CHAPTER 3

GAMMA ORYZANOL CONTENT IN PURPLE RICE VARIETIES

3.1 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 24-Methylene cycloartanyl ferulate, cycloartanyl ferulate, campesteryl ferulate, β-sitosteryl ferulate and campestanyl ferulate have been identified as the major components in rice bran oil which were found to have antioxidant activity 10 times greater than the major tocopherol and tocotrienol components of vitamin E. Ishihara *et al.* (1982) reported that γ-oryzanol supplementation is 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 believe the steroid nature of the ingredients in gamma oryzanol has activity in the body similar to anabolic steroids. This activity includes increased release of growth hormone and increased production and release of testosterone. Studies in animals and humans have shown that γ-oryzanol may help lower elevated cholesterol levels (Scavariello *et al.,* 1998; Nakayama *et al.*, 1987; 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 of total plasma cholesterol and increase of HDL cholesterol levels, inhibition of the platelet aggregation (Cicero and Gaddi, 2001). Kim *et al.,* (1995) reported that γ-oryzanol exhibits antioxidant properties in *in vitro* systems, such as pyrogallol autoxidation, lipid peroxidation and induced in 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 pharmaceutucal purposes.

In purple, the pigment presenting the color is anthocyanin (Hayashi and Abe, 1952). Furthermore, anthocyanins (cyanidin 3-glucoside) from *O. sativa* indica were evidenced by their inhibition on the 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 chapter**,** 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.

3.2 Material and Methods

Thirteen accessions of purple rice genotypes were chosen and grown at the university research field with 25 kg N/ha and 37.5 kg P/ha, two accessions (Kum Doi Saket and Kum Omkoi) were recommended varieties, and other eights were collections over locations in Thailand. Two commercial white rice varieties, Kaow Dok Mali 105 (KDML 105) and Rice Department No.6 (RD6) were used as comparisons. Grains were sampled from the three replicates in RCB. The grain samples from each replicate were dried in the hot air oven at 60^0C 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 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 x 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 were analyzed.

Gamma oryzanol content was determined by calculated from peak area compared with peak area of γ-oryzanol standard (Figure 3.1).

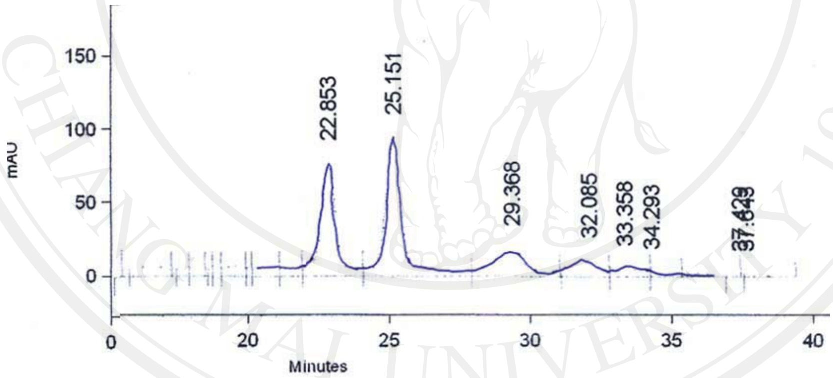

Figure 3.1 Chromatogram of γ-oryzanol standard in the analytical reverse-phase HPLC

3.3 Results

The results in Figure 3.2 show 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 in Figure 3.3. Semi purified γ-oryzanol content ranged between 1.80mg/100g grain to 2.40 mg/100g grain.

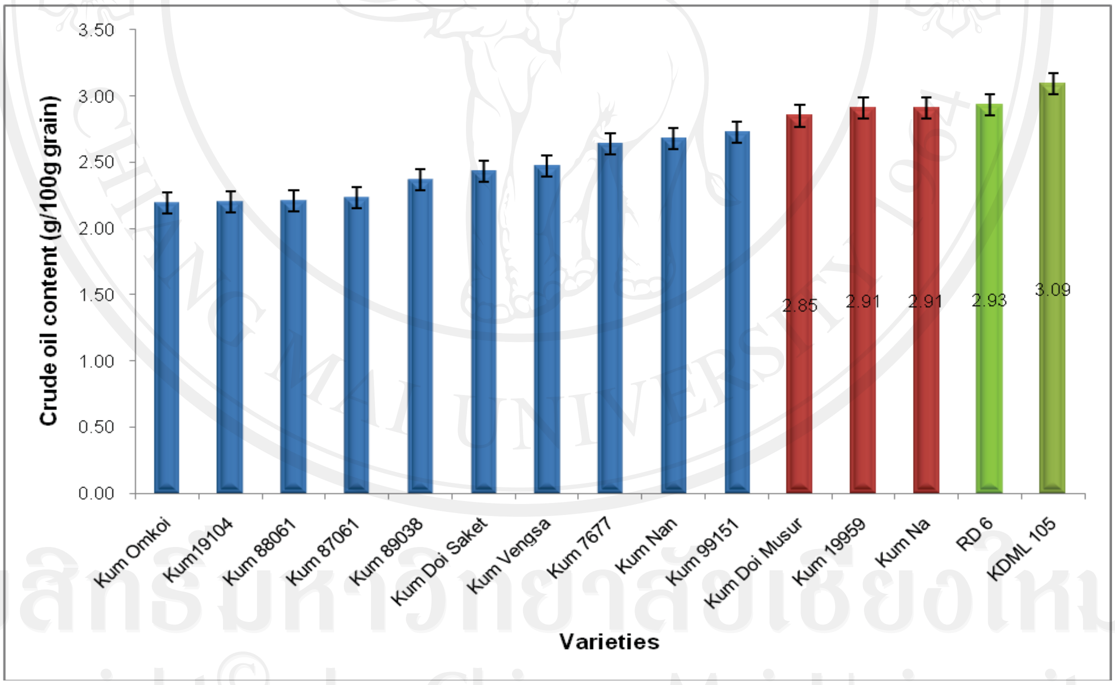

Figure 3.2 Crude oil contents in purple rice and the white rice check varieties

Bar represent mean ± SE, (SE = standard error)

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 showed a content semi purified γ-oryzanol among a higher content of 2.16 to 2.40 mg/100g grain (KDML 105, Kum Doi Muser and Kum Na). With the content of γ-oryzanol, the comparison white rice showed the considerably lower contents than the purple rice genotypes. The contents ranged a lower in RD 6 and KDML 105 (30.44 and 30.89 mg/100g grain, respectively) to a 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) (Figure 3.4).

In fact, although, the purple rice genotypes had on average, a lower crude oil contents than the white rice (KDML 105 and RD 6) they showed a higher values of both semi- purified γ-oryzanol and γ-oryzanol (Table 3.1). However, there was no relationship between crude oil, semi- purified γ-oryzanol and γ-oryzanol (Table 3.2). Furthermore, the 15 rice genotypes showed a higher variance of γ-oryzanol than the variance of both crude oil (value t = 12.82**) and semi- purified γ-oryzanol (value t = 12.98**) (Figure 3.1 and 3.2). This indicates that rice genotypes independently exhibited the three characters. 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. In addition, from the previous chapter, the purple rice which have purple red in apiculus (Kum Doi Musur, Kum Doi Saket, Kum Nan and Kum Vengsa) tend to present high content of γ-oryzanol (Table 2.3; Figure 3.3).

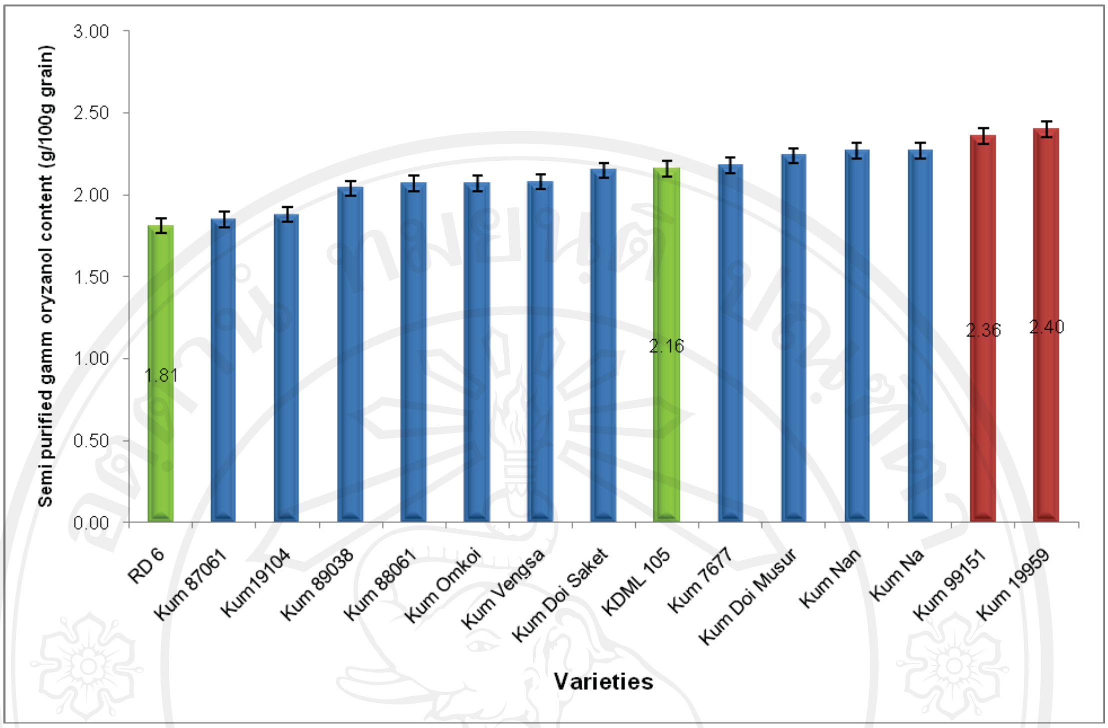

Figure 3.3 Semi purified gamma oryzanol contents in purple rice and the white rice check varieties. Bar represent mean ± SE, (SE = standard error)

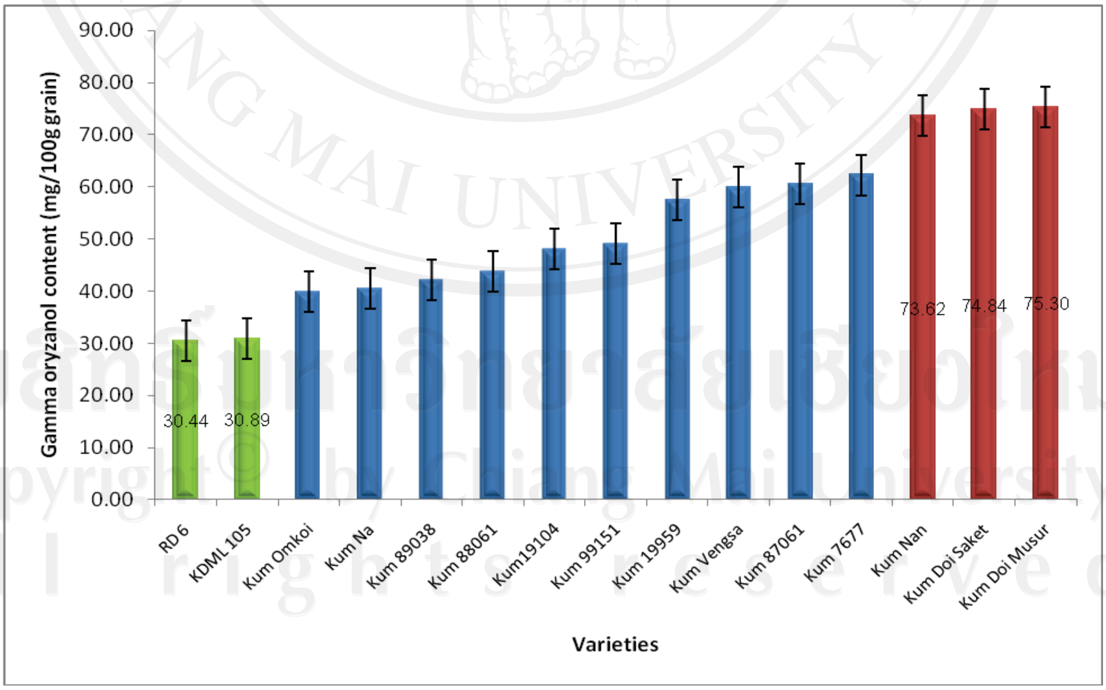

Figure 3.4 Gamma oryzanol contents in purple rice and the white rice check varieties. Bar represent mean ± SE, (SE = standard error)

Table 3.1 The average of crude oil, semi-purified γ-oryzanol and γ-oryzanol contents in purple rice genotypes in comparison to the white rice genotypes

Collection	**Crude oil** (g/100g grain)	**Semi-purified γ-oryzanol** (g/100g grain)	**Gamma Oryzanol** (mg/100g grain)
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 *

ns = non significant, * = significant at *P<0.05*

Table 3.2 Correlation coefficients between oil, semi-purified γ-oryzanol and γ-oryzanol contents in purple rice collection

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

ns = non significant, * = significant at *P<0.05*

3.4 Discussion

Although, γ-oryzanol is one of the minor components of crude oil, its value for health is high because of its advantageous in health properties. Therefore, a rice genotype producing high levels of γ-oryzanol would be commercially valuable. In this results, the amount of extract crude oil, semi- purified γ-oryzanol and of γ-oryzanol content however, varied among the white rice and the purple rice indicated that accumulation of γ-oryzanol in rice is under genotypic determination and its genetic diversity exists naturally in this collected accessions. Miller *et al* (2003) also found variation of γ-oryzanol to be associated with genotype and environment. However, in this result, 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 RD6. This means that although less amount of crude oil was extracted, considerably high quantity of γ-oryzanol extraction could be achieved, indicated either that 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. A higher or a lower γ-oryzanol means a higher or a lower of others health substances 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 would also had a higher amount of others health substances. Furthermore, with the medicinal effect of the purple pigment (cyanidin-3-glucoside) purple rice would be of advantage in medicinal property.

However, some varieties had showed a potential of having high crude oil content and high γ-oryzanol content. An absence in the relation crude oil, semi-purified γ-oryzanol and γ-oryzanol means rice genotypes with equally amount of crude oil could be differ in its amount of γ-oryzanol and that neither crude oil extraction nor semi- purified γ- oryzanol is an appropriate indices for γ-oryzanol. To identify for a high γ-oryzanol content rice genotype, evaluation must be stress directly on the extraction of the γ- oryzanol.