

Effect of Germination on γ -Oryzanol Content of Selected Sarawak Rice Cultivars

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Abstract: Problem statement: Rice is rich in complex carbohydrates and beneficial bioactive compounds such as γ -Aminobutyric Acid (GABA), γ -oryzanol and vitamin. Several bioactive components are known to accumulate in rice during germination process. This study was to investigate the effect of germination process on γ -oryzanol levels in selected Sarawak local rice cultivars. **Approach:** Rice seeds were germinated for 4, 8, 12, 16, 18, 20 or 24 h at 25°C and samples were processed and analyzed for γ -oryzanol content. A partial extraction method was used for quantitative γ -oryzanol analysis. **Results:** Result showed γ -oryzanol concentrations in the rice cultivars varied from 0.099-0.340 mg g⁻¹ on dry weight basis. Cultivars Udang Halus and Silah showed the highest concentration of γ -oryzanol after 16 h germination. **Conclusion:** The germination process exhibited diverse effects on the γ -oryzanol accumulation in rice and it was cultivar dependent.

Key words: rice, *Oryza sativa* L., γ -oryzanol, Sarawak

INTRODUCTION

Rice (*Oryza sativa* L.) is the main cereal food of the world, especially in Asian countries. In recent years, much attention has been on the health benefits of unpolished rice as a source of important bioactive compounds and nutrients. People are encouraged to consume unpolished rice (brown rice) instead of polished rice (white rice). It is because unpolished rice is an important source of bioactive compounds. Brown rice grains contain more nutritional components, such as γ -oryzanol, dietary fibers, phytic acids, vitamin E, vitamin B and γ -aminobutyric acid (GABA), than the ordinary milled rice grains. These bio-functional components exist in the germ and bran layers which are removed during grain polishing or milling^[1].

Research indicates that cereal grains contain special phenolic compounds, such as ferulic acid esters of phytosterols (steryl ferulates) which have been identified in rice, corn, wheat, rye, barley and wild rice. These phenolic compounds have antioxidant, antimutagenic, anticancer and other positive effects as well as play an important role in maintaining health. Among these seeds, rice (*Oryza sativa* L.) exhibits the highest level of steryl ferulates^[2]. The mixture of steryl

ferulates found in rice is termed γ -oryzanol or more exactly is a group of ferulic acid esters of phytosterols and triterpene alcohols^[3]. The sterol components of γ -oryzanol are primarily campesterol and sitosterol. The triterpene alcohol components are cycloartenol and 24-methylene cycloartanol. Since γ -oryzanol is a mixture of ferulate esters with sterols or triterpenes, it can be divided to ferulic acid and triterpenes or sterols. Ferulic acid is initially converted from phenylalanine and tyrosine by enzymes^[4] and much of the ferulic acid occurs as esters in many plants, which may imply the pathway further undergoes conjugation with other molecules.

γ -oryzanol occurs in the unsaponifiable fraction of rice bran oil. It was originally considered a single compound but later was determined to be a mixture of ferulic acids esterified with normal sterols or triterpene alcohols. Ten components of γ -oryzanol identified in rice bran consist of ferulic acid and triterpene derived compounds, which are combined by an ester bond. Cycloartenyl ferulate, 24-methylenecycloartanyl ferulate and campesteryl ferulate are the three major components of γ -oryzanol in rice bran^[5-6].

Several studies on germinated brown rice indicated that the germination process could improve nutritional

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values such as γ -aminobutyric acid (GABA), γ -oryzanol and vitamins^[7,8]. Germinated brown rice or GBR is produced by soaking brown rice grains in water to promote germination. According to Teo^[9], Sarawak has more than 100 varieties of traditional rice and many of these are sold in the market throughout Sarawak. However, no reports have been documented on the effect of germination on bioactive compounds among rice cultivars in Sarawak. In this study, investigations were conducted to look into the effect of germination on γ -oryzanol levels of eight Sarawak rice cultivars.

MATERIALS AND METHODS

Materials: Eight rice cultivars Chelum, Sabak, Silah, Biris, Hitam, Boria, Udang Halus and Mamut2 were collected from southern Sarawak, Malaysia. Pure γ -oryzanol standard was purchased from Tokyo Chemical Industry Co., Ltd. (Product Number: 00172). All chemicals used were analytical grade reagents.

Rice germination: Brown rice grains were soaked in water at 25°C. They were allowed to undergo germination process for 4, 8, 12, 16, 18, 20 or 24 h. The pregerminated brown rice samples were removed from the basin at the respective time intervals, dried, ground and analyzed for γ -oryzanol levels.

Determination of γ -oryzanol: Total γ -oryzanol content in brown rice were analyzed by using partial extraction methodology, as described by Lilitchan^[10]. Rice cultivars were introduced into two identical vials (20 seeds each) and extracted with 4 and 8 mL of n-hexane at room temperature by vigorous agitation on a vortex mixer (VELP SCIENTIFICA) for 1 min. Solid and suspended bran was removed by centrifugation (KUBOTA 3110) at 2,500 rpm for 10 min. The absorbance of the two supernatants was measured at 314 nm using a UV-vis spectrophotometer (Scinco S3100). The γ -oryzanol contents in the extracts were quantified against the standard curve. Total γ -oryzanol was calculated by solving two simultaneous equations:

$$k = \left(\frac{x}{v}\right)\left(\frac{w}{y-x}\right)$$

Where:

k = A constant

x = The amount of γ -oryzanol in the extract (g)

y = The total amount of γ -oryzanol in the bran

v = The volume used for extraction (mL)

w = The weight of bran used for extraction

There are two unknowns (k and y), thus extraction of two identical samples with different volumes of solvent are necessary. In order to simplify the analysis and the calculation, the solvent used for the second extraction is double ($v_2 = 2v_1$) and the amount of solute in the two extracts are x_1 and x_2 . Although the k value for solid extraction is slightly varied, as the amount of solvent used for extractions is different, it is assumed that the change does not affect the accuracy of this study. Thus, by assuming $k_1 = k_2$, total amount of γ -oryzanol (y) can be calculated:

$$\left(\frac{x_1}{v_1}\right)\left(\frac{w}{y-x_1}\right) = \left(\frac{x_2}{v_2}\right)\left(\frac{w}{y-x_2}\right)$$

$$y = \frac{x_1 x_2}{2x_1 - x_2}$$

RESULTS

Results showed that γ -oryzanol was found in all cultivars at all stages of the germination process (Table 1). Among the eight cultivars, cultivar Biris exhibited highest initial γ -oryzanol content (0.270 mg g⁻¹ of dry weight) while the cultivar with lowest γ -oryzanol content was Udang Halus (0.099 mg g⁻¹ of dry weight).

After germination of 24 h, only three cultivars retained or showed slight increases in γ -oryzanol content (Sabak, Silah and Hitam). The rest of the cultivars showed reduction in γ -oryzanol content compared to pre-germination levels. The cultivar which showed highest γ -oryzanol content after 24 h was Silah (0.264 mg g⁻¹ of dry weight) meanwhile the cultivar with lowest γ -oryzanol content was Udang Halus (0.077 mg g⁻¹ of dry weight).

However, the results of analysis at 4-hourly intervals showed some trends in γ -oryzanol content. The γ -oryzanol level in the rice varied with different duration of germination (Fig. 1). Although the γ -oryzanol content after 24 h germination does not show significant increases for all cultivars studied, significant increments in γ -oryzanol content during the 24 h duration did occur except for Boria and Biris where the initial γ -oryzanol values was the highest. Each cultivar had different levels of γ -oryzanol at different germination duration within the 24 h. The γ -oryzanol content of the different cultivars in this study did not increase directly with germination duration but showed significant variation among cultivars (Fig. 1).

Table 1: γ -oryzanol content in eight traditional rice cultivars from Sarawak

Rice cultivar	γ -oryzanol content (mg g ⁻¹)	
	Initial	24 h germination
Chelum	0.182	0.165
Sabak	0.130	0.134
Silah	0.224	0.264
Biris	0.270	0.121
Hitam	0.103	0.132
Boria	0.247	0.077
Udang halus	0.099	0.067
Mamut 2	0.103	0.078

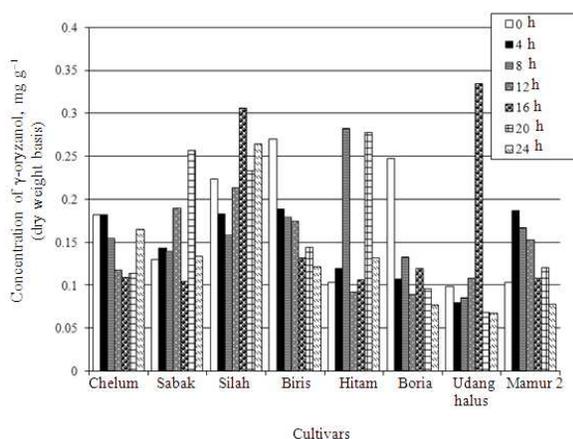


Fig. 1: γ -oryzanol Content in eight rice cultivars at different germination duration

DISCUSSION

Germination time promoted increase in bioactive component concentration in at least five of the eight cultivars studied. Bioactive components such as γ -oryzanol, α -tocopherol, γ -aminobutyric acid (GABA) have been reported at high levels in germinated brown rice^[11]. During the process of germination, saccharification occurs, breaking complex carbohydrates into simple sugars and softening the nutritive tissue surrounding the embryo of plants. Hydrolytic enzymes are thus activated, which increases the amount of digestible vitamins, oligosaccharides, minerals and amino acids by decomposing starch, non starch polysaccharides and proteins. The decomposition of the high molecular weight polymers during germination leads to the generation of bio-functional substances^[12]. From the results obtained, the different rice cultivar had different concentrations of γ -oryzanol within the 24 h duration of germination. Concentrations of γ -oryzanol in GBR are cultivar and germination duration dependent. This may be attributed to genetic and environmental factors. Earlier studies have also

show enormous variation in oryzanol content among genotypes^[13-14]. Furthermore, the trends observed in different cultivars may also be due to different water uptake rates by the different rice seeds^[15].

CONCLUSION

As γ -oryzanol plays an important role health and nutraceutical industry, it is crucial that methods that maximize the extraction of γ -oryzanol from rice be developed. Results do indicate that germination can increase γ -oryzanol content in certain rice cultivars.

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