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# Research Article
# γ-Oryzanol Content Screening in Local Brown Rice Samples from Chiang Mai, Thailand and Comparison Between Uncooked and Cooked Brown Rice

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## Abstract
Brown rice is the rich source of γ-oryzanol, which is important bioactive compounds and nutraceuticals with numerous potential health functions. So, promotion of brown rice consumption among consumer would be having data on γ-oryzanol in rice difference varieties as an alternative for selecting. The aim of present study was to determine γ-oryzanol in brown rice from 4 districts of Chiang Mai province, Northern Thailand and effected of cooking processes by streaming. The brown rice samples were from Mae-Cham, Om-Koi, Kanlayaniwattana and Chiang-Dao district and analyzed by modified HPLC with U/V detection. The γ-oryzanol content in raw brown rice samples were from 34.98-71.13 μg $g^{-1}$ raw brown rice and cooked brown rice were from 12.7-80.79 μg $g^{-1}$ raw brown rice. The most of γ-oryzanol content after cooking by streaming was decreased except some varieties i.e., Jao Luang, Nuk Roo, Kao Pum and Bue Ta Kree. The rice varieties that had increasing content of γ-oryzanol should be promoted to consume as good food and nutraceuticals health product.

Key words: Oryzanol, Chiang Mai, HPLC-UV, brown rice

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Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

There are many types of rice species grown in the every part of Thailand. The rice grown here is nurtured with natural rain water (Nokkoul and Wichitparp, 2009). The most of famers grow in small-household for food security. Rice (*Oryza sativa*) is main food for Thai and Asian people. Brown rice composes of many types of antioxidant compounds such as tocopherol, tocotrienol, oryzanol, polyphenol and anthocyanin (Lerma-Garcia *et al*., 2009; Aguilar-Garcia *et al*., 2007; Heinemann *et al*., 2008). Oryzanol is the one of bioactive compound for increase skin moisture and medical treatment (Juliano *et al*., 2005; Yasukawa *et al*., 1998; Berger *et al*., 2005).

Now a days, health problem are concerned among consumer, the healthy food will be alternative way to supplement such as tablet from extracted from rice or other natural product but not the right way. The most consumers prefer to eat polished rice with less valuable. So, brown rice may is the best way for promoting people to consume the good food with high value and can eat every day. Brown rice has been searched as a rich source of important bioactive compounds and nutraceuticals with numerous potential health functions (Kiing *et al*., 2009; Zubair *et al*., 2012).

The oryzanol in brown rice can be improved glucose metabolism, reduce hypothalamic endoplasmic reticulum stress (Kozuka *et al*., 2012). The previous studies reported ORZ has hold many benefit such a s reduced cholesterol anti-inflamatory, anti-cancer, anti-diabetic and antioxidant compounds (Kozuka *et al*., 2013). Moreover, oryzanol is known in medical treatment, oryzanol can reduce cholesterol in blood, reduce gathering of platelet, increase bile in stools and moderate the activity of disturbed brain (Kozuka *et al*., 2015).

Chiang Mai province located in northern part of Thailand. There are famous area for agriculture where has good source of water and good weather for vegetable and fruit cultivation especially rice cultivation. Several varieties of rice can growing up on upland (less water on the mountain) and low land (much water around river and irrigation area) (FAO., 2015). Because of the previous studied reported time and temperature cooking (Lin *et al*., 2015) have been affected to bioactive compounds such as physiochemical, nutritional and bioactive composition Chiang Mai province has ethnic people who live in hill tribe villages (i.e., Hmong, Lahu and Karen) and growing rice by themselves and have their own seed for cultivating every year. However, they also are cultivated rice for commercial so they had some rice varieties for selling to get the money. Here in, the aim of present study was to determine $\gamma$-oryzanol in brown rice from Chiang Mai province, Northern Thailand by using High performance liquid chromatography with U/V detection (HPLC-UV).

## MATERIALS AND METHODS

**Chemicals:** Oryzanol (purity 97%) was obtained from Sigma, USA. Methanol and acetonitrile were from J.T Baker, USA.

**Rice samples:** The rice seed samples were collected from four district of Chiang Mai provinces, northern Thailand i.e., and Om-Koi, Mae Cham, Kanlayaniwattana and Chiang Dao district, Chiang Mai province northern Thailand. The rice seed samples were kindly given from people who living in these 4 districts and keep in refrigerator 4°C until analyze. The rice varieties were reported in name of local people calling (Table 1).

Table 1: Rice varieties of brown rice sample from four districts of Chiang Mai province, Northern Thailand

| Om Koi | Mae Cham | Chiang Dao | Kanlayaniwattana |
|---|---|---|---|
| **Non-glutinous** | | | |
| Bue Ki | Muey Nawng | Ma Li 105 | Bue Pha Tho |
| Bue Kae | Khao Kai Pa | Ja Tae Nae | Bue Por Mor |
| Bue Pha Tho | Khao Jao Luang | Khao Kra Chee | Kao Pum |
| Bue Chai | Ma Li 105 | Bue Na Ma | Bue Phi |
| Bue Wae | Bue Ki | | |
| Bue Ta Kree | Hog Ra Sek | | |
| Bue Maew | Hog Ra Yang Rang | | |
| Dor Sam Duern | Kao Nuk Roo | | |
| | Kao Bi Yao | | |
| | Leb Nok | | |
| **Glutinous** | | | |
| Kao Lueng | | Ja Fu Ma or Ja Jia | |
| Gor Khor 14 (RD14) | | | |
| Gor Khor 6 (RD6) | | | |
| San-pah-tawng1 | | | |

**Preparation of brown rice samples:** Rice seed was individually separated brown rice from husky by hand press with wood mortar. All individual samples were ground into fine flour by using electronic blender.

**Effect of cooking to γ-oryzanol content**

**Cook rice samples preparation:** Ten grams of brown rice were added 30 mL of distilled water and stirred for dust washing. Then, drained 20 mL of water out and added 15 mL of distillation water and steam by electronic cooker for 40 min.

**Quantification of γ- oryzanol in rice samples**

**γ-oryzanol extraction:** Brown rice samples (raw rice and cooked rice) 2.5 g were exacted with 5 mL of acetonitrile: methanol (1:1 v/v) for 5 min by vortex vigorously and re-extract with the same solvent for 5 mL. The extracted solution were pooled and evaporated to dryness by rotary evaporator on water bath temperature 40°C. The extracted sample was dissolved with 1 mL of acetonitrile: methanol (1:1 v/v) and filtrated through syringe filter (0.45, PVDF). Ten microliter of filtrate was injected to HPLC for γ-oryzanol analyzing.

**High performance liquid chromatography with UV detection (HPLC-UV):** The γ-oryzanol content in extracted brown rice was determined by modifying method from Lee *et al*. (2011). A Reverse Phase High Performance Liquid Chromate (RP-HPLC) graph system (Agilent 1100, Agilent Technologies, Palo Alto, USA) equipped with Discovery C18, 150×4.6 mm, 5 µm, Supelco, USA. and a variable wavelength UV-Vis detector (Agilent 1100, Agilent Technologies, Palo Alto, USA) set at 325 nm was set for anzlyzing. The mobile phase consisted of methanol:acetonitrile in difference ratio were tried for good separation peaks. The flow rate 1.5 mL $min^{-1}$ and column temperature at 40°C was set.

**Method validation:** The difference ratio of methanol and acetonitrile were run as mobile phase for γ-oryzanol analysis and selecting the best one for further. The analytical method was validated in terms of linearity, Limit Of Detection (LOD), Limits Of Quantification (LOQ), recovery, accuracy and precision. The standard γ-oryzanol was added to pooled brown rice samples for method validation at 25, 50, 100 and 200 µg $mL^{-1}$.

**Calculation of γ-oryzanol concentration from rice:** The amount of γ-oryzanol was quantified by the area of γ-oryzanol from brown rice extracted against area of γ-oryzanol standard. The results were calculated on a raw weight of brown rice as following:

$$\text{Oryzanol concentration of raw brown rice (mg g}^{-1}\text{)} = \frac{\text{Concentration from curve}}{\text{Weight of rice}}$$

$$\text{Weight of true rice from cooked brown rice} = \frac{2.5´\text{weight of H}_2\text{O in cooked rice}}{\text{Weight total of cooked rice}}$$

## RESULTS

**Rice seed samples:** The brown rice samples were from four districts of Chiang Mai province. The samples were collected from local farmers who live and cultivate in their own farm. Most of people who gave the samples are ethnic people i.e. Om Koi district was Karen and Lua, Mae Cham district was Karen, Kanlyaniwattata district was Karen and Chiang Dao district was Lahu. The rice samples were difference varieties; they grow both commercial such as San Pa Tong, Mali105, RD6 and RD14, local rice varieties such as Bue Ki, Bue Chai, Ja Fu Ma and Hog Ra Sak and were grown by upland or low land cultivation. The name of local rice varieties were shown in name of their own language Table 1.

**Validation of high performance liquid chromatographic-U/V detection method for detecting γ-oryzanol in rice samples**

**Mobile phase selection:** The difference ratio of methanol and acetonitrile were compared by using four major peaks separation in Fig. 1, then methanol and acetonitrile at ratio 70:30 v/v was selected and used as mobile phase because peak show good separation and high peak area. The mobile phase was used for running all standard and samples. The four major peaks of standard of γ-oryzanol and extracted brown rice sample were used for reported in total γ-oryzanol in unit microgram per gram raw brown rice weigh. Calibration curves were linear with very good correlation coefficients (r>0.996). The concentration range of γ-oryzanol is 10-200 mg $L^{-1}$. The recovery and Precision were calculated by using pooled rice sample added with 25, 50, 100 and 200 µg $L^{-1}$ of γ-oryzanol standard. The range of recovery was 73-95.7%. The precision values were 9.1-11.2%. Limit Of Detection (LOD) and Limit Of Quantitation (LOQ) were 3.0 µg $L^{-1}$ and 10 µg $g^{-1}$, respectively. So, the modified method could be used for sample analyzing.

**γ-oryznol content in raw rice samples:** Four peaks of γ-oryzanol separated via HPLC-UV. The amount of total γ-oryzanol in brown rice range from 34.98-71. The 13 µg $g^{-1}$ raw brown rice. The Leb Nok variety gave the highest γ-oryzanol while Mali 105 gave the lowest concentration. The highest brow rice of Om-Koi, Mae Cham, Chiang Dao and Kanlayaniwatta district were Bue Kae, Leb Nok, Kra Chee and

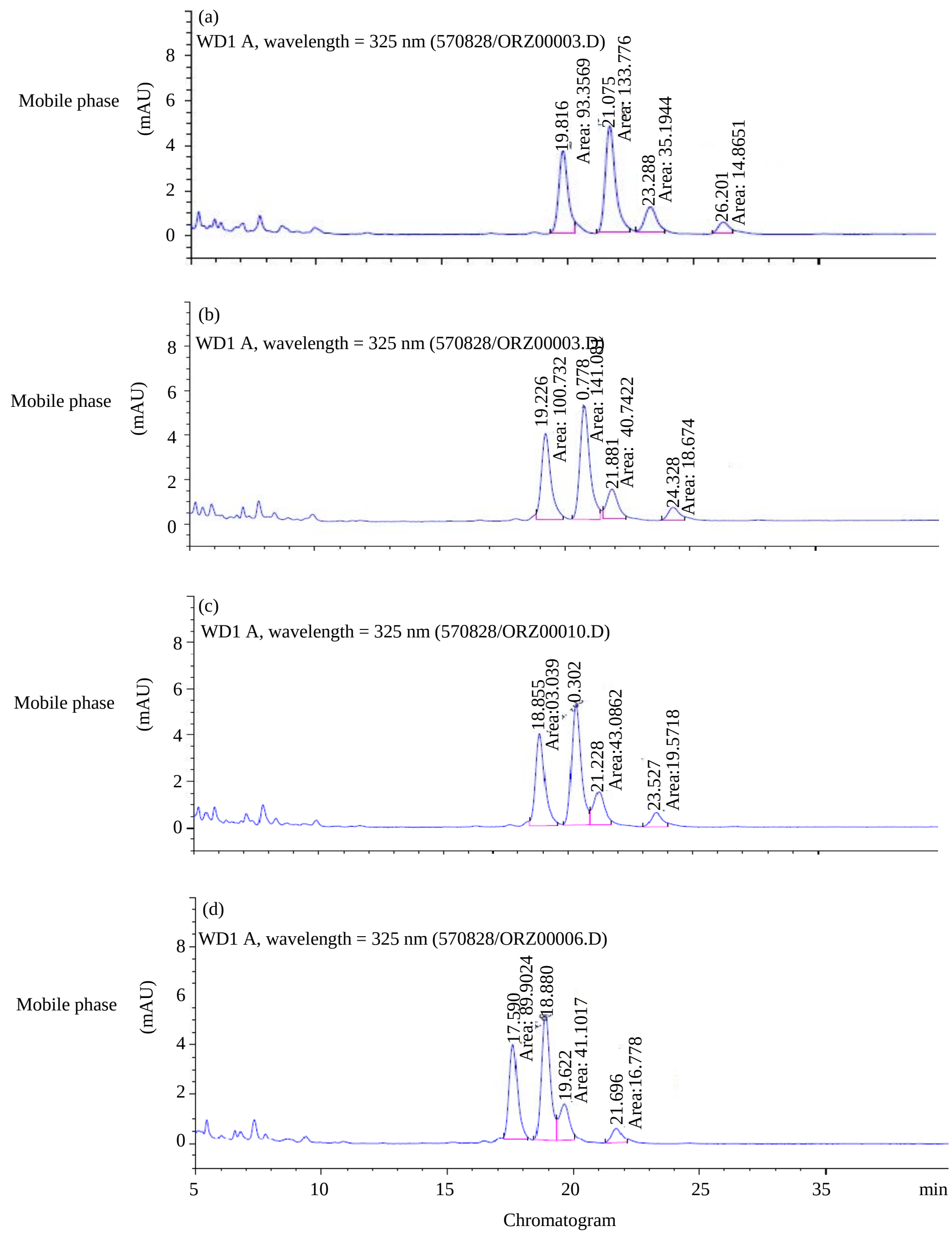


Fig. 1(a-d): Chromatogram of standard γ-oryzanol run by HPLC-U/V at difference ratio of methanol and acetonitrile, (a) MeOH: ACN (70:30), (b) MeOH:ACN (85:15), (c) MeOh:ACN (90:10) and (d) MeOH

Bue Chai, respectively. The γ-oryzanol in both glutinous and non-glutinous rice samples were equally (Table 2).

**Effect of cooking process by streaming in brown rice samples:** The concentrations of γ-oryzanol after cooked by steaming were from 12.7-80.79 μg $g^{-1}$ raw brown rice. The highest γ-oryzanol in brown rice was Jao Luang at concentration 80.79 μg $g^{-1}$ raw brown rice and the lowest was Gor Kor 14 (RD14) at concentration 12.7 μg $g^{-1}$ raw brown rice.

The most of γ-oryzanol in brown rice were decrease after cooking except Jao Luang, Nuk Roo, Kao Pum and Bue Ta Kree (Table 2).

## DISCUSSION

The γ-oryzanol content in rice samples were calculated by 4 major peak against standard compounds (Sakunpak *et al*., 2014). The HPLC-UV method validation of the present studied

Table 2: γ-oryzanol content in uncooked and cooked brown rice from four districts of Chiang Mai, Thailand

| | γ-oryzanol (μg $g^{-1}$ raw brown rice) | | |
|---|---|---|---|
| District and varieties | Uncooked | Cooked | Change (%) |
| **Maecham** | | | |
| Ma Li 105 | 34.98 | 31.79 | -9.12 |
| Muey Nawng | 43.80 | 31.72 | -32.22 |
| Kai Pa | 48.41 | 33.04 | -30.78 |
| San-pah-tawng 1 | 48.57 | 29.75 | -39.35 |
| Kao Bi Yao | 50.79 | 33.46 | -34.12 |
| Hog Ra Yang Rang | 50.96 | 42.42 | -16.76 |
| Jao Luang | 54.83 | 80.79 | 47.35 |
| Gor Khor 6 (RD6) | 55.13 | 24.48 | -55.60 |
| Nuk Roo | 58.17 | 59.19 | 1.75 |
| Kao Lueng | 61.82 | 17.83 | -71.16 |
| Gor Khor 14 (RD14) | 68.36 | 12.70 | -81.42 |
| Leb Nok | 71.13 | 65.10 | -9.60 |
| Hog Ra Sek | 77.20 | 49.77 | -35.53 |
| **Kanlaya-niwattana** | | | |
| Kao Pum | 47.49 | 50.70 | 6.76 |
| Bue Pha Tho | 49.03 | 43.40 | -11.48 |
| Bue Por Mor | 49.48 | 46.06 | -6.91 |
| Bue Phi | 52.06 | 48.21 | -7.40 |
| **Om-koi** | | | |
| Dor Sam Duern | 39.71 | 25.08 | -36.84 |
| Bue Maew | 40.61 | 31.54 | -22.33 |
| Bue Pha Dao | 41.66 | 28.88 | -30.68 |
| Kao Ngew | 47.41 | 18.71 | -60.54 |
| Bue Wae | 48.12 | 32.02 | -32.48 |
| Bue Chai | 49.38 | 42.98 | -12.96 |
| Bue Ki | 50.01 | 47.55 | -4.03 |
| Blue Pha Dao | 57.21 | 35.28 | -38.33 |
| Bue Ta Kree | 59.44 | 61.53 | 3.52 |
| Bue Kae | 69.40 | 25.92 | -62.65 |
| **Chiang Dao** | | | |
| Ma Li 105 | 36.10 | 35.11 | -2.74 |
| Bue Na Ma | 37.73 | 35.32 | -6.39 |
| Ja Fu Ma | 43.15 | 23.09 | -46.49 |
| Kra Chee | 60.84 | 42.01 | -30.95 |
| Ja Tae Nae | 61.71 | 41.44 | -32.85 |

were showed good recovery and low limit of detection and simultaneous to determine γ-oryzanol in brown rice samples.

Brown rice is immediately milled with either an abrasive or friction mill. It has been reported that types of liner significantly affect the husking performance (Shitanda *et al*., 2001; Roy *et al*., 2011). The highest amount of present study compare with others. The γ-oryzanol contents in brown rice samples were range of 34.98-71.The 13 μg $g^{-1}$ raw brown rice. The γ-oryzanol in this study had higher content than IR64 and BPT fine type Jayadeep and Malleshi (2011) and brown rice from European origin (Miller and Engel, 2006). However, the γ-oryzanol contents in brown rice from the present study were lower than wild rice from North America (Aladedunye *et al*., 2013) and 10 time lower color rice when comparison with glutinous purple ricefrom from previous report (Boonsit *et al*., 2010). So, the difference of γ-oryzanol content among these various rice types may be depended on growing environment and rice genotype (Bergman and Xu, 2003).

The effect of cooking by streaming (90-100°C) decreased γ-oryzanol content in brown rice but had some variety showed higher content than raw brown rice. The previous reported showed γ-oryzanol and other bioactive compounds after cooking at 100°C with water higher than raw brown rice (Lin *et al*., 2015) difference from present study may from cooking process and genotype of rice.

## CONCLUSION

The modified HPLC with UV detection was successfully used for analyzing γ-oryzanol in brown rice with good separation of 4 major peaks and suitable ratio of methanol: acetonitrile as mobile phase. The content of γ-oryanol in uncooked and cooked brown rice samples were 34.98-71.13

and 12.7-80.79 µg $g^{-1}$ raw brown rice, respectively. The cooking by streaming was shown effect to γ-oryzanol content to decreasing so selection of rice varieties with no effect from stream process would be alternative consumption.

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## REFERENCES

Aguilar-Garcia, C., G. Gavino, M. Baragano-Mosqueda, P. Hevia and V.C. Gavino, 2007. Correlation of tocopherol, tocotrienol, γ-oryzanol and total polyphenol content in rice bran with different antioxidant capacity assays. Food Chem., 102: 1228-1232.

Aladedunye, F., R. Przybylski, M. Rudzinska and D. Klensporf-Pawlik, 2013. γ-oryzanols of North American wild rice (*Zizania palustris*). J. Am. Oil Chem. Soc., 90: 1101-1109.

Berger, A., D. Rein, A. Schafer, I. Monnard, G. Gremaud, P. Lambelet and C. Bertoli, 2005. Similar cholesterol-lowering properties of rice bran oil, with varied γ-oryzanol, in mildly hypercholesterolemic men. Eur. J. Nutr., 44: 163-173.

Bergman, C.J. and Z. Xu, 2003. Genotype and environment effects on tocopherol, tocotrienol and γ-oryzanol contents of Southern U.S. rice. Cereal Chem., 80: 446-449.

Boonsit, P., P. Pongpiachan, S. Julsrigival and D. Karladee, 2010. Gamma oryzanol content in glutinous purple rice landrace varieties. CMU J. Nat. Sci., 9: 151-157.

FAO., 2015. What are sustainable rice systems? FAO, Rome, Italy. http://www.fao.org/griculture/crops/thematic-sitemap/theme/spi/scpi-home/managing-ecosystems/sustainable-rice-systems/rice-what/en/

Heinemann, R.J.B., Z. Xu, J.S. Godber and U.M. Lanfer-Marquez, 2008. Tocopherols, tocotrienols and γ-oryzanol contents in *Japonica* and *Indica* subspecies of rice (*Oryza sativa* L.) cultivated in Brazil. Cereal Chem., 85: 243-247.

Jayadeep, A. and N.G. Malleshi, 2011. Nutrients, composition of tocotrienols, tocopherols and γ-oryzanol and antioxidant activity in brown rice before and after biotransformation. CyTA-J. Food, 9: 82-87.

Juliano, C., M. Cossu, M.C. Alamanni and L. Piu, 2005. Antioxidant activity of gamma-oryzanol: Mechanism of action and its effect on oxidative stability of pharmaceutical oils. Int. J. Pharm., 299: 146-154.

Kiing, I.C., P.H. Yiu, A. Rajan and S.C. Wong, 2009. Effect of germination on γ-oryzanol content of selected sarawak rice cultivars. Am. J. Applied Sci., 6: 1658-1661.

Kozuka, C., K. Yabiku, S. Sunagawa, R. Ueda and S.I. Taira *et al*., 2012. Brown rice and its component, γ-oryzanol, attenuate the preference for high-fat diet by decreasing hypothalamic endoplasmic reticulum stress in mice. Diabetes, 61: 3084-3093.

Kozuka, C., K. Yabiku, C. Takayama, M. Matsushita, M. Shimabukuro and H. Masuzaki, 2013. Natural food science based novel approach toward prevention and treatment of obesity and type 2 diabetes: Recent studies on brown rice and γ-oryzanol. Obesity Res. Clin. Pract., 7: e165-e172.

Kozuka, C., S. Sunagawa, R. Ueda, M. Higa and H. Tanaka *et al*., 2015. γ-oryzanol protects pancreatic β-cells against endoplasmic reticulum stress in male mice. Endocrinology, 156: 1242-1250.

Lee, J.S., M. Farooq and D.J. Lee, 2011. Relationship of soluble phenolics and γ-oryzanol contents with antioxidant activity in pigmented rice. Crop Environ., 2: 8-14.

Lerma-Garcia, M.J., J.M. Herrero-Martinez, E.F. Simo-Alfonso, C.R.B. Mendonca and G. Ramis-Ramos, 2009. Composition, industrial processing and applications of rice bran γ-oryzanol. Food Chem., 115: 389-404.

Lin, T.C., S.H. Huang and L.T. Ng, 2015. Effects of cooking conditions on the concentrations of extractable tocopherols, tocotrienols and γ-oryzanol in brown rice: Longer cooking time increases the levels of extractable bioactive components. Eur. J. Lipid Sci. Technol., 117: 349-354.

Miller, A. and K.H. Engel, 2006. Content of γ-oryzanol and composition of steryl ferulates in brown rice (*Oryza sativa* L.) of European origin. J. Agric. Food Chem., 54: 8127-8133.

Nokkoul, R. and T. Wichitparp, 2009. Quality of local upland rice seeds produced under organic farming system. Asian J. Food Agro-Ind., 2: S343-S348.

Roy, P., T. Orikasa, H. Okadome, N. Nakamura and T. Shiina, 2011. Processing conditions, rice properties, health and environment. Int. J. Environ. Res. Public Health, 8: 1957-1976

Sakunpak, A., J. Suksaeree, P. Pathompak, T. Charoonratana and N. Sermkaew, 2014. Antioxidant individual γ-oryzanol screening in cold pressed rice bran oil different Thai rice varieties by HPLC-DPPH method. Int. J. Pharm. Pharmaceut. Sci., 6: 592-597.

Shitanda, D., Y. Nishiyama and S. Koide, 2001. PH-postharvest technology: Performance analysis of an impeller husker considering the physical and mechanical properties of paddy rice. J. Agric. Eng. Res., 79: 195-203.

Yasukawa, K., T. Akihisa, Y. Kimura, T. Tamura and M. Takido, 1998. Inhibitory effect of cycloartenol ferulate, a component of rice bran, on tumor promotion in two-stage carcinogenesis in mouse skin. Biol. Pharm. Bull., 21: 1072-1076.

Zubair, M., F. Anwar, M. Ashraf and M.K. Uddin, 2012. Characterization of high-value bioactives in some selected varieties of Pakistani rice (*Oryza sativa* L.). Int. J. Mol. Sci., 13: 4608-4622.