Gamma Oryzanol Content in Glutinous Purple Rice Landrace Varieties

Panita Boonsit[1*], Puntipa Pongpiachan[2], Suthat Julsrigival[1] and Dumnern Karladee[1]

[1]Department of Agronomy, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand
[2]Department of Animal Science, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand

**Corresponding author. E-mail: panita@chiangmai.ac.th*

ABSTRACT

Our objective was to investigate whether purple glutinous rice synthesizes an agricultural nutrition distinguishable from white rice. The grains of ten purple rice landrace and two white rice varieties were examined for the amount of crude oil, semi-gamma oryzanol and gamma oryzanol (γ - oryzanol). Extractions were sampled from unpolished grains. Hexane and ethyl acetate were used as the solvents in extraction of crude oil yield. The amount of γ- oryzanol was analyzed in the HPLC. The results showed that the amounts of crude oil yield extracted from the unpolished rice grain varied from 2.19 to 2.91 g/100 g brown rice. With equal amounts of crude oil fed into the HPLC, the contents of semi purified γ- oryzanol were equal in both rice types, of purple rice (2.08 g/100g grain, on average) and of the white rice (1.99 g/100g grain, on average). In contrast, the contents of γ- oryzanol varied among varieties. The purple rice exhibited the higher content (55.58 mg/100g brown rice, on average) which was greater than the mean of the white rice check varieties (30.68 mg/100g brown rice). Genetic variation of γ- oryzanol apparently existed in the population of purple rice varieties as different contents were found. Among the purple rice, Kum Doi Saket and Kum Doi Musur yielded the greatest amount of γ- oryzanol (72.95, 70.16 mg/100 g brown rice, respectively). There was not any relationship between γ- oryzanol and crude oil content. This indicated that information on crude oil could not be applied as criteria in selection for γ- oryzanol in rice grains.

Key words: Gamma oryzanol, Purple rice

INTRODUCTION

Gamma oryzanol (γ- oryzanol) is a mixture of phytosteryl ferulates which occur in rice bran oil (Scavariello and Arellano, 1998) and function as natural antioxidants in the plants where they occur. Xu and Godber (1999) found that 24-methylene cycloartanyl ferulate, cycloartanyl ferulate, campesteryl ferulate,

β-sitosteryl ferulate and campestanyl ferulate which have been identified as the major components and were found to have antioxidant activity 10 times greater than the major tocopherol and tocotrienol components of vitamin E. Ishihara and Ito (1982) reported that γ- oryzanol supplementation was beneficial in the treatment of menopausal symptoms (Murase et al., 1963; Ishihara et al., 1982), elevated cholesterol (Nakayama et al., 1987; Sakamoto et al., 1987; Seetharamaiah et al., 1990; Scavariello et al., 1998) and numerous gastrointestinal conditions (Mizuta et al., 1978; Ichimaru et al., 1984). Many body builders believed that the steroid nature of the ingredients in γ- oryzanol has activity in the body similar to anabolic steroids. These activities include increased production and release of growth hormone and testosterone. Studies in animal and human have shown that γ- oryzanol may help lower elevated cholesterol levels. (Nakayama et al., 1987; Scavariello et al., 1998; Berger et al., 2004). This benefit is apparently the result of a combination of effects including reduced cholesterol absorption, increased conversion of cholesterol to bile acids, and an increased excretion of those bile acids. (Sakamoto et al., 1987; Seetharamaiah et al., 1990). Moreover, it can reduce total plasma cholesterol and increase HDL cholesterol levels, inhibition of the platelet aggregation (Cicero and Gaddi, 2001). Kim et al., (1995) reported that γ- oryzanol exhibited antioxidant properties in in vitro systems, such as pyrogallol autoxidation, lipid peroxidation and induced porcine retinal homogenate by ferric ion (Hiramitsu and Armstrong, 1991) and cholesterol oxidation accelerated by 2-methylpropionamidine (Xu and Godber, 2001).

Gamma oryzanol has been proposed as a natural antioxidant to improve the stability of foods (Nanua et al., 2000; Kim and Godber, 2001). Moreover, it has been proposed as a UV-A filter in sunscreen cosmetics (Coppini et al., 2001). It seems reasonable to assume that γ-oryzanol can also be used as antioxidant for pharmaceutical purposes.

In purple rice, the pigment presenting the color is anthocyanin (Hayashi and Abe, 1952). Moreover, anthocyanin (cyanidin-3-glucoside) was found to inhibit growth of Lewis lung carcinoma cells in vivo (Chen et al., 2005). Gamma oryzanol and anthocyanin (cyanidin-3-glucoside) are believed to be responsible for the effects. Previous researches on γ-oryzanol have been concentrated on the content in the rice bran (Xu and Godber, 1999; Bergman et al., 2003), which is not useful in human diet.

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

MATERIAL AND METHODS

Thirteen accessions of purple rice landrace genotypes were selected and grown at the research field of the Faculty of Agriculture, Chiang Mai University, two accessions (Kum Doi Saket and Kum Omkoi) were recommended purple rice varieties by the Department of Agriculture, Ministry of Agriculture and

Table 1. Crude oil, semi-purified γ- oryzanol and γ-oryzanol contents in the white rice modern varieties and purple rice landrace varieties.

Collection	**Crude oil** (g/100g grain)	**Semi purified γ-oryzanol** (g/100g grain)	**γ-oryzanol** (mg/100g grain)
Purple rice			
Kum Doi Musur	2.85ab	2.24ab	75.30a
Kum Doi Sa Ket	2.43bc	2.15abc	74.84a
Kum Nan	2.68abc	2.27ab	73.62a
Kum 7677	2.64abc	2.18ab	62.30b
Kum 87061	2.23c	1.85d	60.48b
Kum Vengsa	2.47bc	2.08bcd	59.89b
Kum 19959	2.91ab	2.40a	57.50b
Kum 99151	2.73abc	2.36a	49.14c
Kum19104	2.20c	1.88cd	48.10cd
Kum 88061	2.21c	2.07bcd	43.74de
Kum 89038	2.37bc	2.04bcd	42.21e
Kum Na	2.91ab	2.27ab	40.47e
Kum Omkoi	2.19c	2.07ab	39.84e
White rice			
KDML 105	3.09a	2.16ab	30.89f
RD6	2.93ab	1.81d	30.44f
mean	2.59	2.12	52.58
$LSD_{0.05}$	0.62*	0.27*	5.30*
SE	2.59	0.30	0.13

Cooperatives, Thailand and other eight were accessions collected over locations in Thailand. Two commercial white rice modern varieties, KDML 105 and RD 6 (Rice Department No.6) were used as comparisons. Grains were sampled from the three replicates in Randomize Complete Block Design. The grain samples from each replicate were dried in the hot air oven at 60°C for 48 hours, 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 ground and stored in a cool room at 15°C during the course of lab analysis to retard quality changes due to aging effect and auto oxidation.

The extraction of crude oil, semi purified γ- oryzanol and γ- oryzanol was applied by Xu and Godber method (1999).

Table 2. The average of crude oil, semi-purified γ- oryzanol and γ-oryzanol contents in purple rice genotypes in comparison to the white rice varieties.

Collection	Crude oil	Semi purified	γ-oryzanol
Purple rice	2.52	2.14	55.96
White rice	3.01	1.99	30.67
t-test	0.03*	0.26 ns	0.02*

Table 3. Correlation coefficients between oil, semi-purified γ- oryzanol and γ-oryzanol contents rice genotypes.

	Gamma oryzanol	Semi purified γ-oryzanol
Crude oil (P-value)	-0.068ns 0.248	0.442* 0.013
Semi purified γ-oryzanol	0.306* 0.019	

Extraction of crude oil

Twenty-five grams of brown rice were placed 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 a rotary evaporator with a vacuum and placed in a 60°C water bath for 40 min at 180 rpm. 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 and brown rice residue were separated by filtration. Brown rice residue was extracted a total of three times using this process. The extracts were pooled together and centrifuged at 4,000 g for 10 min. The organic solvent layer was evaporated in a rotary evaporator under vacuum at 60°C to obtain crude oil.

Semipurification of γ-oryzanol using a Low-Pressure silica column

A glass column (2.5x25 cm) packed with 20g of silica (grade 62) was used to remove triglycerides and other lipids. Initially, the crude oil was dissolved in 50 ml of the solvent (hexane/ethyl acetate = 9:1) for flushing through the column. Then, 50 ml of solvent (hexane/ethyl acetate = 7:3) was allowed to flow through the column, and the eluant was collected. The column was then washed with 50 ml of hexane/ethyl acetate (1:1), and the semi purified γ-oryzanol was obtained after the solvent was evaporated.

Separation of individual components of γ-oryzanol in an Analytical Reverse-Phase HPLC

The analytical HPLC system consisted of a Dynatech (Baton Rouge, LA) LC-241 autosampler, a Waters 510 pump, a Hewlett-Packard (San Fernando, CA) UV-vis diode array detector (Series 1050), and a 25 x 4.6 mm diameter column of Microcorb-MV C_{18}. The detector was set at 330 nm. The mobile phase consisted

of methanol, acetonitrile, dichloromethane, and acetic acid (50:44:3:3) and the flow rate was 1.4 ml/min.

RESULTS

The results in Table 1 showed that there was significant difference in the amount of crude oil. The variation ranged from 2.19 g/100g grain in purple rice (Kum Omkoi) to 3.09 g/100g grain in the white rice (KDML 105). Many purple rice genotypes (Kum Na, Kum 19959 and Kum Doi Muser) showed the content of crude oil as high as KDML 105. The significant differences among the content of semi purified γ- oryzanol and of γ- oryzanol were also found. Semi purified γ- oryzanol content ranged between 1.80 g/100g grain to 2.40 g/100g grain. The highest semi purified γ- oryzanol content was in purple rice genotypes (Kum 19959 and Kum 99151). However, the genotypes with high crude oil content also showed a high content of semi purified γ- oryzanol (KDML 105, Kum Doi Muser and Kum Na). With the content of γ- oryzanol, the comparison indicated that white rice showed the considerably lower contents than the purple rice genotypes. The contents ranged lower in RD 6 and KDML 105 (30.44 and 30.89 mg/100g grain, respectively) to higher in purple rice genotypes (Kum Doi Muser, Kum Doi Sa Ket and Kum Nan: 75.30, 74.84 and 73.62 mg/100g grain, respectively). In fact, although the purple rice genotypes had on average, a lower crude oil contents than the white rice (KDML 105 and RD6), they showed a higher values of both semi- purified γ-oryzanol and γ-oryzanol (Table 2). However, none of correlation coefficient between crude oil, semi- purified γ-oryzanol and γ-oryzanol was significant which indicated an absence of relationship among the three characters (Table 3). This indicated that rice genotypes exhibited the three characters independently. While the white rice was a better source of crude oil content, a high γ-oryzanol content was found more regularly in purple rice genotypes.

DISCUSSION

Although γ-oryzanol is one of the minor components of crude oil, its value for health is high because of its advantage in health properties. Therefore, a rice genotype producing high levels of γ-oryzanol would be commercially valuable. In this study, the amount of extracted crude oil, semi- purified γ-oryzanol and γ-oryzanol content varied among the white rice and the purple rice, indicating that the accumulation of γ-oryzanol in rice is under genotypic determination and its genetic diversity exists naturally in these collected accessions. Miller et al., (2003) also found the variation of γ-oryzanol to be associated with genotype and environment. However, the purple rice genotypes that showed the lowest crude oil extractions (Kum Omkoi) showed, in contrast, a higher content of γ-oryzanol than KDML 105 and RD 6. This means that although less amount of crude oil was extracted, considerably high quantity of γ-oryzanol extraction could be achieved. Therefore, consideration should be put forward that either γ-oryzanol is a

major part of crude oil in unpolished rice grains or accumulation of γ-oryzanol is associated with the pigmentation of purple color. If γ-oryzanol is proved to be a major part of oil in unpolished rice grains, then purple rice genotypes would be valuable sources of genetic variation for improving γ-oryzanol content in rice grains and vice versa.

A higher or a lower γ-oryzanol means a higher or a lower of other health substances such as lipids and alpha-tocopherol. Apparently, the higher amount of this substance is presented in the particular purple rice genotypes than in the white rice check genotypes, suggesting that the purple rice genotype with high γ-oryzanol would also have a higher amount of other health substances. Furthermore, with the medicinal effect of the purple pigment (cyanidin-3-glucoside), purple rice would be of advantage in medicinal property and also be a rice genetic resource for improving rice as herbal rice.

However, an absence in the relationship between crude oil, semi- purified γ-oryzanol and γ-oryzanol means rice genotype with equal amount of crude oil could differ in its amount of γ-oryzanol and that many minerals are components of crude oil and semi- purified γ-oryzanol. Therefore, neither crude oil extraction nor semi- purified γ- oryzanol can be the appropriate indices for identifying γ-oryzanol content in rice. To indicate a high γ-oryzanol content rice genotype, evaluation must be stressed directly on the extraction of the γ-oryzanol.

REFERENCES

Berger, A., D. Rein, and A. Schafer.2004. Similar cholesterol-lowering properties of rice bran oil, with varied gamma-oryzanol, in mildly hypercholesterolemic men. Eur J Nutr. May 19. [Epub ahead of print]

Bergman, C.J., and Z. Xu. 2003. Genotype and environment effects on tocopherol, tocotrienol, and [gamma]-oryzanol contents of southern U.S. rice. Cereal Chem. 80(4): 446-449.

Chen, P.N., S.C. Chu, H.L. Chiou, C.L. Chiang, S.F. Yang, and Y.S. Hsieh. 2005. Cyanidin 3-Glucoside and Peonidin 3-Glucoside Inhibit Tumor Cell Growth and Induce Apoptosis In Vitro and Suppress Tumor Growth In Vivo. Nutrition and cancer 53(2): 232-243.

Cicero, A.F., and A. Gaddi. 2001. Rice bran oil and gamma-oryzanol in the treatment of hyperlipoproteinaemias and other conditions. Phytother Res. June; 15(4): 277-289.

Coppini, D., P. Paganizzi, P. Santi, and A. Ghirardini. 2001. Capacità protettiva nei confronti delle radiazioni solari di derivati di origine vegetale. Cosmetic News 136: 15-20.

HayAshi, K., and Y. Abe. 1952. Fundamentals on experimental procedures for the paper-chromatographic survey of naturally occurring anthocyans. Rept. Res. Inst. Natural Resources 28: 1-11.

HayAshi, K., and T. Isaka. 1964. Studies for Anthocyane XIV. Uber Wasserstoffionen konzentration des Pressftes aus der anthocyan fuhrenden Pflazenorganen. Proc. Japan. Acad. 22: 256-258.

Hiramitsu, T., and D. Armstrong. 1991. Preventive effect of antioxidants on lipid peroxidation in the retina, Ophtalmic Res. 23: 196-203.

Ishihara, M., and Y. Ito. 1982. Clinical effect of gamma-oryzanol on climacteric disturbance on serum lipid peroxides. Nippon Sanka Fujinka Gakkai Zasshi 1982 Feb; 34(2): 243-251.

Juliano, B.O., 1993. Grain structure, composition and consumersí criteria for quality. Rice in human nutrition. IRRI and FAO. Rome.

Kim, D.H., K.D. Moon, and J.S. Rhee. 1995. Measurement of superoxide dismutase- like activity of natural antioxidants. Biosci. Biotechnol. Biochem. 59: 822-826.

Kim, J.S., J.S. Godber, J.M. King, W. Prinyawiwatkul, and H.Y. Chung. 2000. Vitamin E Vitamers and g-Oryzanol from Rice Bran Inhibit the Formation of 7-Ketocholesterol in Aqueous Dispersion System. (51E-1). Book of Abstracts: IFT Annual Meeting, Dallas, Texas. June 10-14.

Lichenstein, A.H., L.M. Ausman, J.L. Jenner, W. Carrasco, and E.J. Schaefer. 1994. Arteriosclerosis. 11: 1603 a.

Maclean, J.L., D.C. Dawe, B. Hardy, and G.H. Hettel. 2002. Rice Almanac: Source Book for the Most Important Economic Activity on Earth. IRRI: p.8.

Nakayama, S., A. Manabe, J. Suzuki, K. Sakamoto, and T. Inagake. 1987. Comparative Effects of Two Forms of γ-oryzanol in Different Sterol Compositions on Hyperlipidemia Induced by Cholesterol Diet in Rats. Japan Journal of Pharmacology 44(2): 135-143.

Nanua, J.U., M.C. Gregor, and J.S. Godber. 2000. Influence of high-oryzanol rice bran oil on the oxidative stability of whole milk powder, J. Dairy Sci. 83: 2426-2431.

Scavariello, E.M., and D.B. Arellano. 1998. Gamma-oryzanol: an important component in rice bran oil. Arch Latinoam Nutr. 48(1):7-12.

Seetharamaiah, G.S., and N. Chandrasekhara. 1990. Effect of Oryzanol on cholesterol absorption and biliary and fecal bile acids in rats. Indian J. Med. Res. 92: 471-475.

Seetharamaiah, G.S., T.P. Krishnakantha, and N. Chandrasekhara. 1990. Influence of Oryzanol on platelet aggregation in rats. J. Nutr. Sci. and Vitaminol. 36: 291-297.

Ryu, S.N., S.Z. Park, and H. Chi-tang. 1998. High performance liquid chronatographic of anthocyanin pigments in some varieties. Journal of food and Drug analysis 729-736.

Rong, N., L.M. Ausman, and R.J. Nicolosi. 1997. Oryzanol decreases cholesterol absorption and aortic fatty steaks in hamsters. Lipids 32: 303-309.

Xu, Z., and J. S. Godber. 1999. Purification and Identification of Components of Gamma Oryzanol in Rice Bran Oil. Journal of Agricultural and Food Chemistry 47: 2724-2728.

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