 (107.73 mg/kg) and Taichung-Shien 22 (45.93 mg/kg), respectively.

It is interesting to note that certain rice varieties possessed a significant amount of δ-T3 in rice bran, e.g., Basmati 385 and Taichung-Shien 10. The *Japonica* varieties showed a higher total T, total T3, and γ-oryzanol content than the *Indica* varieties (Tables 5−8). Furthermore, they showed a higher level of α-T

Table 1. Contents of Eight Vitamin E Isomers in Rice Bran of Different Rice Cultivars[a]

	Nonpigmented Rice							
	tocopherols (mg/kg)				tocotrienols (mg/kg)			
type of cultivars	α	β	γ	δ	α	β	γ	δ
				Japonica				
Taikeng 4	85.28 ± 2.81	1.33 ± 0.07	8.97 ± 0.54	0.30 ± 0.01	60.09 ± 0.75	0.48 ± 0.01	104.22 ± 3.75	1.93 ± 0.23
Kaohsiung 139	104.51 ± 3.29	3.27 ± 0.25	14.74 ± 0.77	3.48 ± 0.85	57.75 ± 4.25	0.60 ± 0.04	103.22 ± 8.41	1.69 ± 0.41
Taikeng 14	56.37 ± 1.90	2.11 ± 0.05	7.25 ± 0.10	2.47 ± 0.11	38.04 ± 3.18	5.74 ± 0.47	68.52 ± 1.39	1.34 ± 0.03
Taoyuan 3	97.34 ± 2.39	2.78 ± 0.08	7.68 ± 1.11	0.35 ± 0.02	64.19 ± 1.84	2.31 ± 1.45	109.88 ± 2.28	1.75 ± 0.08
Tainan 11	87.66 ± 2.59	2.50 ± 0.12	11.29 ± 1.24	1.59 ± 0.86	54.55 ± 2.44	0.62 ± 0.23	97.32 ± 7.16	2.16 ± 0.42
Tainung 71	107.73 ± 1.06	3.26 ± 0.06	10.99 ± 0.92	1.53 ± 0.76	67.01 ± 2.91	2.80 ± 1.70	118.98 ± 3.99	2.66 ± 0.11
average	89.82 ± 17.0	2.54 ± 0.68	10.15 ± 2.55	1.62 ± 1.12	56.94 ± 9.38	2.09 ± 1.86	100.36 ± 15.7	1.92 ± 0.41
				Indica				
Tainung-Shien 22	28.16 ± 3.11	0.92 ± 0.20	45.93 ± 5.12	1.76 ± 0.52	8.44 ± 1.32	1.14 ± 1.71	132.80 ± 10.8	2.26 ± 0.18
OS4	65.27 ± 10.2	2.56 ± 0.44	20.63 ± 3.21	2.57 ± 0.54	52.40 ± 5.74	0.25 ± 0.10	106.35 ± 10.9	2.79 ± 0.33
Jasmine 85	33.48 ± 1.51	2.70 ± 0.07	6.09 ± 0.22	0.26 ± 0.04	16.19 ± 1.27	N.D.	132.11 ± 11.0	1.67 ± 0.13
Basmati 385	68.38 ± 4.04	2.31 ± 0.15	22.04 ± 1.37	1.30 ± 0.50	38.95 ± 1.40	0.34 ± 0.04	99.64 ± 6.58	3.53 ± 0.22
Taichung-Shien 10	27.66 ± 0.93	1.61 ± 0.69	37.95 ± 1.54	2.72 ± 1.02	9.42 ± 1.33	0.35 ± 0.16	98.26 ± 4.22	3.26 ± 0.49
Long Glutinous	34.95 ± 0.54	1.27 ± 0.15	37.68 ± 1.05	1.71 ± 0.03	18.30 ± 0.62	0.24 ± 0.05	151.09 ± 2.31	1.75 ± 0.10
average	42.98 ± 17.1	1.90 ± 0.67	28.39 ± 13.4	1.72 ± 0.82	23.95 ± 16.2	0.46 ± 0.34	120.04 ± 19.8	2.54 ± 0.71

	Pigmented Rice							
	tocopherols (mg/kg)				tocotrienols (mg/kg)			
type of cultivars	α	β	γ	δ	α	β	γ	δ
				Black-Colored				
Ch'o-Tzu	62.10 ± 1.41	2.68 ± 0.07	10.55 ± 0.38	1.10 ± 0.50	43.56 ± 0.14	0.73 ± 0.03	97.79 ± 1.65	2.16 ± 0.16
Kuroo-Mochi	35.40 ± 1.85	2.43 ± 0.75	39.22 ± 0.45	3.27 ± 0.93	12.52 ± 3.39	0.48 ± 0.84	130.64 ± 8.70	1.68 ± 0.33
average	48.75 ± 13.4	2.56 ± 0.13	24.89 ± 14.3	2.19 ± 1.09	28.04 ± 15.5	0.61 ± 0.13	114.22 ± 16.4	1.92 ± 0.24
				Red-Colored				
Hung-No	54.56 ± 3.97	2.24 ± 0.16	15.51 ± 0.55	1.34 ± 0.13	38.92 ± 3.66	N.D.	75.98 ± 3.64	1.45 ± 0.85
Ai-Chueh-Shien-Hung-No	23.27 ± 0.40	0.83 ± 0.06	33.38 ± 0.46	1.67 ± 0.68	11.80 ± 0.13	0.06 ± 0.10	120.26 ± 3.19	1.78 ± 0.07
average	38.92 ± 15.7	1.54 ± 0.71	24.45 ± 8.94	1.51 ± 0.17	25.36 ± 13.6	0.06 ± 0.10	98.12 ± 22.1	1.62 ± 0.17

[a] Values are the mean ± SD of three analyses (n = 3).

and α-T3 but a lower level of γ-T and γ-T3 than the *Indica* varieties (Tables 1−4). Besides the cultivar Hung-No, which contained much higher γ-oryzanol content than other pigmented rice varieties, there was no obvious difference between these cultivars in total T, total T3, and γ-oryzanol content.

Variation between Grain Parts. Results showed that the total vitamin E content varied from 181.23−314.96 mg/kg in rice bran, 18.18−62.31 mg/kg in brown rice, 0.41−33.67 mg/kg in rice husk, and 1.06−24.60 mg/kg in polished rice (Tables 5−8). Regardless of the rice variety, the order of total T3 content was rice bran (111.29−191.44 mg/kg) > brown rice (11.94−50.59 mg/kg) > polished rice (0.85−21.62 mg/kg) > rice husk (0.15−18.13 mg/kg). The highest total T content was in rice bran (42.53−126.00 mg/kg), followed by brown rice (4.62−29.23 mg/kg), rice husk (0.18−15.54 mg/kg), and polished rice (0.21−5.34 mg/kg). In rice bran, the average total T3 content was 1.85 times higher than that of the total T content.

The concentrations of γ-T3, the main T3 isomer in the rice grain, were 68.52−151.09 mg/kg in rice bran, 8.60−46.95 mg/kg in brown rice, 0.03−11.59 mg/kg in rice husk, and 0.52−24.84 mg/kg in polished rice (Tables 1−4). For T, α-T was the predominant form whose concentrations were 23.27−107.73 mg/kg in rice bran, 2.80−18.99 mg/kg in brown rice, 0.06−10.77 mg/kg in rice husk, and 0.03−3.79 mg/kg in polished rice. δ-T, α-T3, and β-T3 were noted to be present in trace amounts in rice husk, whereas β-T, δ-T, and β-T3 were not detected in polished rice of certain varieties.

The order of γ-oryzanol present in the different grain parts was rice bran (1811.81−3133.14 mg/kg) > brown rice (269.53−1727.44 mg/kg) > rice husk (38.18−653.01 mg/kg) > polished rice (32.64−625.97 mg/kg) (Tables 5−8). The highest γ-oryzanol content was observed in the bran fraction, while the lowest was in polished rice.

DISCUSSION

The present study has demonstrated that regardless of the rice varieties, the rice bran contained the highest levels of T, T3, and

Table 2. Contents of Eight Vitamin E Isomers in Brown Rice of Different Rice Cultivars[a]

	Nonpigmented Rice							
	tocopherols (mg/kg)				tocotrienols (mg/kg)			
type of cultivars	α	β	γ	δ	α	β	γ	δ
Japonica								
Taikeng 4	10.06 ± 1.59	0.89 ± 0.06	1.54 ± 0.31	0.03 ± 0.01	6.81 ± 0.85	N.D.	11.05 ± 7.85	0.44 ± 0.12
Kaohsiung 139	4.07 ± 0.57	0.33 ± 0.03	0.78 ± 0.30	1.59 ± 1.33	2.57 ± 1.05	0.24 ± 0.34	8.60 ± 0.65	0.77 ± 0.65
Taikeng 14	7.41 ± 3.35	0.58 ± 0.21	2.93 ± 1.31	0.50 ± 0.38	5.84 ± 3.43	0.50 ± 0.58	15.99 ± 6.85	0.52 ± 0.06
Taoyuan 3	5.40 ± 1.57	0.29 ± 0.19	1.19 ± 0.13	0.10 ± 0.07	3.90 ± 0.69	0.48 ± 0.47	9.78 ± 2.27	0.44 ± 0.11
Tainan 11	5.17 ± 0.63	0.42 ± 0.26	1.68 ± 0.27	0.16 ± 0.10	3.30 ± 0.90	N.D.	9.65 ± 0.46	0.46 ± 0.10
Tainung 71	9.11 ± 2.70	0.78 ± 0.38	1.07 ± 0.31	0.08 ± 0.09	5.66 ± 1.59	1.22 ± 0.29	16.31 ± 3.74	0.58 ± 0.13
average	6.87 ± 2.17	0.55 ± 0.22	1.53 ± 0.69	0.41 ± 0.55	4.68 ± 4.52	0.61 ± 0.37	11.9 ± 3.09	0.54 ± 0.12
Indica								
Tainung-Shien 22	4.58 ± 0.43	0.28 ± 0.03	7.72 ± 0.80	0.83 ± 0.53	2.22 ± 0.49	0.03 ± 0.03	31.55 ± 2.65	0.81 ± 0.00
OS4	7.39 ± 0.87	0.53 ± 0.08	3.16 ± 0.24	0.79 ± 0.06	6.39 ± 0.71	N.D.	14.71 ± 1.43	0.75 ± 0.06
Jasmine 85	3.29 ± 0.25	0.08 ± 0.14	1.09 ± 0.06	0.16 ± 0.14	1.85 ± 0.56	0.26 ± 0.13	24.09 ± 1.94	0.57 ± 0.07
Basmati 385	9.99 ± 0.60	0.62 ± 0.39	4.36 ± 0.34	0.56 ± 0.29	6.05 ± 0.51	0.04 ± 0.04	18.83 ± 1.12	0.90 ± 0.09
Taichung-Shien 10	2.80 ± 0.43	0.29 ± 0.15	4.90 ± 1.02	0.63 ± 0.38	1.12 ± 0.14	0.04 ± 0.04	26.73 ± 4.03	1.28 ± 0.06
Long Glutinous	4.25 ± 0.33	0.30 ± 0.05	6.33 ± 0.41	0.87 ± 0.45	1.74 ± 0.19	N.D.	24.32 ± 0.61	0.50 ± 0.06
average	5.38 ± 2.52	0.35 ± 0.18	4.59 ± 2.13	0.64 ± 0.24	3.23 ± 2.14	0.09 ± 0.10	23.37 ± 5.40	0.80 ± 0.25

	Pigmented Rice							
	tocopherols (mg/kg)				tocotrienols (mg/kg)			
type of cultivars	α	β	γ	δ	α	β	γ	δ
Black-Colored								
Ch'o-Tzu	12.57 ± 0.37	0.88 ± 0.08	1.88 ± 1.35	0.35 ± 0.32	6.12 ± 0.21	0.11 ± 0.01	17.51 ± 1.22	0.76 ± 0.14
Kuroo-Mochi	4.53 ± 0.43	0.67 ± 0.07	8.91 ± 0.57	0.92 ± 0.54	2.03 ± 0.36	N.D.	31.98 ± 4.80	0.65 ± 0.04
average	8.55 ± 4.02	0.78 ± 0.11	5.40 ± 3.52	0.64 ± 0.29	4.08 ± 2.05	0.11 ± 0.01	24.75 ± 7.24	0.71 ± 0.06
Red-Colored								
Hung-No	18.99 ± 0.57	1.06 ± 0.06	6.81 ± 0.37	2.38 ± 0.33	11.41 ± 0.76	N.D.	19.71 ± 0.17	1.51 ± 0.11
Ai-Chueh-Shien-Hung-No	4.32 ± 0.42	0.34 ± 0.04	6.89 ± 0.20	0.17 ± 0.00	3.07 ± 0.13	N.D.	46.95 ± 1.87	0.58 ± 0.07
average	11.66 ± 7.34	0.70 ± 0.36	6.85 ± 0.04	1.28 ± 1.11	7.24 ± 4.17	N.D.	33.33 ± 13.6	1.05 ± 0.47

[a] Values are the mean ± SD of three analyses (n = 3).

γ-oryzanol, followed by brown rice and rice husk. The polished rice contained the lowest amount of all these phytochemicals. Among the vitamin E components, γ-T3 was the highest vitamin E isomer present in all rice varieties, while β-T, β-T3, δ-T, and δ-T3 were present in trace amounts. Results demonstrated that the *Japonica* varieties possessed a higher total T, total T3, and γ-oryzanol than the *Indica* varieties. They also showed a higher level of α-T and α-T3, but a lower level of γ-T and γ-T3 than the *Indica* varieties. In the pigmented rice, Hung-No was found to contain much higher γ-oryzanol than other species. Interestingly, certain rice varieties in Taiwan were noted to possess a significant amount of δ-T3, e.g., Basmati 385 and Taichung-Shein 10.

Consistent with findings of Heinemann et al.,[10] the average content of total vitamin E in the *Japonica* varieties was higher than that in the *Indica* varieties. Aguilar-Garcia et al.[11] reported that the level of total vitamin E content in rice bran of three Venezuelan rice cultivars was 196−219 mg/kg, whereas the contents in brown rice of 32 cultivars in Brazil was 10.4−32.5 mg/kg.[10] Studies showed that the γ-oryzanol content in rice bran of different rice varieties in the USA was 3400−3900 mg/kg,[12] in rice bran of five long-grained varieties in Thailand was 560−1080 mg/kg,[13] in brown rice of 32 cultivars in Brazil was 130−280 mg/kg,[10] and those in Canada were 1500−2700 mg/kg.[11] In this study, rice bran of selected commercial rice varieties in Taiwan appeared to have a higher total vitamin E but not γ-oryzanol content than those reported by others. The discrepancies in the contents of these compounds between different studies could be due to the differences in genotypes and growing environmental conditions of the analyzed rice samples.[14] Furthermore, the lack of a standardized method for the extraction and analysis of these phytochemicals may have also led to a wide variation in the data reported. In certain studies, the presence of γ-T3, the predominant form of vitamin E isomer in rice, was omitted by some authors.[13,15] It has been reported that RP-HPLC is unable to resolve β and γ isomers of T and T3,[8,12,15] whereas some normal phase HPLC methods failed to separate γ-T and β-T3;[16,17] this poor separation may have led to a misinterpretation in the identification of the different vitamin E isomers.

Table 3. Contents of Eight Vitamin E Isomers in Rice Husk of Different Rice Cultivars[a]

	Nonpigmented Rice							
	tocopherols (mg/kg)				tocotrienols (mg/kg)			
type of cultivars	α	β	γ	δ	α	β	γ	δ
Japonica								
Taikeng 4	2.13 ± 0.04	0.12 ± 0.05	0.41 ± 0.09	0.04 ± 0.00	0.04 ± 0.05	0.57 ± 0.02	1.75 ± 0.24	0.04 ± 0.03
Kaohsiung 139	0.32 ± 0.24	0.04 ± 0.01	0.15 ± 0.09	0.08 ± 0.07	0.08 ± 0.27	0.25 ± 0.07	0.40 ± 0.19	0.06 ± 0.02
Taikeng 14	0.73 ± 0.04	0.07 ± 0.04	0.10 ± 0.07	0.06 ± 0.01	0.06 ± 0.00	0.30 ± 0.31	0.33 ± 0.34	0.06 ± 0.01
Taoyuan 3	1.72 ± 0.10	0.01 ± 0.01	0.31 ± 0.18	0.06 ± 0.02	0.06 ± 0.02	0.06 ± 0.06	1.02 ± 0.08	0.08 ± 0.04
Tainan 11	1.31 ± 0.04	0.12 ± 0.03	0.94 ± 0.04	0.17 ± 0.08	0.17 ± 0.00	N.D.	0.28 ± 0.07	0.02 ± 0.00
Tainung 71	0.72 ± 0.05	0.41 ± 0.08	0.07 ± 0.03	0.08 ± 0.00	0.08 ± 0.22	0.07 ± 0.02	0.57 ± 0.20	0.07 ± 0.06
average	1.16 ± 0.63	0.13 ± 0.13	0.33 ± 0.30	0.08 ± 0.04	0.08 ± 0.04	0.25 ± 0.19	0.73 ± 0.52	0.06 ± 0.02
Indica								
Tainung-Shien 22	0.06 ± 0.03	0.12 ± 0.05	0.05 ± 0.01	0.03 ± 0.03	0.03 ± 0.06	N.D.	0.03 ± 0.03	0.02 ± 0.02
OS4	0.20 ± 0.17	0.01 ± 0.01	0.05 ± 0.03	0.05 ± 0.01	0.05 ± 0.06	N.D.	0.08 ± 0.04	0.04 ± 0.02
Jasmine 85	0.08 ± 0.01	0.20 ± 0.03	0.09 ± 0.02	0.07 ± 0.05	0.07 ± 0.02	0.12 ± 0.05	0.32 ± 0.10	0.05 ± 0.02
Basmati 385	0.16 ± 0.08	0.03 ± 0.02	0.12 ± 0.07	0.06 ± 0.03	0.06 ± 0.02	0.10 ± 0.06	0.11 ± 0.06	0.05 ± 0.02
Taichung-Shien 10	0.07 ± 0.06	0.02 ± 0.02	0.04 ± 0.03	0.06 ± 0.02	0.06 ± 0.01	0.06 ± 0.05	0.11 ± 0.10	0.05 ± 0.03
Long Glutinous	0.13 ± 0.02	0.14 ± 0.08	0.06 ± 0.07	0.07 ± 0.05	0.07 ± 0.22	0.09 ± 0.04	0.08 ± 0.14	0.02 ± 0.01
average	0.12 ± 0.05	0.09 ± 0.07	0.07 ± 0.03	0.06 ± 0.01	0.06 ± 0.01	0.09 ± 0.02	0.12 ± 0.09	0.04 ± 0.01

	Pigmented Rice							
	tocopherols (mg/kg)				tocotrienols (mg/kg)			
type of cultivars	α	β	γ	δ	α	β	γ	δ
Black-Colored								
Ch'o-Tzu	0.24 ± 0.03	0.03 ± 0.01	0.08 ± 0.04	0.11 ± 0.08	0.11 ± 0.11	0.14 ± 0.05	0.12 ± 0.02	0.05 ± 0.01
Kuroo-Mochi	0.25 ± 0.08	0.06 ± 0.03	0.34 ± 0.05	0.24 ± 0.12	0.24 ± 0.07	0.09 ± 0.11	0.43 ± 0.34	0.13 ± 0.08
average	0.25 ± 0.01	0.05 ± 0.02	0.21 ± 0.13	0.18 ± 0.07	0.18 ± 0.07	0.12 ± 0.03	0.28 ± 0.16	0.09 ± 0.04
Red-Colored								
Hung-No	10.77 ± 1.28	0.33 ± 0.33	3.49 ± 0.61	0.95 ± 0.54	0.95 ± 0.77	N.D.	11.59 ± 0.78	0.48 ± 0.11
Ai-Chueh-Shien-Hung-No	2.75 ± 1.28	0.27 ± 0.10	2.64 ± 0.20	0.70 ± 0.39	0.70 ± 0.23	N.D.	8.33 ± 2.91	0.36 ± 0.08
average	6.76 ± 4.01	0.30 ± 0.03	3.07 ± 0.42	0.83 ± 0.13	0.83 ± 0.13	N.D.	9.96 ± 1.63	0.42 ± 0.06

[a] Values are the mean ± SD of three analyses (n = 3).

In this study, the average concentration of total vitamin E in rice bran (232.72 mg/kg) was 5.5 times higher than that in brown rice (42.61 mg/kg), and more than 20 times higher than that in rice husk (9.91 mg/kg) and polished rice (11.94 mg/kg). On the basis of the results from over 250 rice samples in Japan, rice bran was reported to contain the highest amount of total T and T3 contents (352−1408 mg/kg).[18] In a Yu et al.[19] study, the bran fraction was divided into rice bran and germ, whereas in our study, the results of rice bran included the germ. The level of vitamin E in rice germ was reported to be five times greater than that in rice bran. These results indicated that lipophilic compounds are more concentrated in regions closer to the bran fraction than to the endosperm, especially the germ part,[8,20] suggesting that the lipid fractions are mainly distributed in the outer layers of rice grains. Consistently, γ-oryzanol was found significantly higher in the outer bran layer than in other grain parts.[21] This indicates that γ-oryzanol, similar to T and T3, is mainly concentrated in regions closer to the pericarp than to the endosperm.

Among the T3 isomers, the highest α-T3 (67.01 mg/kg) and γ-T3 (151.09 mg/kg) levels were detected in the bran fraction of Tainung 71 and Long Glutinous cultivars, respectively. According to Yu et al.,[19] rice bran and germ have significantly different profiles of vitamin E isomers. The major vitamin E component in rice germ was α-T and in rice bran was γ-T3. Consistently, our findings showed that γ-T3 was the prevalent form of the vitamin E isomer in rice, which accounts for an average of 51.18% of the total vitamin E content, followed by α-T (21.13%), α-T3 (12.87%), and γ-T (11.89%). In this study, no definite trend was discernible between colored and noncolored rice varieties in T and T3 contents. However, studies showed that black rice varieties were rich in anthocyanin and polyphenolic compounds,[22,23] and a significant positive correlation was noted between their extracts and antioxidant activity. This suggests that in addition to T, T3, and γ-oryzanol, polyphenolic compounds may also play an important role in the antioxidant properties of colored rice varieties.

Table 4. Contents of Eight Vitamin E Isomers in Polished Rice of Different Rice Cultivars[a]

	Nonpigmented Rice							
	tocopherols (mg/kg)				tocotrienols (mg/kg)			
type of cultivars	α	β	γ	δ	α	β	γ	δ
				Japonica				
Taikeng 4	0.40 ± 0.09	0.03 ± 0.02	0.10 ± 0.07	0.04 ± 0.02	0.54 ± 0.04	0.07 ± 0.03	2.28 ± 0.22	0.20 ± 0.05
Kaohsiung 139	0.03 ± 0.01	0.03 ± 0.01	0.06 ± 0.05	0.08 ± 0.02	0.13 ± 0.04	0.10 ± 0.02	0.52 ± 0.17	0.11 ± 0.09
Taikeng 14	1.24 ± 0.10	0.18 ± 0.10	0.60 ± 0.29	0.05 ± 0.05	1.11 ± 0.41	0.12 ± 0.11	3.28 ± 0.52	0.25 ± 0.10
Taoyuan 3	0.33 ± 0.07	0.02 ± 0.01	0.05 ± 0.02	0.03 ± 0.00	0.41 ± 0.08	0.04 ± 0.04	1.60 ± 0.18	0.14 ± 0.01
Tainan 11	0.24 ± 0.07	0.10 ± 0.07	0.09 ± 0.03	0.08 ± 0.02	0.24 ± 0.04	0.14 ± 0.01	1.03 ± 0.04	0.18 ± 0.05
Tainung 71	0.72 ± 0.07	0.13 ± 0.09	0.36 ± 0.13	0.08 ± 0.02	0.57 ± 0.07	0.24 ± 0.15	2.86 ± 0.13	0.22 ± 0.17
average	0.49 ± 0.39	0.08 ± 0.06	0.21 ± 0.02	0.06 ± 0.02	0.50 ± 0.31	0.12 ± 0.06	1.93 ± 0.98	0.18 ± 0.05
				Indica				
Tainung-Shien 22	0.29 ± 0.15	0.07 ± 0.06	0.87 ± 0.28	0.04 ± 0.04	0.53 ± 0.21	0.16 ± 0.11	11.17 ± 2.64	0.56 ± 0.22
OS4	0.50 ± 0.01	0.03 ± 0.01	0.37 ± 0.06	0.13 ± 0.13	1.38 ± 0.08	0.15 ± 0.03	4.47 ± 0.24	0.34 ± 0.05
Jasmine 85	0.80 ± 0.08	0.03 ± 0.00	0.10 ± 0.08	N.D.	0.98 ± 0.09	0.16 ± 0.02	17.13 ± 3.89	0.27 ± 0.18
Basmati 385	0.92 ± 0.21	0.04 ± 0.04	4.95 ± 6.01	0.04 ± 0.05	1.57 ± 0.37	0.43 ± 0.16	9.27 ± 4.96	0.53 ± 0.07
Taichung-Shien 10	0.45 ± 0.24	0.02 ± 0.03	0.63 ± 0.34	0.03 ± 0.03	0.70 ± 0.30	0.12 ± 0.13	12.90 ± 0.48	0.78 ± 0.16
Long Glutinous	0.39 ± 0.11	0.07 ± 0.08	0.61 ± 0.53	0.06 ± 0.02	0.43 ± 0.20	0.10 ± 0.13	9.28 ± 8.11	0.27 ± 0.05
average	0.56 ± 0.23	0.04 ± 0.02	1.26 ± 1.67	0.06 ± 0.04	0.93 ± 0.42	0.19 ± 0.11	10.70 ± 3.86	0.46 ± 0.18

	Pigmented Rice							
	tocopherols (mg/kg)				tocotrienols (mg/kg)			
type of cultivars	α	β	γ	δ	α	β	γ	δ
				Black-Colored				
Ch'o-Tzu	0.33 ± 0.02	0.15 ± 0.01	0.42 ± 0.11	0.08 ± 0.02	0.68 ± 0.07	0.20 ± 0.10	3.97 ± 0.17	0.35 ± 0.02
Kuroo-Mochi	0.94 ± 0.63	0.26 ± 0.32	1.66 ± 0.59	0.12 ± 0.13	1.44 ± 1.37	0.12 ± 0.13	19.01 ± 6.62	1.06 ± 0.55
average	0.64 ± 0.31	0.21 ± 0.06	1.04 ± 0.62	0.10 ± 0.02	1.06 ± 0.38	0.16 ± 0.04	11.49 ± 7.52	0.71 ± 0.36
				Red-Colored				
Hung-No	3.78 ± 0.52	0.13 ± 0.13	1.38 ± 0.24	0.05 ± 0.09	4.14 ± 0.28	N.D.	12.55 ± 5.54	0.40 ± 0.14
Ai-Chueh-Shien-Hung-No	1.34 ± 0.60	N.D.	2.04 ± 0.73	N.D.	1.62 ± 1.19	N.D.	24.82 ± 4.33	0.28 ± 0.02
average	2.56 ± 1.22	0.20 ± 0.07	1.71 ± 0.33	0.38 ± 0.33	2.88 ± 1.26	N.D.	18.68 ± 6.14	0.34 ± 0.06

[a] Values are the mean ± SD of three analyses (n = 3).

Table 5. Total Vitamin E (Vit E), Total Tocopherols (T), Total Tocotrienols (T3), and γ-Oryzanol in Rice Bran of Different Rice Cultivars[a]

	Nonpigmented Rice			
	concentration (mg/kg)			
type of cultivars	total vit E	total T	total T3	γ-oryzanol
		Japonica		
Taikeng 4	262.61 ± 3.01	95.89 ± 2.13	166.72 ± 3.79	2022.62 ± 150.08
Kaohsiung 139	289.27 ± 12.76	126.00 ± 2.98	163.27 ± 9.98	2723.15 ± 81.59
Taikeng 14	181.84 ± 4.91	68.20 ± 1.59	113.64 ± 3.32	2232.32 ± 77.67
Taoyuan 3	286.28 ± 2.35	108.15 ± 2.92	178.13 ± 1.47	2631.84 ± 117.58
Tainan 11	257.70 ± 8.74	103.05 ± 2.91	154.65 ± 6.70	3133.14 ± 16.53
Tainung 71	314.96 ± 5.07	123.52 ± 0.91	191.44 ± 4.17	2212.72 ± 146.68
		Indica		
Tainung-Shien 22	221.42 ± 15.48	76.77 ± 6.39	144.65 ± 9.68	1811.87 ± 96.40
OS4	252.84 ± 25.13	91.03 ± 11.24	161.80 ± 13.91	2193.52 ± 143.07
Jasmine 85	192.49 ± 9.39	42.53 ± 1.45	149.97 ± 10.10	1824.86 ± 42.92

Table 5. Continued

Nonpigmented Rice	concentration (mg/kg)			
type of cultivars	total vit E	total T	total T3	γ-oryzanol
Basmati 385	236.51 ± 3.99	94.04 ± 4.45	142.47 ± 6.71	2206.97 ± 159.45
Taichung-Shien 10	181.23 ± 7.14	69.94 ± 2.55	111.29 ± 4.74	1728.36 ± 79.46
Long Glutinous	246.99 ± 3.81	75.60 ± 1.44	171.38 ± 2.37	1915.61 ± 99.34

Pigmented Rice	concentration (mg/kg)			
type of cultivars	total vit E	total T	total T3	γ-oryzanol
		Black-Colored		
Ch'o-Tzu	220.68 ± 2.23	76.44 ± 0.97	144.24 ± 1.45	2636.39 ± 144.08
Kuroo-Mochi	225.65 ± 10.03	80.33 ± 2.36	145.33 ± 9.33	2071.44 ± 86.93
		Red-Colored		
Hung-No	190.01 ± 2.35	73.65 ± 3.73	116.35 ± 5.31	2663.79 ± 49.73
Ai-Chueh-Shien-Hung-No	193.05 ± 3.53	59.15 ± 0.92	133.90 ± 2.76	1844.91 ± 25.27

[a] Values are the mean ± SD of three analyses (n = 3).

Table 6. Total Vitamin E (Vit E), Total Tocopherols (T), Total Tocotrienols (T3), and γ-Oryzanol in Brown Rice of Different Rice Cultivars[a]

Nonpigmented Rice	concentration (mg/kg)			
type of cultivars	total vit E	total T	total T3	γ-oryzanol
		Japonica		
Taikeng 4	30.82 ± 4.22	12.52 ± 1.48	18.30 ± 5.62	593.32 ± 97.47
Kaohsiung 139	18.18 ± 1.31	6.24 ± 0.72	11.94 ± 0.60	492.78 ± 92.32
Taikeng 14	34.27 ± 11.83	11.42 ± 3.66	22.85 ± 8.17	360.49 ± 29.27
Taoyuan 3	21.57 ± 3.63	6.98 ± 1.47	14.59 ± 2.16	656.12 ± 36.74
Tainan 11	20.85 ± 1.72	7.43 ± 1.01	13.43 ± 0.72	788.59 ± 99.84
Tainung 71	34.81 ± 6.97	11.03 ± 2.62	23.77 ± 4.52	494.18 ± 46.46
		Indica		
Tainung-Shien 22	48.02 ± 3.50	13.41 ± 1.22	34.62 ± 2.53	700.07 ± 90.66
OS4	33.72 ± 2.58	11.86 ± 0.88	21.86 ± 1.70	583.40 ± 4.16
Jasmine 85	31.39 ± 1.80	4.62 ± 0.19	26.77 ± 1.63	608.58 ± 70.13
Basmati 385	41.36 ± 2.53	15.53 ± 1.25	25.83 ± 1.33	676.43 ± 66.95
Taichung-Shien 10	37.79 ± 2.33	8.61 ± 1.57	29.18 ± 3.23	656.48 ± 18.41
Long Glutinous	38.32 ± 1.24	11.75 ± 0.97	26.56 ± 0.50	574.38 ± 72.18

Pigmented Rice	concentration (mg/kg)			
type of cultivars	total vit E	total T	total T3	γ-oryzanol
		Black-Colored		
Ch'o-Tzu	40.18 ± 1.34	15.68 ± 1.31	24.50 ± 0.81	794.45 ± 54.83
Kuroo-Mochi	49.72 ± 4.16	15.02 ± 0.74	34.70 ± 4.11	885.52 ± 34.04
		Red-Colored		
Hung-No	61.86 ± 1.69	29.23 ± 1.01	32.63 ± 0.70	1727.44 ± 28.68
Ai-Chueh-Shien-Hung-No	62.31 ± 2.03	11.72 ± 0.47	50.59 ± 1.66	269.53 ± 8.42

[a] Values are the mean ± SD of three analyses (n = 3).

Table 7. Total Vitamin E (Vit E), Total Tocopherols (T), Total Tocotrienols (T3), and γ-Oryzanol in Rice Husk of Different Rice Cultivars[a]

	Nonpigmented Rice concentration (mg/kg)			
type of cultivars	total vit E	total T	total T3	γ-oryzanol
		Japonica		
Taikeng 4	5.50 ± 0.54	2.69 ± 0.08	2.81 ± 0.46	134.96 ± 12.17
Kaohsiung 139	1.80 ± 0.56	0.60 ± 0.32	1.20 ± 0.29	139.79 ± 4.57
Taikeng 14	1.65 ± 0.29	0.96 ± 0.03	0.69 ± 0.27	137.89 ± 7.56
Taoyuan 3	3.67 ± 0.24	2.09 ± 0.16	1.58 ± 0.11	158.71 ± 16.40
Tainan 11	2.85 ± 0.06	2.55 ± 0.02	0.30 ± 0.05	72.82 ± 0.83
Tainung 71	2.30 ± 0.29	1.28 ± 0.08	1.02 ± 0.24	135.56 ± 8.32
		Indica		
Tainung-Shien 22	0.41 ± 0.16	0.26 ± 0.09	0.15 ± 0.11	53.97 ± 20.65
OS4	0.53 ± 0.12	0.30 ± 0.17	0.23 ± 0.12	56.56 ± 9.98
Jasmine 85	1.08 ± 0.07	0.44 ± 0.03	0.64 ± 0.08	90.77 ± 2.94
Basmati 385	0.64 ± 0.09	0.36 ± 0.14	0.28 ± 0.07	55.06 ± 13.26
Taichung-Shien 10	0.51 ± 0.02	0.18 ± 0.02	0.33 ± 0.01	88.95 ± 5.15
Long Glutinous	0.84 ± 0.12	0.40 ± 0.07	0.45 ± 0.06	87.60 ± 12.06
	Pigmented Rice concentration (mg/kg)			
type of cultivars	total vit E	total T	total T3	γ-oryzanol
		Black-Colored		
Ch'o-Tzu	0.88 ± 0.23	0.46 ± 0.10	0.42 ± 0.12	38.18 ± 7.84
Kuroo-Mochi	1.74 ± 0.37	0.88 ± 0.16	0.86 ± 0.28	182.05 ± 21.52
		Red-Colored		
Hung-No	33.67 ± 3.46	15.54 ± 2.17	18.13 ± 1.32	653.01 ± 125.53
Ai-Chueh-Shien-Hung-No	16.13 ± 2.20	6.37 ± 1.42	9.76 ± 2.24	446.48 ± 34.42

[a] Values are the mean ± SD of three analyses (n = 3).

Table 8. Total Vitamin E (Vit E), Total Tocopherols (T), Total Tocotrienols (T3), and γ-Oryzanol in Polished Rice of Different Rice Cultivars[a]

	Nonpigmented Rice concentration (mg/kg)			
type of cultivars	total vit E	total T	total T3	γ-oryzanol
		Japonica		
Taikeng 4	3.67 ± 0.21	0.58 ± 0.07	3.09 ± 0.23	95.60 ± 7.44
Kaohsiung 139	1.06 ± 0.24	0.21 ± 0.05	0.85 ± 0.25	32.64 ± 18.11
Taikeng 14	6.83 ± 0.55	2.07 ± 0.29	4.76 ± 0.26	81.69 ± 7.10
Taoyuan 3	2.62 ± 0.29	0.43 ± 0.08	2.19 ± 0.21	83.62 ± 4.93
Tainan 11	2.11 ± 0.04	0.51 ± 0.04	1.60 ± 0.02	75.06 ± 3.83
Tainung 71	5.19 ± 0.32	1.29 ± 0.23	3.89 ± 0.14	71.94 ± 4.24
		Indica		
Tainung-Shien 22	13.69 ± 2.90	1.27 ± 0.37	12.42 ± 2.53	85.67 ± 9.99
OS4	7.36 ± 0.36	1.02 ± 0.11	6.34 ± 0.31	79.74 ± 2.23
Jasmine 85	19.46 ± 2.66	0.93 ± 0.11	18.54 ± 2.54	279.24 ± 203.35
Basmati 385	8.24 ± 1.18	1.45 ± 0.11	6.79 ± 1.07	521.35 ± 14.51
Taichung-Shien 10	15.63 ± 1.23	1.13 ± 0.51	14.49 ± 0.73	517.68 ± 136.88
Long Glutinous	11.20 ± 7.34	1.13 ± 0.56	10.07 ± 6.85	76.33 ± 17.21

Table 8. Continued

	Pigmented Rice			
	concentration (mg/kg)			
type of cultivars	total vit E	total T	total T3	γ-oryzanol
		Black-Colored		
Ch'o-Tzu	6.19 ± 0.33	0.99 ± 0.11	5.20 ± 0.26	123.51 ± 3.49
Kuroo-Mochi	24.60 ± 8.06	2.97 ± 1.21	21.62 ± 6.94	625.97 ± 57.98
		Red-Colored		
Hung-No	22.43 ± 5.29	5.34 ± 0.76	17.09 ± 4.55	564.87 ± 25.53
Ai-Chueh-Shien-Hung-No	21.99 ± 15.99	2.64 ± 1.99	19.35 ± 14.01	69.11 ± 7.84

[a] Values are the mean ± SD of three analyses (n = 3).

Among the 16 rice varieties analyzed, Tainung 71 rice bran showed the highest total T3 content, while Kaohsiung 139 rice bran has the highest total T content; and in brown rice, Ai-Chueh-Shien-Hung-No showed the highest total T3 content, while Hung-No had the highest total T content. This suggests that these rice varieties are attractive sources of T3 or vitamin E and could be selected for mass cultivation. The T3-rich rice varieties may have the genetic characteristics responsible for homogentistic acid geranylgeranyl transferase (HGGT, T3 synthetase) or even enzymes related to the biosynthesis of vitamin E precursors (i.e., acetyl CoA, geranylgeranyl diphosphate and homogentistic acid).[24] Stress factors such as drought, low temperature, and high light intensity have been shown to affect the formation and degradation of T and T3.[25−28] T, T3, and γ-oryzanol have antioxidant properties and have been suggested to prevent lipid peroxidation in plant membranes.[29]

In conclusion, we have demonstrated that the contents of T, T3, and γ-oryzanol in 16 selected commercial rice varieties in Taiwan are mainly present in the bran fraction, followed by the husk, and the lowest was in polished rice. Rice bran was the best source of vitamin E as it contains a significant amount of all eight isomers. Regardless of the rice cultivars and grain parts, γ-T3 was the major vitamin E isomer in rice, followed by α-T and α-T3. Brown rice, being the major component of rice grain and rich in bioactive phytochemicals, would be an excellent source of health-maintaining food for daily consumption. Results on the distribution of bioactive phytochemicals in the different grain parts of rice varieties have provided useful information for the food industry because of their potential to serve as nutraceutical and functional ingredients.

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