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# Individual $\gamma$ -Oryzanol (Steryl Ferulates) in brown grain and bran of jasmine rice and riceberry (*Oryza sativa* L.)

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**ABSTRACT:**  $\gamma$ -oryzanol (steryl ferulates), has been shown to be a major bioactive compound in rice. In this work, content of individual  $\gamma$ -oryzanols in brown rice and bran of jasmine rice and riceberry were determined by high performance liquid chromatography (HPLC). Four main steryl ferulates were identified as cycloartenyl ferulate (1), 24-methylenecycloartanyl ferulate (2), campesteryl ferulate (3), and sitosteryl ferulate (4). The total  $\gamma$ -oryzanol content (mg/100 g) in brown jasmine rice and jasmine rice bran ( $53.2 \pm 3.0$  and  $494.5 \pm 15.6$ ) was higher than that found in brown riceberry and riceberry bran ( $39.5 \pm 4.8$  and  $222.6 \pm 11.4$ ). The content (mg/100 g) of 1 ( $6.0 \pm 0.3$  and  $64.7 \pm 1.3$ ), 2 ( $28.5 \pm 1.5$  and  $280.3 \pm 8.1$ ) and 4 ( $7.2 \pm 0.5$  and  $60.2 \pm 4.0$ ) in brown jasmine rice and jasmine rice bran was higher than that observed in brown riceberry and riceberry bran 1 ( $4.5 \pm 0.6$  and  $27.6 \pm 1.5$ ), 2 ( $19.2 \pm 2.1$  and  $109.4 \pm 6.2$ ) and 4 ( $4.3 \pm 0.6$  and  $24.2 \pm 1.1$ ). However, the content of 3 in the brown jasmine rice and brown riceberry samples did not differ significantly.

**KEYWORDS:**  $\gamma$ -oryzanol, jasmine rice, riceberry, steryl ferulates.

## 1. INTRODUCTION

Rice (*Oryza sativa*), is the most widely consumed staple food for a large part of the world's human population. It is the main export of Thailand, especially white jasmine rice 105 (Dok Mali 105). Thailand has a large number of rice varieties, 3,500 kinds with different characters. Jasmine rice is a long-grain variety of fragrant rice (also known as aromatic rice). Its fragrance, reminiscent of pandan (*Pandanus amaryllifolius*) and popcorn, results from the rice plant's natural production of aromatic compounds, of which 2-acetyl-1-pyrroline is the most salient. Riceberry is a registered rice variety from Thailand, a cross-breed of Jao Hom Nin (JHN), a local non-glutinous purple rice and Khoa Dawk Mali 105. The outcome is a deep purple whole grain rice with softness and a delicious aftertaste. Riceberry has been favorite brown rice due to its health-promoting properties. Brown rice is more abundant in nutrients such as phytic acids, vitamins (thiamine, riboflavin, niacin, folic acid, and tocopherol), and minerals (magnesium, manganese, and zinc) than white rice.  $\gamma$ -oryzanol (steryl ferulates), which esterifies trans-ferulic acid and sterols or triterpenols, is well known to be the primary bioactive compound in brown rice [1]. Since  $\gamma$ -oryzanol was first identified in rice bran oil in 1954 [2], several other  $\gamma$ -oryzanol constituents and their derivatives have been found in rice. The four most predominant  $\gamma$ -oryzanol components in rice were determined to be cycloartenyl ferulate, 24-methylenecycloartanyl ferulate, campesteryl ferulate, and sitosteryl ferulate [3].  $\gamma$ -Oryzanol has been previously shown to display various biological activities, including antioxidant [4-6], anti-inflammatory [7], and anti-tumor activities. In this study, the total and individual content of  $\gamma$ -oryzanol (1-4, as shown in fig.1) in riceberry and jasmine rice samples was quantitatively determined by HPLC. The total and individual content of  $\gamma$ -oryzanol between whole grain and rice bran was compared.

## II. MATERIAL AND METHODS

### Materials

A jasmine rice and riceberry variety (*O. sativa*) were used to compare the total and individual content of  $\gamma$ -oryzanol between brown rice and bran of jasmine rice and riceberry.

**Chemicals**

The  $\gamma$ -oryzanol mixture was purchased from Sigma-Aldrich Co., Ltd. HPLC grade acetonitrile (MeCN), methanol were purchased from Merck Co., Ltd.

**Extraction of  $\gamma$ -oryzanol from brown rice and bran**

The  $\gamma$ -oryzanol of brown rice and bran were extracted according to the method described by Xu and Godber [8], with slight modifications. Briefly, the brown rice and bran were ground. The brown rice and bran powder (1 g) were placed in a thermostable screw cap test tube, and n-hexane (9 mL) and ethanol (EtOH, 1 mL) were added. The mixture was then sonicated for 5 min. After extraction using a heating block at 70 °C for 30 min, the mixture was centrifuged at 3000 rpm and cooled to 15 °C.

**HPLC analysis of  $\gamma$ -oryzanol**

Individual  $\gamma$ -oryzanol in brown rice was qualitatively and quantitatively analyzed using a photodiode array (PDA) HPLC system (Ultimate 3000, Thermo Scientific) operating at wavelengths ranging from 190 to 800 nm according to the modified method described by Xu and Godber [8], with slight modifications. Individual steryl ferulates ( $\gamma$ -oryzanol) were separated on an ODS-Hypersil column (4.6 mm  $\times$  250 mm, 5  $\mu$ m). Elution was achieved by using a solvent mixture of MeOH /MeCN 35:65 (v/v) as the mobile phase and the flow rate was 1.0 mL/min.

**III. RESULTS AND DISCUSSIONS**

To quantify the  $\gamma$ -oryzanol content in brown rice and rice bran, the  $\gamma$ -oryzanol fraction obtained from commercial  $\gamma$ -oryzanol mixture and rice extract were analyzed by HPLC at an analytical scale. The four main peaks were detected at retention times of 14.79 (peak 1), 16.04 (peak 2), 17.04 (peak 3), 19.64 (peak 4) min on the HPLC chromatogram (Fig. 2-3).

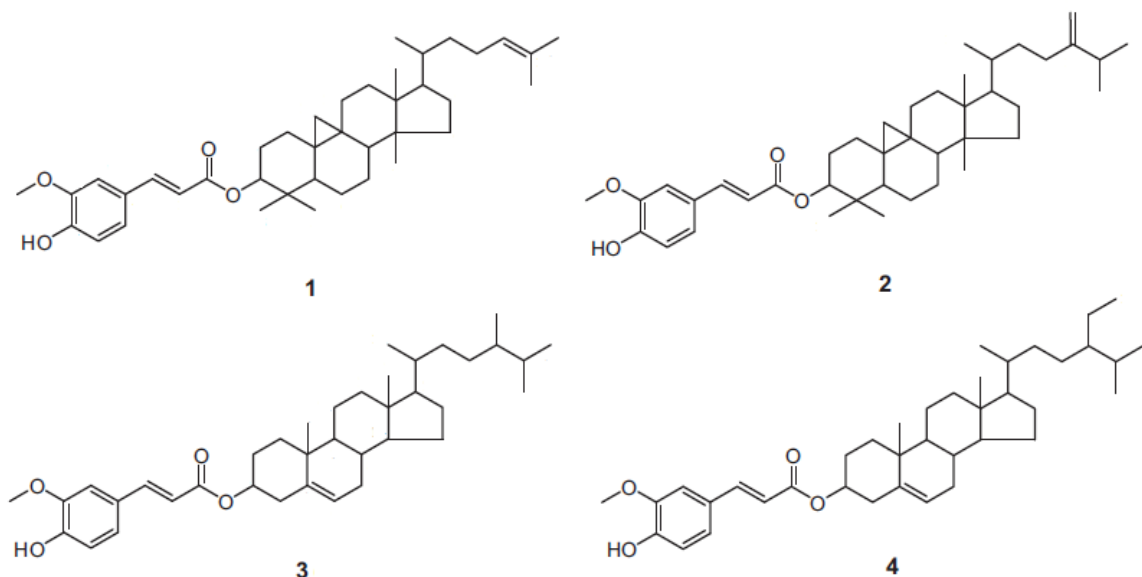


Fig. 1 Chemical structures of the steryl ferulates identified from the commercial  $\gamma$ -oryzanol mixture and rice extract

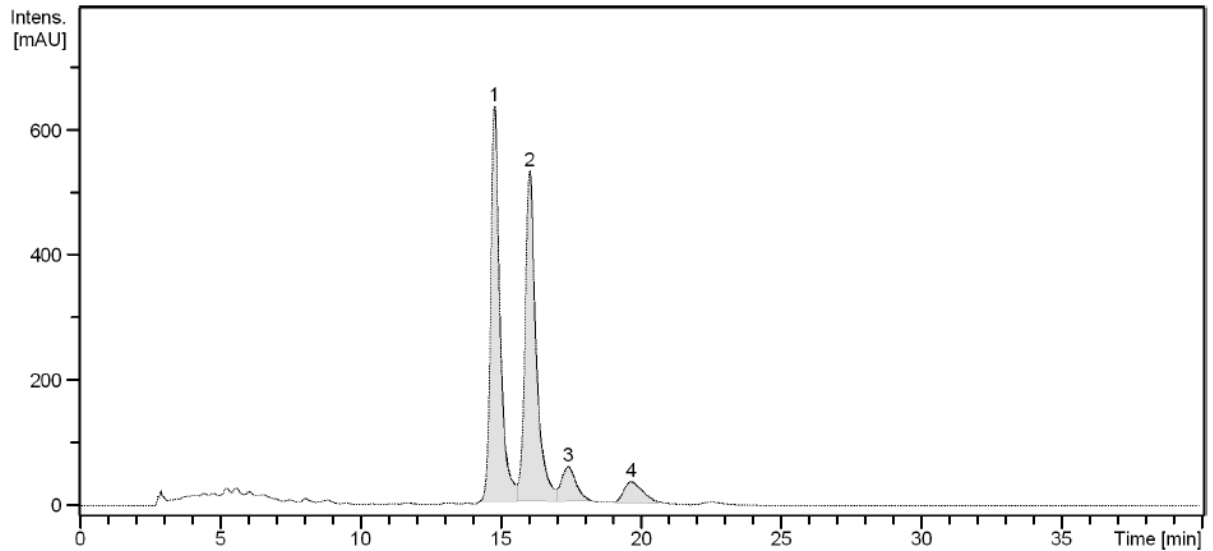
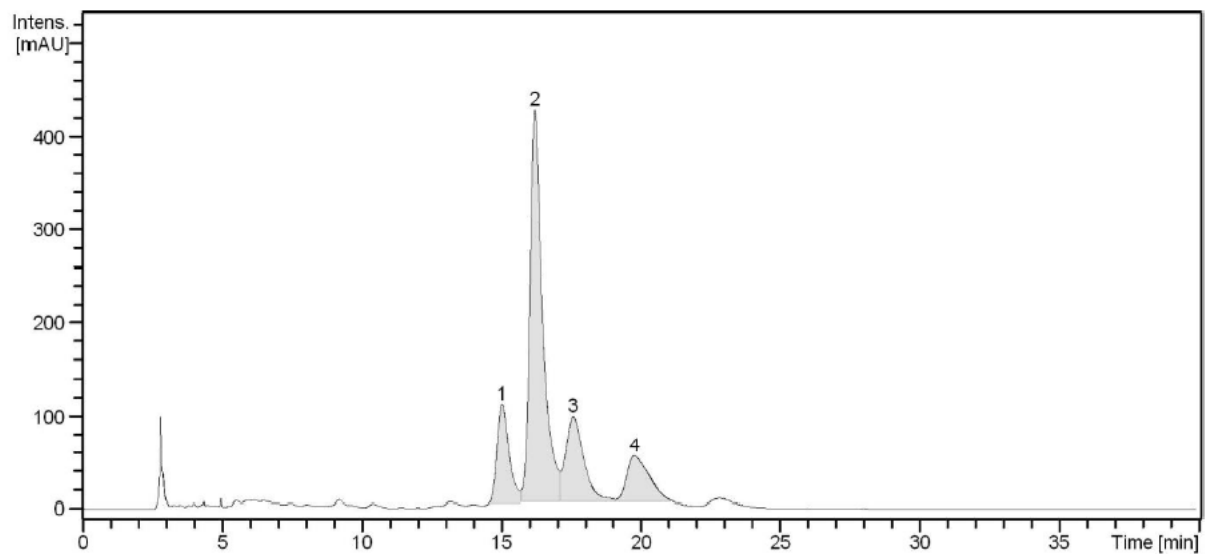
Fig. 2 HPLC chromatograms of the commercial  $\gamma$ -oryzanol mixture.

Fig. 3 HPLC chromatograms of the bran of jasmine rice extract.

Individual  $\gamma$ -oryzanol (1-4) in brown rice and bran of jasmine rice and riceberry were quantitatively analyzed by HPLC. In the present study, fig. 4 shows the content of compound 1 (mg/100 g) were as follows: jasmineRB ( $64.72 \pm 1.31$ ) > riceberryRB ( $27.67 \pm 1.60$ ) > jasmine ( $6.09 \pm 0.31$ ) > riceberry ( $4.51 \pm 0.64$ ).

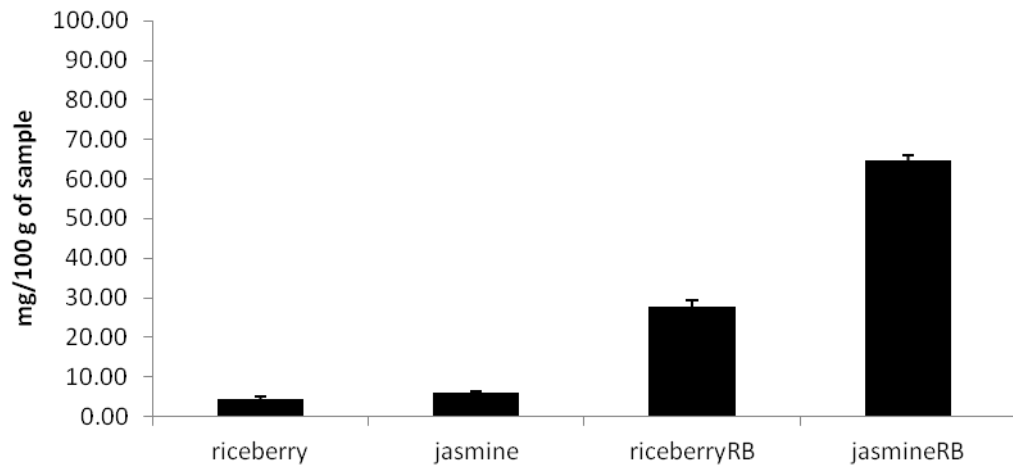
**compound 1**

Fig. 4 The amount of compound 1 in brown rice and rice bran of riceberry (riceberryRB) and jasmine (jasmineRB). Data points shown are averages of three measurement  $\pm$  SE

Fig. 5 shows the content of compound 2 (mg/100 g) were as follows: jasmineRB ( $280.36 \pm 8.15$ ) > riceberryRB ( $109.46 \pm 6.25$ ) > jasmine ( $28.57 \pm 1.53$ ) > riceberry ( $19.22 \pm 2.19$ ).

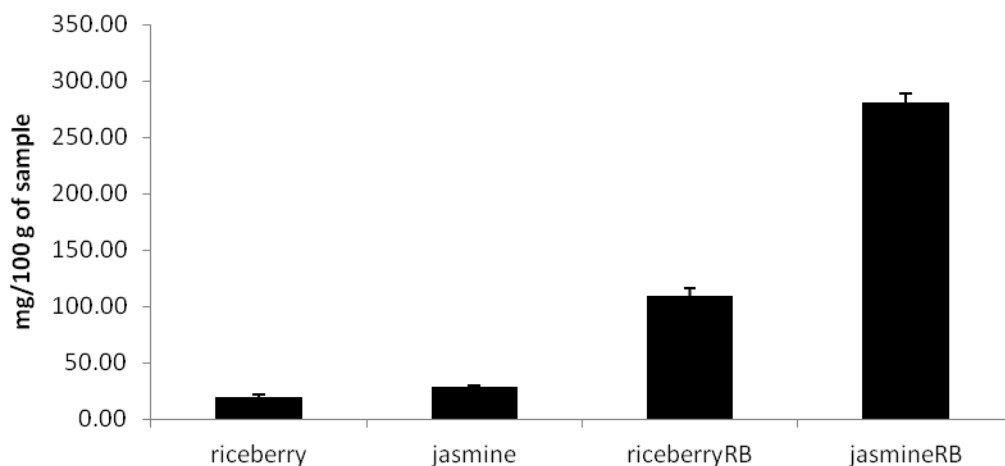
**compound 2**

Fig. 5 The amount of compound 2 in brown rice and rice bran of riceberry (riceberryRB) and jasmine (jasmineRB). Data points shown are averages of three measurement  $\pm$  SE

Fig. 6 shows the content of compound 3 (mg/100 g) were as follows: jasmineRB ( $89.26 \pm 3.50$ ) > riceberryRB ( $61.29 \pm 3.18$ ) > riceberry ( $11.48 \pm 1.43$ ) > jasmine ( $11.39 \pm 0.73$ ).

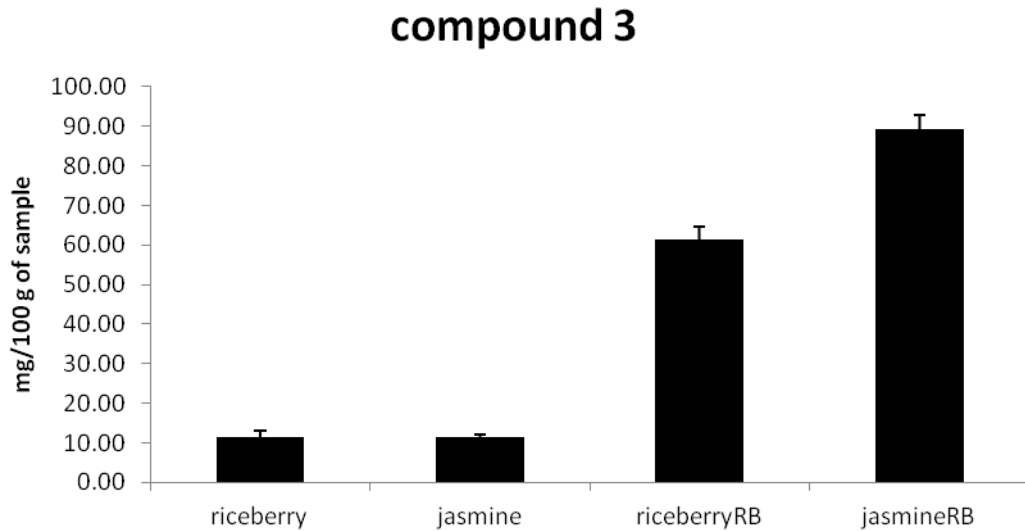


Fig. 6 The amount of compound 3 in brown rice and rice bran of riceberry (riceberryRB) and jasmine (jasmineRB). Data points shown are averages of three measurement  $\pm$  SE

Fig. 7 shows the content of compound 4 (mg/100 g) were as follows: jasmineRB ( $60.21 \pm 4.03$ ) > riceberryRB ( $24.24 \pm 1.18$ ) > jasmine ( $7.22 \pm 0.55$ ) > riceberry ( $4.34 \pm 0.68$ ).

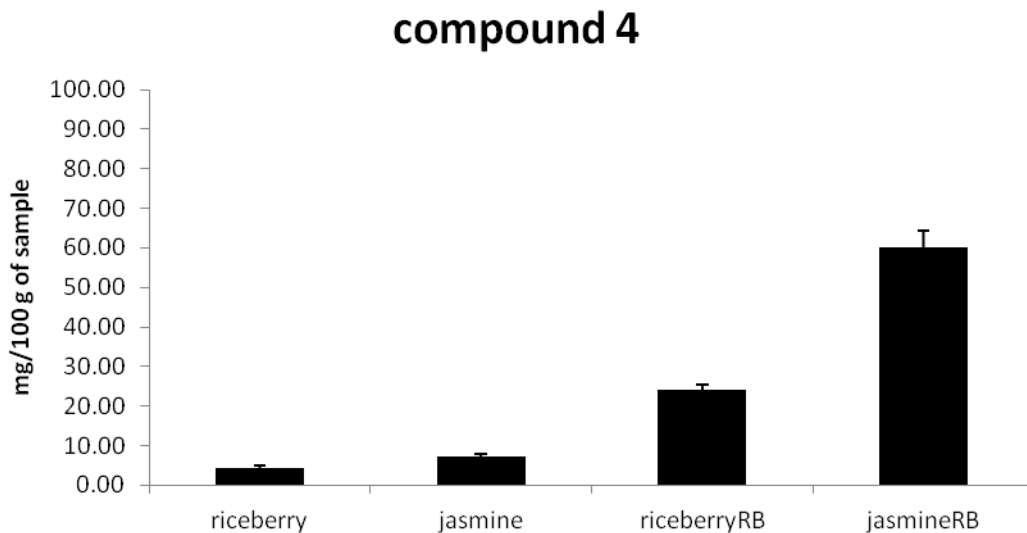


Fig. 7 The amount of compound 4 in brown rice and rice bran of riceberry (riceberryRB) and jasmine (jasmineRB). Data points shown are averages of three measurement  $\pm$  SE

#### IV. CONCLUSION

The content of individual  $\gamma$ -oryzanols in brown rice and bran of jasmine rice and riceberry were determined by high performance liquid chromatography (HPLC). Four main sterol ferulates were identified as cycloartenyl ferulate (1), 24-methylenecycloartenyl ferulate (2), campesterol ferulate (3), and sitosterol ferulate (4). The total  $\gamma$ -oryzanol content (mg/100 g) in brown jasmine rice and jasmine rice bran ( $53.2 \pm 3.0$  and  $494.5 \pm 15.6$ ) was higher than that found in brown riceberry and riceberry bran ( $39.5 \pm 4.8$  and  $222.6 \pm 11.4$ ). The content (mg/100 g) of 1 ( $6.0 \pm 0.3$  and  $64.7 \pm 1.3$ ), 2 ( $28.5 \pm 1.5$  and  $280.3 \pm 8.1$ ) and 4 ( $7.2 \pm 0.5$  and  $60.2 \pm 4.0$ ) in brown jasmine rice and jasmine rice bran was higher than



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that observed in brown riceberry and riceberry bran 1 ( $4.5\pm 0.6$  and  $27.6\pm 1.5$ ), 2 ( $19.2\pm 2.1$  and  $109.4\pm 6.2$ ) and 4 ( $4.3\pm 0.6$  and  $24.2\pm 1.1$ ). However, the content of 3 in the brown jasmine rice and brown riceberry samples did not differ significantly.

## V. ACKNOWLEDGEMENTS

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