Accumulation of Gamma-Oryzanol in Purple Rice Grain

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Abstract

Our main objective of this inquiry was to determine whether purple glutinous rice (*Oryza sativa* L.) synthesizes an agricultural nutritional substance distinguishable from the white rice (*Oryza sativa* L.). In this report, the grains of four local purple rice cultivars (Kum Omkoi, Kum 87061, Kum Nan and Kum Doisaket) and white rice (which was used as a control) were examined for the amount of crude Oil and Gamma Oryzanol (γ - Oryzanol). Extractions were sampled from the bran and brown rice, using n-hexane and ethyl acetate as the solvents for extraction of crude Oil yield. The peak area of γ- Oryzanol component was read in the HPLC. The exact amount of γ- Oryzanol was calculated using an equation developed from the standard solution, which is:

$$\gamma\text{- Oryzanol (ppm)} = \left\{(2.527\times10^{-3})\times\text{peak area of sample}\right\}/\text{mass of crude Oil sample}$$

The results show that the amounts of crude Oil yield extracted from bran or brown rice, were equal among the purple rice cultivars and also that of the white rice. This differed from the amounts of crude Oil yield extracted from the bran, as it was an equal quantity among the tested purple rice but were higher than the white rice. With the different amount on average (lesser in brown rice 0.6602 g) of crude Oil fed into the HPLC, the peak area read in purple rice of brown rice (4,344,657. on average) was higher than in the bran (824,292.8 on average). In consequence, the calculated amount of γ- Oryzanol was higher (17035.17 ppm. in brown rice to 670.19 ppm. in the bran). As a consequence of coexistence with anthocyanin in the pericarp layer, the purple rice exhibited the higher amount of crude Oil and γ- Oryzanol than the white rice control variety. Genetic variation of γ- Oryzanol apparently exists in the population of purple rice cultivars as different amounts were found. Among the purple rice cultivars, Kum Nan yielded the highest amount of γ- Oryzanol (900.033 ppm) in its bran ($LSD_{0.05}$= 238.74*) while Kum 87061 was the highest (32929.63 ppm) in brown rice ($LSD_{0.05}$= 18141.0*).

There was not any relationship between crude Oil and γ- Oryzanol in either brown rice or bran, indicating that information on crude Oil could not be applied as a criterion for selection of γ- Oryzanol in rice grains. The lower amount of crude oil in brown rice but higher in γ- Oryzanol extraction, inferring the significant of brown rice grain as a better source of γ- Oryzanol.

Keywords: Purple rice, Anthocyanin, Gamma-Oryzanol

Introduction

One of the unsaponifiable components of rice bran oil is Gamma Oryzanol (γ- Oryzanol). This substance is composed of several kinds of ferulic acids and has an effect similar to that of Vitamin E in accelerating human growth, facilitating blood circulation and stimulating hormonal secretion. Its abilities include reducing plasma-cholesterol (Lichenstein *et al*., 1994), reducing cholesterol absorption and decreasing early atherosclerosis (Rong *et al*., 1977), as well as

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inhibiting platelet aggregation (Seetharamaiah *et al*., 1990) and increasing fecal bile acid excretion (Seetharamaiah and Chandrasekhara, 1990). Much evidence has also indicated that it increases testosterone levels, the release of endorphins and the growth of lean muscle tissue in humans. Previous researches on γ- Oryzanol have been concentrated mainly on bran of the white rice. Yet, γ- Oryzanol in the purple rice' bran has not been investigated. In this report, the grains of four local purple rice cultivars (Kum Omkoi, Kum 87061, Kum Nan and Kum Doisaket) and white rice (which was used as a control) were examined for the amount of crude Oil and γ - Oryzanol. Since γ- Oryzanol is not an essential nutrient; it is not associated with any deficiency state. Therefore, knowledge gained in this experiment could benefit the health attentive individual and athletes or other physical active individuals who involved in competitive training.

Materials and Method

Four cultivars of purple glutinous rice; Kum Omkoi, Kum 87061, Kum Nan, Kum Doisaket, were chosen, having white non glutinous rice; Kao Dok Mali 105 as a control. All rice samples were collected from the same condition of planting. After harvesting and threshing, paddies were put into the hot air oven at 60^0c for 48hrs. There were milled unpolished by using SATAKE milling machine and the final yields of separation were the rice bran and brown rice (inner seed). Finally, the samples were grounded using a small-scale cereal grinding machine 1 mm. sieve, then kept in dark plastic bags and stored in a cool room at 15^0c during the course of experimentation to retard quality changes due to aging effect and autooxidation. The Xu and Godber method (1999) was applied in extracting crude oil. After extraction, crude oil weight was calculated as:

Crude oil (g) = weight of conical flask after extraction – weight of empty conical flask

Analysis of γ- Oryzanol component from crude oil was performed using Thin Layer Chromatography. The mobile phase using in the HPLC technique was the mixture solvent of 4:1 (v/v) ethyl acetate in hexane. Standard γ- Oryzanol was provided by Boso Co., Ltd, Japan. It appearances were white powder, non-smelling, water insoluble. But, it can completely dissolved in the mixture of n-hexane and ethyl acetate. The regression equation used to calculate the amount of γ- Oryzanol was defined as:

$$\gamma\text{- Oryzanol (ppm)} = \frac{(\text{Concentration of std.}\mu\text{g/ml}) \times (\text{Peak area of sample}) \times (\text{Dilution factor}) \times 10}{(\text{Peak area of standard } \gamma\text{- Oryzanol}) \times (\text{Mass of crude oil sample (g)})}$$

In preparation, the standard γ- Oryzanol was weighed and diluted in solvent mixture (95%) of n-hexane and ethyl acetate (50:50). This solution was injected into the Liquid Chromatography model CLASS-LC10/M10A, Shimadzu, Japan with the control conditions at mixture solvent of 95% n-hexane and ethyl acetate (95.5:0.5) as mobile phase, flow rate of 1.5 ml/min, pressure at 54-65 kgf/cm^2 , oven temperature at 25^0 C, wavelength at 292 nm and 20 μl injection. From HPLC technique, γ- Oryzanol peak should appear around 14.300-15.700 minutes retention time. But, γ- Oryzanol naturally existed in 5 isomer forms, so at high concentration, peak can present in a wide range between 15.000-20.000 minutes retention time (Moustapha Diack, *et al*., 1994). Finally the peak area of γ- Oryzanol was recorded. The average peak area of samples was 2821755. Since, the standard concentration (ppm) was 713, the γ- Oryzanol (ppm) is:

$$= \frac{(713 \times (\text{Peak area of sample}) \times 1) \times 10}{(2821755 \times (\text{Mass of crude oil sample}))}$$

Therefore, the exactly amount of γ- Oryzanol in crude oil sample was calculated as:

$$\gamma\text{- Oryzanol (ppm)} = (2.527\times10^{-3}) \times \frac{\text{peak area of sample}}{\text{mass of crude Oil sample}}$$

Statistic method of one-way analysis of variance (SXW) was applied in statistical comparison.

Results

The results showed that there was not any significant difference in the amount of crude oil when a comparison was made among the purple rice cultivars, either for the extraction from bran ($LSD_{0.05}$ =1.588ns) or from the brown rice ($LSD_{0.05}$ =1.581ns). However, the amount of crude oil extracted from bran was higher than from brown rice (average of 3.099 g and 0.660 g respectively) (Table1 and 2). While the amounts of crude oil fed into the HPLC either of bran or of brown rice was in the same quantity, the amount of γ- Oryzanol of crude oil extracted however, varied from cultivar to cultivar, ranging from 428.3 to 900.0 ppm in bran oil and from 3638.2 to 32929.6 ppm in brown rice. The highest amount of γ- Oryzanol in brown rice was found in Cultivar Kum 87061 and Kum Nan (Table 2) while in bran Kum Nan was the highest one (Table1). When compared to the check (Kaow Dok Mali 105), the amounts of crude oil extracted from the brown rice did not differ (Figure 2), only that extracted from the bran of the purple rice were higher than of the check white rice (Figure 1). In contrast, γ- Oryzanol in bran and also in brown rice of purple glutinous rice were both higher than of the check white non-glutinous rice (Figure 3 and 4). It was found that there was not a relationship between the amount of crude oil and the peak area (r = 0.383ns for bran and – 0.254ns for brown rice)

Table 1 Amount of γ- Oryzanol in crude oil (ppm) extracted from Bran of various purple glutinous rice cultivars by using Hexane

Cultivar	Weight of Extract oil (g)	Peak area 0f γ - Oryzanol component	Amount of γ - Oryzanol in μg. per 1 g of crude oil (ppm.)
Kum Omkoi	3.1311	530746	428.346
Kum 87061	2.9401	830611	713.906
Kum Nan	3.2618	1161744	900.033
Kum Doisaket	3.0636	774070	638.489
Average	3.0992	824292.75	670.194
	$LSD_{0.05}$= 1.588 ns	**r (crude oil vs peak area) = 0.383ns**	**$LSD_{0.05}$= 238.74***
	SE.= 0.649		**SE. = 97.569**

Table 2 Amount of γ- Oryzanol in crude oil (ppm) extracted from Brown Rice of various purple glutinous rice cultivars by using Hexane

Cultivar	Weight of Extract oil (g)	Peak area 0f γ - Oryzanol component	Amount of γ - Oryzanol in μg. per 1 g of crude oil (ppm.)
Kum Omkoi	0.6788	1395328	5194.45
Kum 87061	0.5797	7554137	32929.63
Kum Nan	0.7156	7469867	26378.36
Kum Doisaket	0.6663	959299	3638.22
Average	0.6601	4344657.75	17035.17
	$LSD_{0.05}$= 1.581ns	**r (crude oil vs peak area) = - 0.254ns**	**$LSD_{0.05}$= 18141.00***
	SE. = 0.646		**SE. = 7414.00**

Figure 1 Comparison of the amount of crude oil (g) extracted from Bran of various purple glutinous rice cultivars and of controlled white rice (Kaow Dok Mali 105)

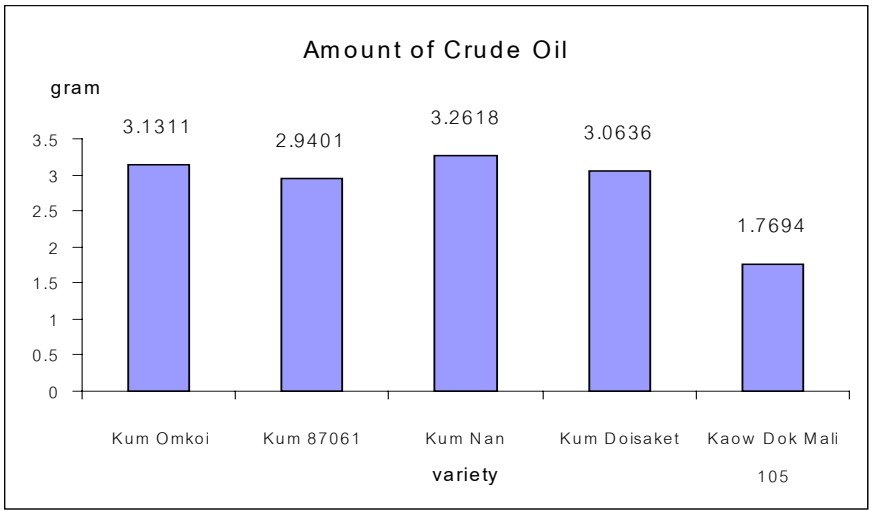


Figure 2 Comparison of the amount of crude oil (g) extracted from Brown rice of various purple glutinous rice cultivars and of controlled white rice (Kaow Dok Mali 105)

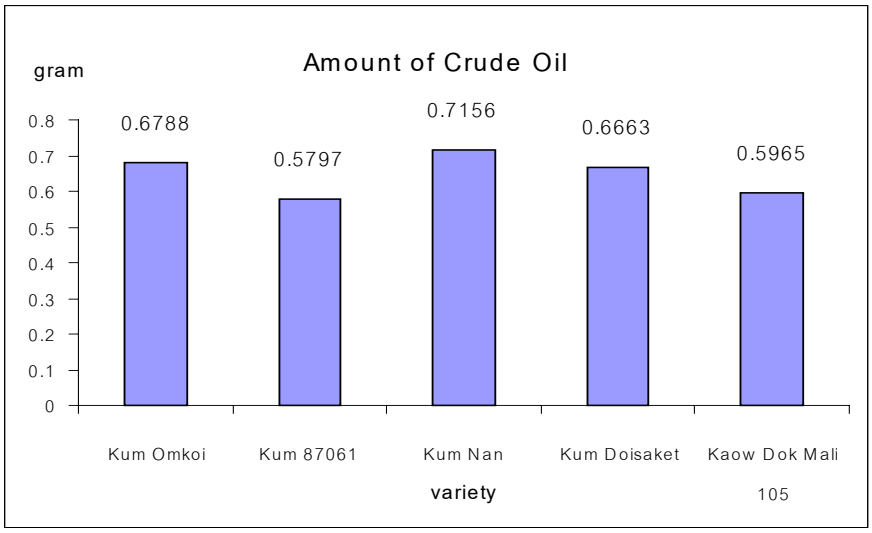


Figure 3 Comparison of the amount of γ- Oryzanol in crude oil (ppm) extracted from Bran of various purple glutinous rice cultivars and of controlled white rice (Kaow Dok Mali 105)

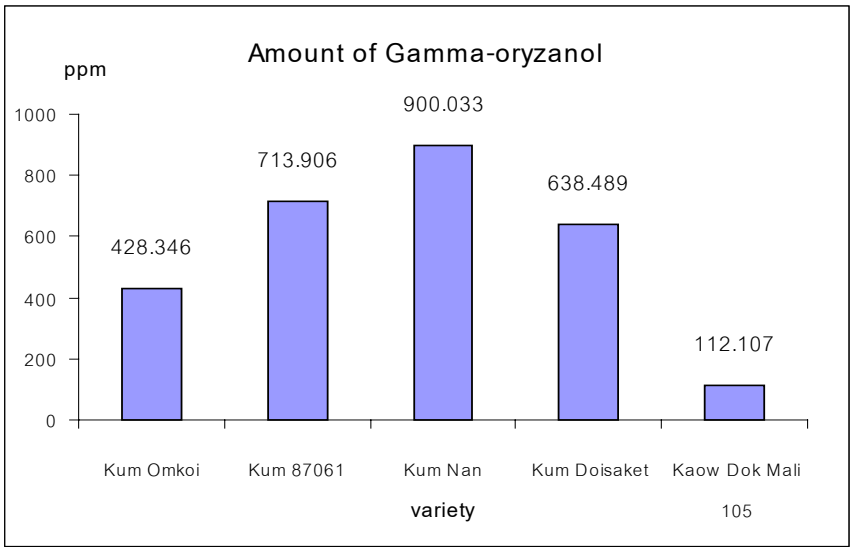
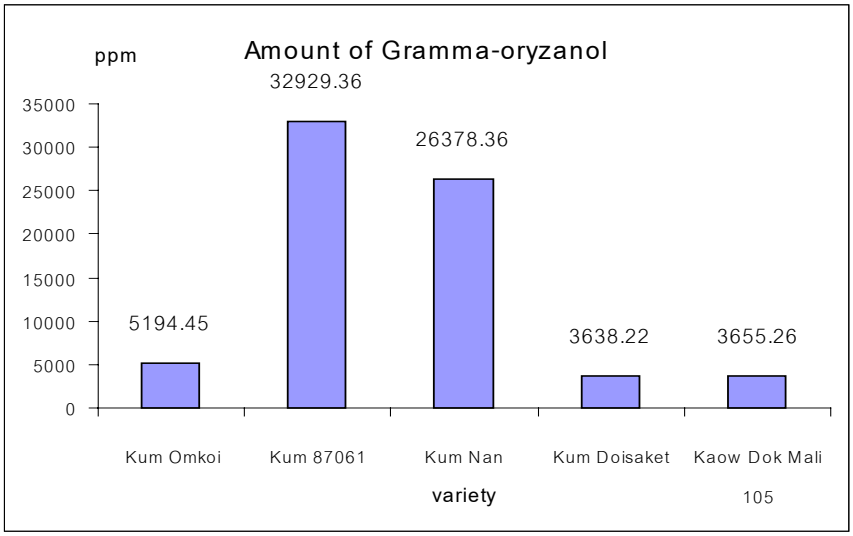


Figure 4 Comparison of the amount of γ- Oryzanol in crude oil (ppm) extracted from Brown rice of various purple glutinous rice cultivars and of controlled white rice (Kaow Dok Mali 105)



Discussion

Gamma Oryzanol is one of the minor components of crude oil. But at present, its cost is high (around 150 US$ to lb.) because of its advantageous nutraceutical properties. Therefore, the rice genotype with genes for γ- Oryzanol accumulation would be commercially valuable.

Variation in ability to accumulate γ- Oryzanol found in this experiment indicated that γ- Oryzanol is under genotype determination and its genetic diversity exists in the rice biodiversity. Since the darkness of the purple pigment (amount of anthocyanin) characterized the husk (bran) and the pericarp of the brown rice (unpolished inner seed), the variation of anthocyanin pigmentation existed (Takahashi, 1997) may has association with the variation of γ- Oryzanol accumulation. If so, the darkness levels of the purple pigment could be used as a genetic marker to identify a high γ- Oryzanol accumulation genotype. More screening should be done within purple rice natural variation and biodiversity.

Another exciting result was that the crude oil extracted from the brown rice was even though less quantity than from the bran, more quantity of γ- Oryzanol could be extracted. This indicated that γ- Oryzanol is a major component of crude oil in brown rice but only a minor component one in the bran. Higher or lower γ- Oryzanol means higher or lower of other components such as Lipids and Alpha-tocopherol (α-tocopherol).

An absence in the relationship between crude oil and peak area of γ- Oryzanol component means that, rice cultivars with equally amount of crude oil yield could be differ in its accumulated amount of γ- Oryzanol.

The higher amount of this antioxidant property substance was found in the purple rice than in the white rice may suggest that purple glutinous rice has some properties of preventing the development of cancer (Princess Sirinthorn, Her Royal Highness, 1985).

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