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Gamma Oryzanol Content in Purple Rice Thailand Local Genotypes

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Abstract

Gamma oryzanol (γ-oryzanol) is an unsaponifiable component of rice oil, composed of several kinds of ferulic acids and has an effect similar to vitamin E. Previous researches on γ-oryzanol have been concentrated on the content in the rice bran, which is not useful in human diet. Therefore, our objective in this research was to determine the content of γ-oryzanol in the unpolished rice grain. Ten purple rice and three white rice genotypes were used in determination for crude oil, semi-gamma oryzanol and γ-oryzanol contents. Crude oil was extracted from the unpolished grains using n-hexane and ethyl acetate. A reverse-phase HPLC column of ODS C_{18} was applied in analyzing the contents. The results show that the contents of crude oil extracted from brown rice grains of the white rice genotypes were not differ significantly from the contents extracted from purple rice grain of the purple rice genotypes. The overall mean was 2.60 g/100g grain. This led to a non significant correlation coefficient of crude oil to semi purify γ-oryzanol and γ-oryzanol. The differences among the contents of semi purify γ-oryzanol were significant which also were the differs among the contents of γ-oryzanol. The higher content of γ-oryzanol was found in two purple rice genotypes (72.95 and 70.16 mg/100g grain). These two genotypes exhibited also a higher semi purify γ-oryzanol content (2.15 and 2.24 g/100g grain). While the purple rice genotypes with a lower γ-oryzanol content exhibited also a lower semi purify γ-oryzanol, one of the white rice genotype (KDML105) that showed a lower γ-oryzanol, exhibited semi purify γ-oryzanol among the higher group. However, the relation between this two characters was still significant. Comparison on the three characters between the two rice groups indicated, the purple rice group exhibited a higher mean of γ-oryzanol content (55.58 mg/100g grain) than the white rice group mean (30.67 mg/100g grain).

Introduction

Purple rice (*Oryza sativa* L.) is rice with purple pigment in the husk (hull) and pericarp as a unique characteristis. The pigment is anthocyanin (Hayashi and Abe, 1952). In Thailand, purple rice genotypes are local races; the type is a glutinous *indica*, grown widely in different geographical areas across the kingdom and varied in their phenotype pigmentation. As the local ancient wisdom, the rice was considered to have an herbal property and has been used as a traditional Thai herb for various kinds of a medicinal treatment. At present, it was proved that gamma oryzanol (γ-oryzanol), an extraction from rice crude oil is an effective medicinal substance in reducing plasma cholesterol (Lichenstein *et al.,* 1994), cholesterol absorption and decreasing early atherosclerosis (Rong *et al.,* 1997) and used in treatment for nerve imbalance and disorders of menopause (Nakayama *et al.,*1987). Moreover its anthocyanin (cyanidin-3-glucoside) was evidenced by its inhibition on the growth of Lewis lung carcinoma cells *in vivo* (Pei- Ni-Chen *et al*., 2005)

In this report**,** the objective was to investigate the content of γ-oryzanol in purple rice genotypes collected over locations in Thailand. The results could indicate the diversity in γ-oryzanol content among local genotypes in Thailand rice germplasm.

Material and Methods

Ten samples of purple rice genotypes were grown at the university research field, two were recommend varieties (Kumdoisaket and KumOmkoi) the other eight were collected over locations in Thailand. Two commercial rice cultivars, Kaow Dok Mali 105 (KDML 105) and Rice Department No.6 (RD 6) were used as comparison white rice varieties. Grains were sampled from the three replicates RCB. The grain samples from each replicate were dried in the hot air oven at 60^0c for 48 hrs, then milled to an unpolished purple rice grains (from purple rice) and brown rice grains (from the white rice) by SATAKE milling machine. The purple rice grains and brown rice grains were grounded and stored in a cool room at 15^0c during the course of lab analysis to retard quality changes due to aging effect and auto oxidation.

The Xu and Godber method (1999) was applied in extraction crude oil. Twenty-five grams of sample were place in a 500 ml round-bottom flask with 1 g. of ascorbic acid, 35 ml of hexane and 15 ml of ethyl acetate. The flask was attached to rotary evaporator, with vacuum and placed in a 60^0c water bath for 40 min with 180 rpm. Then 25 ml of distilled water was added to the flask. The flask was placed on the rotary evaporator at the same temperature and rotation speed for 10 min. Solvent were separated by filtration and was extracted a total of three times using this process. The extracts were pools and centrifuged at 4000 g for 10 min. The organic solvent layer was evaporated in a rotary evaporator under vacuum at 60^0c to obtain crude oil.

As extraction of Semi purification of γ-oryzanol a Low-Pressure silica column was used. Fifty ml of solvent (hexane/ethyl acetate = 7:3) was allowed to flow through the column, and the eluant was collected. The semi purified γ-oryzanol was obtained after the solvent was evaporated. Then, Separation of individual components of γ-oryzanol in an Analytical Reverse-Phase HPLC system consisted of a 25 x 4.6 mm diameter column of Microcorb-MV C_{18}. The detector was set at 330. Three replications for each sample was analyzed.

Results

The results in table 1 show that, there was not any significant difference in the amount of crude oil with the overall mean at 2.60 g/100 g grain. This led to the correlation coefficient of crude oil to semi purified γ- oryzanol and γ- oryzanol to be non significant (Table 2). The differences among the content of semi purified γ- oryzanol were significant, as were the differences among the content of γ- oryzanol. Semi purified γ- oryzanol content exhibited between 2.15 – 2.40 g/100 g grain in the highest genotypes. Kum Col. No. 026 has the lowest content (1.56 g/100 g grain). The highest genotypes of γ-oryzanol content present 72.16 mg/100 g grain and 72.95 mg/100 g grain. The white rice, KDML 105 and RD 6 were the lowest content (30.89 and 30.44 mg/100 g grain, respectively). While the purple rice genotypes with a lower γ- oryzanol content exhibited also a lower semi purified γ- oryzanol, one of the white rice genotype (KDML 105) also showed a lower γ- oryzanol, exhibited semi purified γ-oryzanol among the higher group. The relationship, however, between these two characters was still significant (Table 2).

Table 1 Crude oil, semi gamma oryzanol and gamma oryzanol content

Genotype	Crude oil (g/100g grain)	Semi purified gamma oryzanol (g/100g grain)		Gamma Oryzanol (mg/100g grain)	
Purple rice:					
Kumdoisaket	2.68	2.15	ab	72.95	a
Kum Col. No. 002	2.23	2.24	ab	70.16	a
Kum Col. No. 003	2.43	2.27	ab	61.50	b
Kum Col. No. 001	2.91	1.85	b	60.48	b
Kum Col. No. 027	2.47	2.40	a	57.49	bc
Kum Col. No. 029	2.85	2.08	b	54.18	c
Kum Col. No. 028	2.19	2.27	ab	49.77	c
Kum Col. No. 026	2.91	1.56	c	48.10	c
Kum Col. No. 008	3.09	1.88	b	41.31	d
KumOmkoi	2.32	2.07	b	39.83	d
White rice:					
KDML 105	2.20	2.16	ab	30.89	e
KD 6	2.93	1.81	bc	30.44	e
Mean	**2.61**	**2.06**		**51.43**	
LSD (0.05)	0.68 ns	0.28	*	6.09	*
SD	0.33	0.24		14.00	
SE	0.33	0.14		2.94	

Table 2 Correlation coefficient of crude oil, seme gamma oryzanol and gamma oryzanol content

	Semi purified gamma oryzanol	**Gamma oryzanol**
Crude oil	0.514 ns	0.146 ns
Semi purified gamma oryzanol		0.684 *

Discussion

Although γ-oryzanol is one of the minor components of crude oil, its cost is high because of its advantageous properties. Therefore, a rice genotype producing high levels of γ-oryzanol would be commercially valuable. In this experiment, the amount of extract crude oil did not differ but the amount of γ-oryzanol varied among genotypes indicated that accumulation of γ-oryzanol in rice is under genotypic determination and its genetic diversity exists in Thailand germplasm. Miller *et al* (2003) also found variation of γ-oryzanol to be associated with genotype and environment. The amount of crude oil extracted that found non significant different, could be resulted from a smaller amount of oil component in the pericarp of purple rice grains and brown rice grains than the other part of the grains. Although less crude oil was extracted from this part of the rice grain, a considerable high quantity of γ-oryzanol could be extracted. This indicated γ-oryzanol is a major part of crude oil in unpolished rice grains. Higher or lower γ-oryzanol means higher or lower of others components such as lipids and alpha-tocopherol. Apparently, the higher amount of this substance showed in the particular purple rice genotypes than in the white rice check genotypes, suggested the purple rice genotype with high gamma oryzanol along with the medicinal effect of the purple pigment (cyanidin-3-glucoside) would be of advantage in medicinal property.

An absence in the relation crude oil and γ-oryzanol means rice genotypes with equally amount of crude oil could be differ in its amount of γ-oryzanol.

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