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Effect of Bio-Liquid Fertilizer from Saltpan Residual on the γ-Oryzanol Content of Germinated Brown Rice (Thai Pigmented Rice)

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Abstract

γ-Oryzanol has been identified from rice grains. The γ-oryzanol accumulation had affected by genotype and environmental factors. Salinity conditions had affected the growth and bioactive compounds in rice. Saltpan residual is the mix of microbiome, algae, and salt that produced from the saline harvest period. In this study, we produced the Bio-liquid fertilizer from saltpan residual. The γ-oryzanol content of Thai pigmented rice cultivars treated and un-treated with Bio-liquid fertilizer was investigated. The results revealed that the γ-oryzanol content of black and purple pigmented rice cultivars have was higher than red rice cultivars. The highest γ-oryzanol was obtained from Khao Hom Mali-Nin (black rice) with 475.03 ± 13.96 µg/g. Germination times had affected the γ-oryzanol content of germinated pigmented rice but variable depending on the rice cultivars. The pigmented rice seeds were treated with Bio-liquid fertilizer and the germination times were 12 to 24 h for Khao RiceBerry, 12 to 36 h for Khao Mali-Komen Surin and 48 h for Khao Hom Mali-Nin had affected the γ-oryzanol content higher than the rice seeds un-treated with Bio-liquid fertilizer. The results indicate that the potential of Bio-liquid fertilizer from saltpan residual can be used for application in the fertilizer industry and for enhancing the bioactive compound in rice grains.

Keywords: Pigmented Rice Cultivars, Bio-Liquid Fertilizer, Saltpan Residual, γ-Oryzanol

1. Introduction

Rice is the leading staple food globally, especially in Asia (1). The people in Asia have consumed pigmented rice and non-pigmented rice (2). Interestingly, pigmented rice has the rich in bioactive compounds related to human health than non-pigmented rice (3, 4). Moreover, brown rice or husked rice of pigmented rice and non-pigmented rice has also more bioactive compounds than white rice (white rice refer to the rice grain without the outer layer and rice husk) (5). γ-Oryzanol is one of the bioactive compounds had identified from pigmented rice and non-pigmented rice, especially in brown rice (6, 7). These bioactive compounds have been revealed to have some biological activity, such as antioxidant activity, anti-cancer activity, anti-diabetes and anti-inflammatory (8). γ-Oryzanol is a mixture of ferulic acid esters of plant compounds and first purified from rice (9, 10). In rice, the amount of γ-oryzanol had a variation depending on many factors such as rice cultivars, cultivated region and environment (11-13). The soil salinity also affected the γ-oryzanol of rice grains at the reproductive stage (14). Thammapat et al. (15) also reported that the soaking contention with NaCl solution had affected the γ-oryzanol of the glutinous rice.

Saltpan residual or Kee Dad Na Kluer (in Thai) is a mix of marine bacterial, algae and salt (16, 17). During the saline harvest period, the field dry will occur, and the mix of marine bacterial, algae and salt perched along the ground will dry. Then the thin brown plate (Saltpan residual or Kee Dad Na Kluer) was occurred and approximately 2-5 mm thick. Saltpan has been reported to the plant growth depending on the salt concentration. *Salicornia rubra* is a salt-tolerant species and abundantly found in the salinity area. This highest plant growth was recorded at 200 mM salt concentration (18). Moreover, the halotolerant bacteria related to plant growth have been identified from soil salinity, such as *Swaminathania salitolerans* and *Azotobacter chroococcum*. These are the N_2-fixing and phosphate-solubilizing bacterium (19). Especially for the *Swaminathania, halotolerant* has been identified from wild rice (*Porteresia coarctata* Tateoka) (20).

Thus, this study investigated the γ-oryzanol content of pigmented rice (Thai rice cultivars). We also determined the γ-oryzanol content of germinated rice (pigmented rice) under the wet condition of bio-liquid fertilizer from saltpan residual (Kee Dad Na Kluer). These findings might be helpful for the study of the changing of bioactive compounds of plants under salinity conditions. Moreover, the bio-liquid fertilizer from saltpan residual might be used for plant bioactive compounds production in the agriculture industry.

2. Materials and Experiment

2.1 Chemical reagent, materials and rice samples

Standard γ-oryzanol were obtained from Tokyo Chemical Industry (Tokyo, Japan). Seeds of rice samples including Khao Hom Mali-Nin, Khao RiceBerry and Khao Mali-Komen Surin were harvested from Pathum Thani province (rice season 2021). The rice seeds were sterilized with 0.1% NaClO and then rinsed with distilled water. Then the sterilized rice seeds were dried at 40 °C until the moisture of rice seeds had less than 13%. The Kee Dad Na Kluer (in Thai) was harvested from Samut Songkhram province, Thailand (Figure 1).

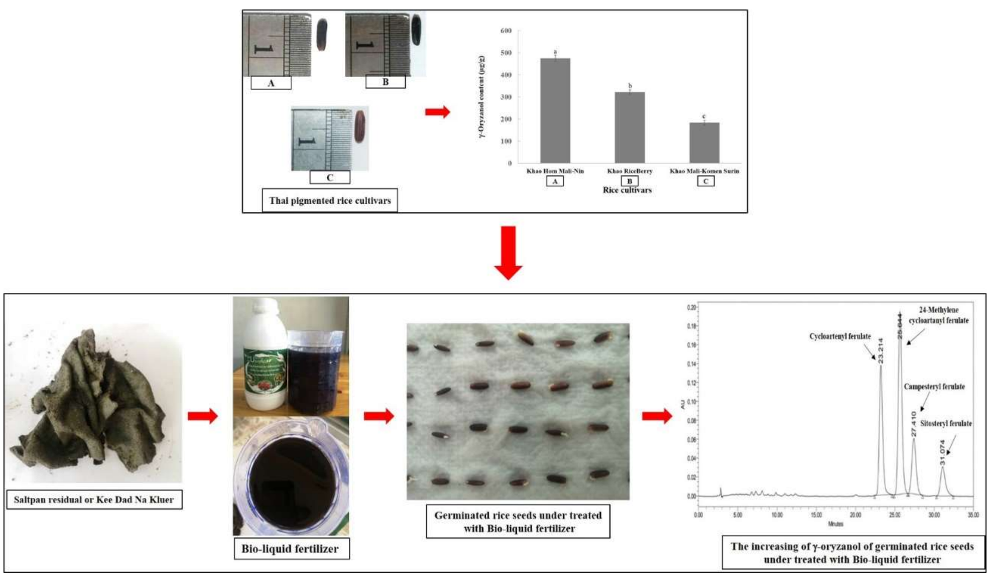

Figure 1 The overall methodology of the research. Three pigmented rice cultivars including Khao Hom Mali-Nin, Khao RiceBerry and Khao Mali-Komen Surin. The characteristic of saltpan residual or Kee Dad Na Kluer (in Thai) harvested from Samut Songkhram province, Thailand and the bio-liquid fertilizer produced from saltpan residual.

2.3 Seeds germination

Approximately one hundred grams the of rice seeds were used for the germination experiment. The seeds coat of rice seeds was taken out before germination. Then the length and width of rice seeds (without seeds coats) were measured. Two hundred rice seeds of equal quality were treated under two different conditions. First, one hundred rice seeds were treated with deionized water for 24 h. Second, the other one hundred rice seeds were treated with 1% bio-liquid fertilizer for 24 h. The treated rice seeds were germinated for 12, 24, 36 and 48 h. at 37 °C. The 500 mL of deionized water was spayed to the germination experiment every 12 h. The germinated rice seeds were harvested 12, 24, 36 and 48 h. The harvested rice seeds were dried at 65 °C for 24 h. Then the germinated rice seeds were ground with liquid nitrogen to produce rice powder. The rice powder samples were kept at desiccator until extraction.

2.4 Rice extraction

The 1.50 grams of samples were mixed with diatomite in the ratio of (1:4). The mixed samples were dissolved with isopropanol. Then the mixing solution was transferred to the Accelerated Solvent Extraction (Dionex™ ASE™ 350, Thermo Fisher Scientific, US) with the condition of 80 °C, 1,500 psi, 30 min and two cycles. After that, the extraction solvent was evaporated at 45 °C to produce crude extracts. The crude extracts were kept at 4 °C until γ-oryzanol quantification.

2.5 γ-Oryzanol analysis

The crude extracts were dissolved with 7.0 mL of isopropanol. The samples solution was centrifuged at 6,000 rpm for 15 min. The γ-oryzanol of germinated rice samples was determined by High Performance Liquid Chromatography (HPLC)- Waters 2996 photodiode array detector (Water model Water 600, USA) with Luna C18 HPLC column. The mobile phase consisted of methanol and acetonitrile with a ratio of 60:40 v/v. The diode array detector was set to monitor at 325 nm for γ-oryzanol content. The γ-oryzanol range of samples was calculated by using the standard calibration curve ($y = 17{,}325x - 2{,}913.6$, $R^2 = 0.9999$). The calibration curve was constructed with the external standard.

2.6 Statistical analysis

The γ-oryzanol content of germinated rice samples was presented as the mean ± standard deviation (SD) of the experiment performed in triplicate. Statistical differences were determined by one-way analysis of variance (ANOVA), and the differences were considered significant at P values less than 0.05.

3. Results and Discussion

3.1 Rice characteristic of Thai pigmented rice

The characteristic of pigmented Thai rice cultivars, including Khao Hom Mali-Nin, Khao RiceBerry and Khao Mali-Komen Surin was shown in Figure 2 and Table 1. The pigmented rice (brown rice) was identified as black for Khao Hom Mali-Nin, purple for Khao RiceBerry and red for Khao Mali-Komen Surin, respectively. The seeds length and seeds width of Khao Hom Mali-Nin and Khao RiceBerry had shown the similar size. In comparison, the length and width of the seeds of Khao Mali-Komen Surin had shown a shorter than Khao Hom Mali-Nin and Khao RiceBerry (Table 1).

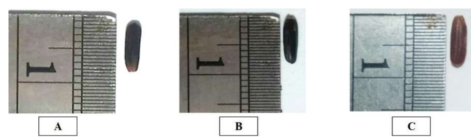

Figure 2 The rice seed without seed coat of three pigmented Thai rice cultivars A; Khao Hom Mali-Nin, B; Khao RiceBerry and C; Khao Mali-Komen Surin.

Table 1 The characteristic of rice seed without seed coat of pigmented Thai rice cultivars

Rice cultivars	Pigmented characteristic	Seeds length (cm)	Seeds width (cm)
Khao Hom Mali-Nin	Black	0.69± 0.011	0.40± 0.020
Khao RiceBerry	Purple	0.68± 0.015	0.37± 0.025
Khao Mali-Komen Surin	Red	0.63± 0.010	0.32± 0.010

3.2 The amount of γ-oryzanol of Thai pigmented rice

The amount of γ-oryzanol of Thai pigmented rice, including Khao Hom Mali-Nin, Khao RiceBerry and Khao Mali-Komen Surin was identified by the HPLC-photodiode array as shown in Figure 3. The highest γ-oryzanol content was obtained from Khao Hom Mali-Nin (475.03 ± 13.96 µg/g) followed by Khao RiceBerry (322.29 ± 10.29 µg/g). In comparison, the lowest γ-oryzanol content was obtained from Khao Mali-Komen Surin (183.71 ± 10.89 µg/g). The results indicated that the γ-oryzanol range of black and purple pigmented rice cultivars seems higher than red rice cultivars. The γ-oryzanol has been identified in many plants and was first reported from rice bran (9). This compound has also been reported in pigmented and non-pigmented rice (21). However, the pigmented rice's γ-oryzanol seem to have higher than non-pigmented rice's γ-oryzanol (13, 22). Boonsit et al. (6) also reported that the γ-oryzanol of purple rice had higher than non-pigmented rice. Furthermore, some pigmented rice cultivars (purple) from Malaysia, including Hitam, Hitam Keladi and Keladi seem to have higher γ-oryzanol than some red rice cultivars, including Bario Merah and Merah (23). Tsuzuki et al. (24) also reported that the Japanese black-purple rice varieties had γ-oryzanol content higher than Japanese red rice varieties. Our result showed that the black and purple rice cultivars, including Khao Hom Mali-Nin and Khao RiceBerry had γ-oryzanol range higher than red rice cultivars Khao Mali-Komen Surin (Figure 2 and 3). The previous report and our results indicated that the γ-oryzanol content of black and purple rice cultivars seems to be higher than red rice and non-pigmented rice cultivars.

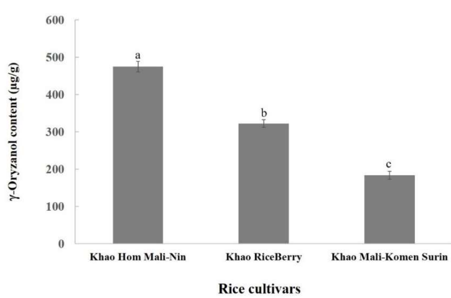

Figure 3 The γ-oryzanol content of three Thai pigmented rice cultivars, including Khao Hom Mali-Nin, Khao RiceBerry and Khao Mali-Komen Surin. The differences letters above the error bar indicate significant differences between the γ-oryzanol content of Thai pigmented rice cultivars ($P < 0.05$).

3.3 Effect of germination times and bio-liquid fertilizer on the γ-oryzanol content of germinated brown rice (Thai pigmented rice)

The γ-oryzanol content of germinated brown rice, treated with 1% (v/v) Bio-liquid fertilizer and water was identified by HPLC (Figure 4) and shown in Table 2. The γ-oryzanol content of germinated brown rice of Khao Hom Mali-Nin was in the range of 410.04 ± 29.15 to 521.43 ± 6.21 µg/g for treated with 1% (v/v) Bio-liquid fertilizer and 483.84 ± 7.4 to 536.10 ± 4.39 µg/g for treated with water. The γ-oryzanol content of 356.83 ± 8.11 to 383.83 ± 11.13 µg/g and 319.27 ± 3.06 to 412.68 ± 3.52 µg/g were obtained from germinated Khao RiceBerry which were treated with 1 % (v/v) Bio-liquid fertilizer and water, respectively. While the γ-oryzanol content of germinated Khao Mali-Komen Surin which were treated with 1% (v/v) Bio-liquid fertilizer and water was in the range of 202.54 ± 0.96 to 208.61 ± 0.27 µg/g and 184.64 ± 4.26 to 205.30 ± 1.12 µg/g respectively. Moreover, the γ-oryzanol content of Khao RiceBerry and Khao Mali-Komen Surin treated with water was increased when the germination increased at 12 to 48 h. Similar results were observed in Khao Hom Mali-Nin treated with 1 % (v/v) Bio-liquid fertilizer by the γ-oryzanol content increasing when germination times increased at 12 to 48 h. While the highest γ-oryzanol content of Khao RiceBerry and Khao Mali-Komen Surin treated with 1 % (v/v) Bio-liquid fertilizer was obtained from the germination times of 36 h for both rice cultivars and the highest γ-oryzanol content of Khao Hom Mali-Nin treated with water was also observed in 36 h.

The germination times affected the γ-oryzanol accumulation in rice during the germination process. Kiing et al. (25) reported that the γ-oryzanol content of germinated Sarawak rice cultivars from Malaysia was affected by the germination time from 4 to 24 h and the highest γ-oryzanol obtained from 24 h for germination duration. Our results showed the γ-oryzanol accumulation in Khao RiceBerry and Khao Mali-Komen Surin treated with water had increased when the germination duration increased from 12 to 48 h. At the same time, the γ-oryzanol accumulation in Khao Hom Mali-Nin increased when the germination duration increased from 12 to 36 h and slightly decreased at 48 h (Table 2). However, Kiing et al. (25) reported that the γ-oryzanol accumulation in some rice cultivars decreased after 24 h, which is similar to Khao Hom Mali-Nin of our results. The previous reports and our results indicate that the increasing germination duration affected the increase of γ-oryzanol accumulation in rice. Nevertheless, in some rice cultivars, the expansion of γ-oryzanol accumulation had limited by the rise of germination duration.

Salinity condition has been reported to affect the γ-oryzanol accumulation in rice (14). Shahzad et al. (26) reported that the ferulic acid of rice (*Oryza sativa* L.) had decreased under salt stress conditions. The alleviation of salt stress has been reported in rice plants (Shahzad et al. (26). Inoculation of fungi species such as *Glomus etunicatum*, *Glomus geosporum*, and *Glomus mosseae* in the salt stress condition had improved some bioactive compounds in some upland rice cultivars (27). The Bio-liquid fertilizer produced from saltpan residual affected the γ-oryzanol accumulation in rice. The γ-oryzanol of Khao RiceBerry treated with Bio-liquid fertilizer at germination times of 24 and 36 h had higher than the rice seeds treated with water. While at the germination duration of 48 h of Khao Hom Mali-Nin treated with Bio-liquid fertilizer had higher content than rice seeds treated with water. Moreover, the higher γ-oryzanol content was observed from germinated Khao Mali-Komen Surin treated with Bio-liquid fertilizer at the germination times of 12 to 36 h compared with rice seed treated with water at the same germination duration. The saltpan residual is the mix of salt, algae and microbiome species. Some species have been reported as the N_2-fixing and phosphate-solubilizing bacterium (19). The salt-tolerant bacteria such as *Swaminathania* halotolerant have been identified from wide rice and showed N_2-fixing and phosphate-solubilizing properties (20). Bio-liquid fertilizer was produced from saltpan residual and mixed with *Trichoderma* during the assembling process. Nitrogen has been reported effect to the growth and bioactive compounds in plants (28). Amarowicz et al. (29) reported that the phenolic compounds content of *Helianthus tuberosus* L. had been affected by N fertilization. Bio-liquid fertilizer consisted of 2.70-3.40 % of total Nitrogen, 1.05-1.40 % of total Phosphorus (P_2O_5) and 1.70-1.74 Potassium (K_2O). Thus, the results suggesting that plant nutrition and microbiome species in Bio-liquid fertilizer might explain why the γ-oryzanol content increased in pigmented rice cultivars under Bio-liquid fertilizer treatment.

4. Conclusions

The γ-oryzanol content of three Thai pigmented rice cultivars was investigated. The highest γ-oryzanol content was obtained from black rice cultivars. Germination times had affected the γ-oryzanol range of Thai pigmented rice cultivars. The increasing germination times seem to effectively increase γ-oryzanol accumulation in Thai pigmented rice cultivars. Bio-liquid fertilizer also affected the γ-oryzanol accumulation in Thai pigmented rice cultivars. The γ-oryzanol accumulation at 48 h germination times of Khao Hom Mali-Nin treated with Bio-liquid fertilizer had higher than this rice cultivar treated with water. Khao RiceBerry treated with Bio-liquid fertilizer and germinated at 12 to 24 h had higher γ-oryzanol content than germinated rice seeds treated with water. While Khao Mali-Komen Surin treated with Bio-liquid fertilizer and germinated at 12 to 36 h had the γ-oryzanol content higher than germinated rice seeds un-treated with Bio-liquid fertilizer.

Table 2 The γ-oryzanol content of germinated brown rice of three pigmented rice cultivars

Germination times (hr)	γ-Oryzanol content (µg/g)					
	Khao Hom Mali-Nin		Khao RiceBerry		Khao Mali-Komen Surin	
	1% (v/v) Bio-liquid fertilizer	Water	1% (v/v) Bio-liquid fertilizer	Water	1% (v/v) Bio-liquid fertilizer	Water
12	410.04 ± 29.15^{d}	483.84 ± 7.40^{b}	356.83 ± 8.11^{c}	319.27 ± 3.06^{c}	202.54 ± 0.96^{b}	184.64 ± 4.26^{b}
24	421.86 ± 20.37^{c}	491.23 ± 7.03^{b}	362.72 ±16.22^{bc}	322.03 ± 26.22^{c}	202.74 ± 3.95^{b}	185.37 ± 10.24^{b}
36	468.23 ± 10.42^{b}	536.10 ± 4.39^{a}	383.83 ± 11.13^{a}	389.58 ±28.92 ^{b}	208.61 ± 0.27^{a}	188.08 ± 0.39^{b}
48	521.43 ± 6.21^{a}	479.57 ± 12.03^{b}	364.13 ± 15.28^{b}	412.68 ± 3.52^{a}	200.80 ± 0.71^{b}	205.30 ± 1.12^{a}

The differences superscript letters between the column indicate significant differences between the γ-oryzanol content of three pigmented rice cultivars ($P < 0.05$).

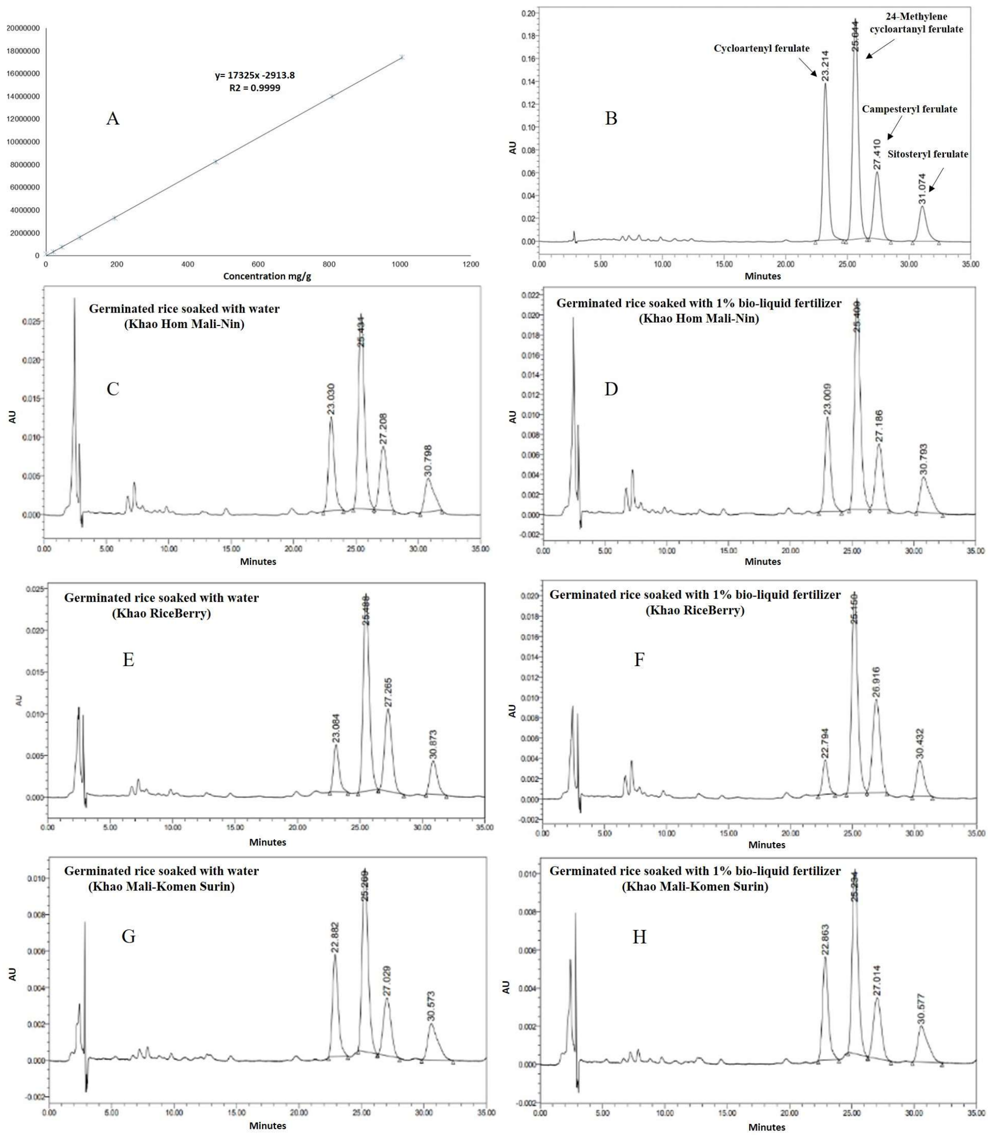

Figure 4 Calibration curve of standard γ-oryzanol (A); HPLC chromatogram of standard γ-oryzanol, (B); HPLC chromatogram of germinated rice treated with water (C, E and G) and germinated rice treated with 1% bio-liquid fertilizer (D, F and H).

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Declaration of conflicting interests

The authors declared that they have no conflicts of interest in the research, authorship, and this article's publication.

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