

Research Article

Comparison of proximate composition, bioactive compounds and antioxidant activity of rice bran and defatted rice bran from organic rice and conventional rice

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Abstract

Rice bran and defatted rice bran is a by-product from a rice milling and the production of rice bran oil which is mostly obtained from paddy rice grown under conventional farming systems. Currently, organic farming systems have been gaining in popularity. However, this agricultural system which strictly limits using chemicals may affect chemical composition and antioxidant activity of rice kernel, but no investigation has been documented on the effect of the growing system of rice on bioactive compounds and antioxidant activity of rice bran and defatted rice bran, therefore, this study was conducted. Organic rice bran was obtained from Khao Dawk Mali-105 cultivar grown under organic system whereas conventional rice bran was from rice grown under conventional farming system. Rough rice was dehulled and milled to yield the rice bran with degree of milling of 8%. Rice bran was extracted using two different methods, hexane extraction and enzymatic extraction. The oil fraction was separated whilst the residue was defatted rice bran (DRB). Defatted rice bran of organic and conventional rice bran extracted by hexane was also prepared for comparison. The results revealed that the growing system had significant effect on some chemical components. The ORB and ODRB contained higher levels of crude fibre, ash carbohydrate, total phenolic compounds and α -tocopherol. The oil extraction using enzymes yielded a higher concentration of α -tocopherol and the strongest antioxidant activity evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging as well as total antioxidant activity.

Keywords: defatted rice bran, antioxidant activity, oryzanol, organic farming, Thailand

Introduction

Organic agriculture is an ecological production management system that promotes and enhances biodiversity, biological cycles and soil biological activity. It is based on minimal use of off-farm inputs and on management practices that restore, maintain and enhance ecological harmony [1]. The organic farming is characterized by the prohibition of a majority of synthetic chemicals in both crop and livestock production [2]. The term “conventional” farming is also referred to as regular farming system which is widely applied to any non-organic farming system and relies on external inputs to achieve high production yields [2]. The organic agricultural is wide ranging and overall seek to promote the development of a food production system that is socially, ecologically, and economically sustainable. The key principles and practices of organic food production aim to encourage and enhance biological cycles within the farming system to maintain and increase long-term fertility of soils, to minimize all forms of pollution, to avoid the use of synthetic fertilizers and pesticides, to maintain genetic diversity of the production system, to consider the wider social and ecological impact of the food production and processing system, and to produce food of high quality in sufficient quantity [3].

Consumers demand organic products because they believe they are more flavorful and respectful to the environment and human health. However, there is still having some controversies over the perceived quality advantage of organically grown foods. Several studies with organic and conventional food productions have been conducted including potato, wheat, rye, and a number of fruits and vegetables as well as meat products as shown in Table 1 [3].

Barrett et al., [4] studied organically grown tomatoes focusing on commercial production of processing tomatoes, with compared organic and conventional fields. The results indicated that tomato juice prepared from organic on some farms was significantly higher in soluble solids (degrees Brix), higher in consistency, and titratable acidity. Mitchell et al., [5] reported a ten years research on the influence of organic and conventional crop management practices on the content of flavonoids in tomatoes. Comparisons of analyses of archived samples from conventional and organic production systems demonstrated statistically higher levels ($P < 0.05$) of quercetin and kaempferol aglycones in organic tomatoes. Perez-Lopez et al., [6] indicated the effects of conventional, integrated, and organic farming on color, minerals, and carotenoids of sweet pepper fruits (*Capsicum annuum*), cv. Almuden. The experimental results proved that organic farming provided peppers with the highest intensities of red and yellow colors, contents of minerals, total carotenoids, and finally, organic red peppers was considered as those having the highest antioxidant activity of all studied peppers (agricultural farming and development stage).

Table 1. Summary of studies comparing the nutritional value and general quality of organically and conventionally grown food as purchased from retailers.

Study	Products tested	Study design	Nutrients analyzed	Key results
Anon	Green bean, tomatoes, capsicum, silver-beet	Samples from certified organic farm and supermarket	Ca, K, mg, Na, Fe, Zn, Vitamin C, carotene	Vitamin C and carotene levels similar in organic and conventional produce; mineral levels were higher in all organic products
Cooklin and Thomson	Tomatoes, potatoes, sweet pepper, carrots, lettuce, apples, grapes	organic and conventional samples from 5 retail outlets each week (18 week period), over 80% of organic products labeled as certified	Visible quality characteristics (bruises, tearing, insect damage, discoloration)	Visible quality of organic and conventional products was frequently indistinguishable. Organic carrot, leaf lettuce, pepper, and potatoes had more defects.
Pither and Hall	Apples, carrots, green cabbage, potatoes, tomatoes	Products were purchased from variety of retailers, 30 sample each of organic and conventional	Moisture, total solids, vitamin C, sugar, starch, Fe, Zn, K	Results were variable, apples: vitamin C higher in organic, Sugars and vitamin C higher in conventional carrots, green cabbage, potatoes, tomatoes
Smith	Apples, pears, potatoes, wheat, sweetcorn, baby food	Samples were purchased over two years from stores	Range of minerals	Higher levels of some minerals in all organic products except baby food.

Source: [3]

Recently the popularity of organic rice has increased in many regions around the world even though the organic rice market remains relatively immature. The decreased cost of production per acre and environmentally-friendly production methods have become appealing to some producers. However the effect of farming practice on production yields, chemical compositions, bioactive components compared to conventional production have been still a question for most producers. Rice is consumed as a whole kernel which commonly produced by milling and leaving a rice bran as a by-product. It corresponds to approximately 10 % of the total rice grain [7,8] and the potential of producing rice bran at the global level is 27.3 million tonnes [3]. Although rice bran is a good source of proteins, dietetic fibers and functional compounds such as oryzanol and vitamin E [7,9,10], rice bran is at present underutilized as a food material. It basically is used for animal feed; only small portion is used for human consumption and mainly used for rice bran oil production. Rice bran is the source of a high quality vegetable oil (rice bran oil, RBO), which has attracted much medical attention due to its strong hypocholesterolemic properties primarily attributable to its balanced fatty acid composition and high levels of antioxidant phytochemicals such as oryzanols, tocopherols and tocotrienols [11,12]. Rice bran oil extraction produces deoiled or defatted meal as the by-product in large quantities per year. Defatted rice bran, a by-product of rice bran oil extraction, is a good source of insoluble dietary fibre [13], protein, phytic acid, inositol and vitamin B [14,15]. The main objective of the present study was to investigate the effect of growing system on chemical compositions and antioxidant efficacy of rice bran and defatted rice bran.

Materials and Methods

Chemicals

Standard α -tocopherol was purchased from Sigma-Aldrich Chemical Co., (St. Louis, Mo, USA). HPLC grade methanol, acetonitrile, hexane, ethyl acetate and ethanol were purchased from BHD (Poole, UK). γ -Oryzanol standard was purchased from Tsuno food industrial Co., Ltd. (Wakayama, Japan) Gallic acid, 1, 1-diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) were obtained from Fluka Chemical (Buchs, Switzerland). All chemicals and reagents were an analytical grade.

Rice bran samples

The samples of rice bran were obtained from the milling of rough rice of *Oryza stiva* L. CV. Khao Dawk Mali-105. The organic rice bran (ORB) was from organic rough rice grown in certified organic farming in Surin province, Thailand. The conventional rice bran (CRB) was from rough rice grown under a conventional practice. Both types of samples were harvested in 2008 crop year. Rough rice was de-hulled and milled to yield the rice bran with degree of milling of 8%.

Preparation of rice bran

Rice bran was stabilized using ohmic heating was applied by following the method described by [16,17] with some modifications. The bran (180g) were added with deionized water to reach moisture content of 30 % wet basis and then placed in the ohmic heating unit (lab scale). The electrical field strength of 150 V/cm at a frequency of 50 Hz were applied to obtain the temperature at 105°C and then rice bran was cooled to room temperature and kept in polyethylene(zipper-top) at -25°C until use.

Rice bran oil extraction and defatted rice bran preparation

Rice bran oil extraction using hexane

The hexane extraction was carried out by using the Soxhlet method following the method of [18]. The rice bran was placed into thimble paper cone using n-hexane solvent in soxhlet extractor and extracted for 2 h. The oil was separated and then defatted rice bran remained in the chamber was collected and dried in oven. Defatted rice bran low temperature (-25°C) until analysis.

Rice bran oil extraction using enzymes

The extraction was done according to the method reported by [19] with few modifications. The rice bran was added with distilled water (rice bran to water ratio 1:5 w/v), the pH was adjusted to 4.75 with 0.1 N HCL and then cellulose and hemicellulase were added. The mixture was incubated at 37°C for 3 hr in shaking water bath at 80 rpm [20]. After that, the pH was adjusted to 7.0 with 0.1 N NaOH, and then α -amylase and protease were added into the slurry (40°C) with shaking for 18 h (overnight). The oil was recovered by heating on hot plate at 50°C for 30 min, centrifuged at 8000 rpm (4°C) for 20 min and frozen prior to scraping and collecting the crude rice bran oil. The residual meal was defatted rice bran, which was dried overnight at 85±2°C in hot air oven. The defatted rice bran was kept in low temperature (-25°C) until use. The rice bran and defatted rice bran samples were used to analyze proximate components, bioactive compounds and antioxidant activity.

Determination of proximate compositions

The proximate composition analyses were determined by following the methods of AOAC [18]. Reducing sugar was determined by the method reported by Miller [21].

Extraction of rice bran

Finely ground bran samples (5.0g) were extracted in 80% methanol (25 mL) by placing the mixture in a sonicator for 10 minutes. The mixture was filtered and residue was subjected to the same procedure twice further. The residue was extracted with 0.15 mol/L hydrochloric acid and the extracts were combined and filtered through filter paper, evaporated to dryness under reduced pressure at 45°C by a rotary evaporator, and stored at -25°C until use.

Determination of bioactive compounds**Determination of total phenolic content [9]**

The reaction was initiated by mixing 0.2 mL of appropriate diluted rice bran extract, 0.8 mL of freshly prepared diluted Folin-Ciocalteu reagent and 2 mL of 7.5% sodium carbonate. The volume of the resulting mixture was adjusted to 7 mL by deionized water and placed in dark for 2 h to ensure completion of reaction. The absorbance of resulting blue-colored mixture was measured at 765 nm by spectrophotometer (Shimadzu, Japan). Gallic acid was used as calibration standard and results were calculated as gallic acid equivalent (mg) per gram (g) of bran.

Determination of α -Tocopherol γ -Oryzanol [22,23]

Rice bran (1g) was extracted by following the method reported by [23]. Prior to HPLC analysis, the extracts were filtered through a 0.45 mm syringe filter. An analysis of γ -oryzanol and α -tocopherol was performed, using the reversed phase high performance liquid chromatography (RP-HPLC), according to the method reported by Chen and Bergman (2005), with some modifications. The Shimadzu HPLC system (model L-6200A), equipped with a Photo diode array detector (Shimadzu, Japan) and a computer system, was applied. Detection was operated at 292 and 325 nm, simultaneously. The spectra, from 250 to 600 nm, were recorded for all peaks. The extracted samples were injected through a guard-column and separated on a C₁₈ column (4.60 x 150mm, 4 μ m) (Phenomenex, USA). Gradient elution was then applied. Mobile phases A, B, and C were methanol, water and buthanol, respectively. The gradient was as follows: 0-12 min 92% A, 4% B and 4% C: 12-25 min linear gradient, from 4% B to 3 % B and 4% C to 5 % C, with flow rate of 1.5 mL /min and injection volume of 20 μ L. The α -tocopherol was detected at 292 nm and γ -oryzanol was detected at 325 nm. Chromatograms were recorded, and peak areas were used to calculate the content of γ -oryzanol and α -tocopherol, against the standard curve of standards.

Evaluation of antioxidant activity**DPPH radical scavenging activity [24]**

Aqueous extract (0.1 mL) was added to 3 mL of a 0.004% MeOH solution of DPPH. Absorbance at 517 nm was determined after 30 min, and the percent inhibition activity was calculated.

$$\% \text{ Inhibition} = \frac{[(1 - Ae)]}{Ao} \times 100$$

Where Ao = Absorbance without extract.

Ae = Absorbance with extract.

Total antioxidant capacity [24]

The extract and gallic acid (0.3 mL) was combined with 3 mL of reagent solution (0.6M sulfuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate). The mixture were incubated at 95°C for 90 min and cooled to room temperature. The absorbance of the solution was measured at 695 nm against a blank. The antioxidant activity is expressed as the number of equivalents of gallic acid (GAE).

Statistical analysis

All measurements were triplicated on triplicated samples. The results were statistically analysed by analysis of variance (ANOVA) and Duncan's multiple range test (DMRT). Statistical significance was accepted at a level of $P < 0.05$

Results and Discussion***Effect of growing system on proximate compositions of rice bran***

Chemical composition analysis included moisture content, crude protein, crude fat, crude fiber, ash, carbohydrate and reducing sugar as illustrated in Table 2. There was no significant difference ($p < 0.05$) of crude protein and crude fat between organic and conventional rice bran whereas the contents of fiber and ash of organic rice bran was significant higher ($p < 0.05$) than those of conventional rice bran. Carbohydrate and reducing sugar contents also showed a statistical difference ($p < 0.05$) between conventional rice bran and organic rice bran. The proximate compositions of rice bran obtained in this study were similar to those reported by [25] The proximate compositions of conventionally and organically grown foods is varied among study to study, however, several studies have been documented that organic grown food contained more minerals than those of conventional growing system as indicated in Table 1. A number of factors can affect plant composition, and for this reason, it is often difficult to isolate the effect of growing plant system. The main factors that can influence the nutritive value of crops include genetics (i.e., plant crop and cultivar), environment, soil type and structure fertilizer type, climate (light, temperature, rainfall, humidity), soil microbial populations, management practices e.g., crop rotation, use of pesticides, irrigation, growth regulators, cultivation practices, post-harvest practices (harvest time (crop maturity), handling and storage, processing methods and conditions) [3].

Table 2. Proximate composition of rice bran.

Component (%)	Conventional rice bran	Organic rice bran
Moisture ^{ns}	9.99 ± 0.09	9.85 ± 0.11
Crude Protein ^{ns}	11.01 ± 0.65	11.77 ± 0.10
Crude Fat ^{ns}	15.17 ± 0.20	14.92 ± 0.09
Crude Fibre	16.47 ± 0.64 ^b	19.947 ± 1.08 ^a
Ash	13.28 ± 0.26 ^b	16.63 ± 0.10 ^a
Carbohydrate	34.08 ± 1.22 ^a	26.89 ± 0.78 ^b
Reducing sugar	6.13 ± 0.14 ^a	5.52 ± 0.29 ^b

For crude protein determination, the conversion factor of 5.95 was used.

Values superscripted with dissimilar letters (^a, ^b, ^c, ^d) are significantly different ($p < 0.05$).

Each Mean ± SD represents three replications.

Means within a row with different superscript letters (a, b, c, d) are different ($p < 0.05$).

^{ns} = Means within a row are not different

Carbohydrate (nitrogen free extract) = $100 - (\% \text{moisture} + \% \text{crude protein} + \% \text{crude fat} + \% \text{ash} + \% \text{crude fibre})$

Effect of growing system on bioactive compounds of rice bran

Total phenolic content was estimated by the Folin–Ciocalteu colorimetric method, using gallic acid as a standard phenolic compound. Total phenolic content of organic rice bran and conventional rice bran were 1.88 and 1.39 mg/g, respectively (Table 3). This result was similar to that reported by [26], in which the TPC values ranged from 2.20 to 3.20 mg gallic acid equivalent/g rice bran, in different cultivars of raw rice bran in Thailand.

The concentration of α -tocopherol of organic rice bran was higher than that of conventional but the level of γ -oryzanol was higher. However the levels of both compounds were similar to that reported by [9].

Table 3. Bioactive components of rice bran (mg/g).

Bioactive components	Conventional rice bran (CRB)	Organic rice bran (ORB)
Total phenolic	1.39 ± 0.04 ^b	1.88 ± 0.08 ^a
α -tocopherol	0.025 ± 0.006 ^b	0.034 ± 0.002 ^a
γ -oryzanol	1.61 ± 0.02 ^a	1.10 ± 0.01 ^b

Each Mean ± SD represents three replications.

Means within a row with different superscript letters (a, b, c, d) are different ($p < 0.05$).

^{ns} = Means within a row are not different

Effect of growing system on antioxidant activity

DPPH radical scavenging activity

The proton radical scavenging action is known to be one of the various mechanisms for measuring antioxidant activity. DPPH is one of the compounds that possess a proton free radical. Antioxidant activity on interaction with DPPH, either transfer an electron or hydrogen atom to DPPH, is neutralization its free radical character. The colour changes from purple to yellow and its absorbance at wavelength 517 decreases [24]. The antioxidant activity can be expressed by calculating the concentration of sample required to scavenge 50% free radical (IC_{50}). The lowest IC_{50} indicates the strongest ability of the extracts to act as DPPH scavengers. The scavenging activity of rice bran was significant difference ($p < 0.05$), organic rice bran showed stronger antioxidant activity than that of conventional rice bran (Fig. 1).

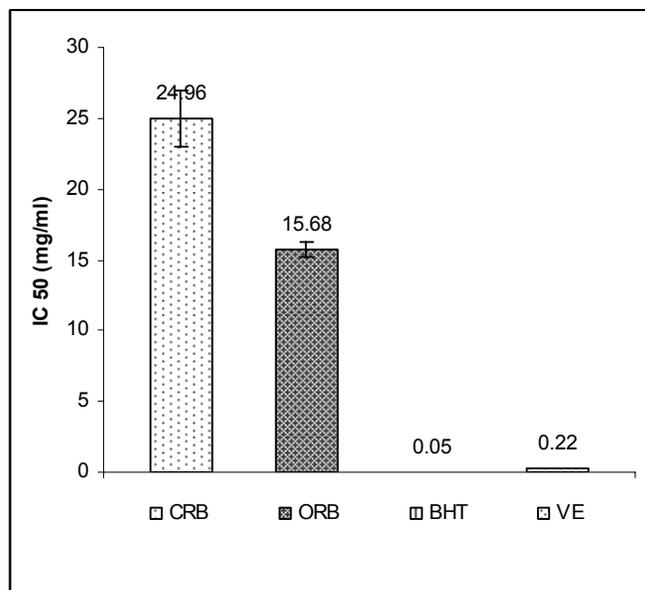


Figure 1. Scavenging activity of rice bran on DPPH radicals (50% inhibition concentration, IC₅₀).

Total antioxidant capacity

The total antioxidant activity of rice bran is expressed as the number of equivalent of gallic acid. Organic rice bran had a higher capacity than conventional rice bran with the amount of 16.35 µg GAE/mg whilst that of conventional was 9.97 µg GAE/mg. The extracts were found to have different levels of antioxidant activity in the systems tested.

Proximate compositions, bioactive compounds and antioxidant activity of defatted rice bran

Effect of growing system on proximate compositions

The composition of defatted rice bran is shown in Table 4. The oil fraction was separated whilst the residue was organic defatted rice bran (ODRB) and conventional defatted rice bran (CDRB). The effect of extraction methods, hexane extraction (Soxhlet method) and enzymatic extraction, was also compared. Therefore, organic defatted rice bran extracted by hexane (ODRBH) and enzyme (ODRBE), conventional rice bran extracted by hexane (CDRBH) and enzyme (CDRBE) were obtained. The results of this study revealed that the growing system was no effect on the level of protein, fat, fiber, and carbohydrate whereas the extraction method was significant effect on the level of the proximate compositions.

Table 4. Proximate composition of rice bran.

Component (%)	CDRB		ODRB	
	Hexane	Enzyme	Hexane	Enzyme
Moisture	8.37±0.09 ^{ab}	8.28±0.05 ^b	8.61±0.09 ^a	8.17± 0.13 ^b
Crude Protein	14.27± 0.36 ^a	6.37±0.49 ^b	12.17±1.69 ^a	5.83± 0.58 ^b
Crude Fat	2.65± 0.03 ^c	8.54± 0.61 ^a	2.91±0.41 ^c	6.86±0.20 ^b
Crude Fibre ^{ns}	1.80±0.83	2.06±0.03	2.09±0.02	2.17±0.23
Ash	14.59± 0.24 ^d	15.45±0.08 ^c	17.39± 0.07 ^b	18.28± 0.11 ^a
Carbohydrate ^{ns}	58.41± 2.74	59.30±1.62	56.83± 1.74	58.69± 1.76
Reducing sugar	12.44± 0.28 ^a	6.33± 0.29 ^b	11.95±0.48 ^a	3.10± 0.04 ^c

CDRB stands for conventional defatted rice bran; ORB stands for organic defatted rice bran

Values superscripted with dissimilar letters (^a, ^b, ^c, ^d) are significantly different ($p < 0.05$).

Each Mean ± SD represents three replications.

Means within a row with different superscript letters (a, b, c, d) are different ($p < 0.05$).

^{ns} = Means within a row are not different

Effect of growing system on bioactive compounds of defatted rice bran

The total phenolic compound of rice bran extracts was reported in Table 5. The conventional defatted rice bran extracted using enzyme (CDRBE) showed the highest total phenolic content (3.64 mg/g) while conventional defatted rice bran using hexane (CDRBH) indicated the lowest amount of total phenolic compound (1.60 mg/g). Table 5 also lists the concentrations of α -tocopherol and γ -oryzanol of defatted rice bran extracted by hexane and enzyme. Results indicate that the amounts of α -tocopherol and oryzanols significantly higher ($p < 0.05$) in the organic defatted rice bran using enzyme (0.051 and 2.59 mg/g, respectively)

Table 5. Bioactive components of defatted rice bran obtained from different growing system and extractions (mg/g).

Bioactive Component	CDRB		ODRB	
	Hexane	Enzyme	Hexane	Enzyme
Total phenolic	1.60± 0.14 ^c	3.64± 0.05 ^a	2.07± 0.05 ^b	2.26± 0.02 ^b
α - tocopherol	0.031±0.004 ^c	0.046±0.005 ^b	0.037±0.008 ^c	0.051±0.006 ^a
γ -oryzanol	0.39±0.02 ^d	1.90± 0.04 ^b	0.73± 0.03 ^c	2.59± 0.04 ^a

CDRB stands for conventional defatted rice bran; ORB stands for organic defatted rice bran
 Values superscripted with dissimilar letters (^a, ^b, ^c, ^d) are significantly different ($p < 0.05$).
 Each Mean \pm SD represents three replications.
 Means within a row with different superscript letters (a, b, c, d) are different ($p < 0.05$).
^{ns} = Means within a row are not different

Effect of growing system on antioxidant activity of defatted rice bran

DPPH radical scavenging assay

The antioxidant effect of defatted rice bran is preventing the free radical of DPPH. The decrease in absorption is taken as a measure of the extent of radical scavenging. DPPH assay shows that the scavenging activity of defatted rice bran was significant differences ($p < 0.05$). IC₅₀ values were 9.19 mg/mL for CDRBE which showed the highest activity.

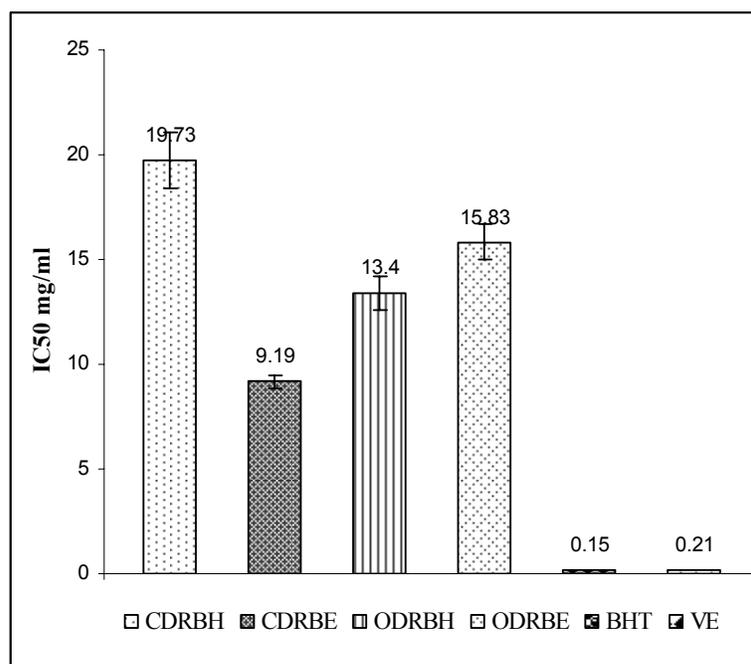


Figure 2. Scavenging activity of defatted rice bran extracted by hexane and enzyme on DPPH radicals (50% inhibition concentration. IC₅₀).

Total antioxidant capacity

Total antioxidant capacity of defatted rice bran using enzyme from both growing system indicated significant higher than those of defatted rice bran using hexane extraction. The enzyme extracts were found to have different levels of antioxidant activity in the systems tested. The results of the present study indicate the presence of compounds possessing high antioxidant activity in defatted rice bran extract using enzyme.

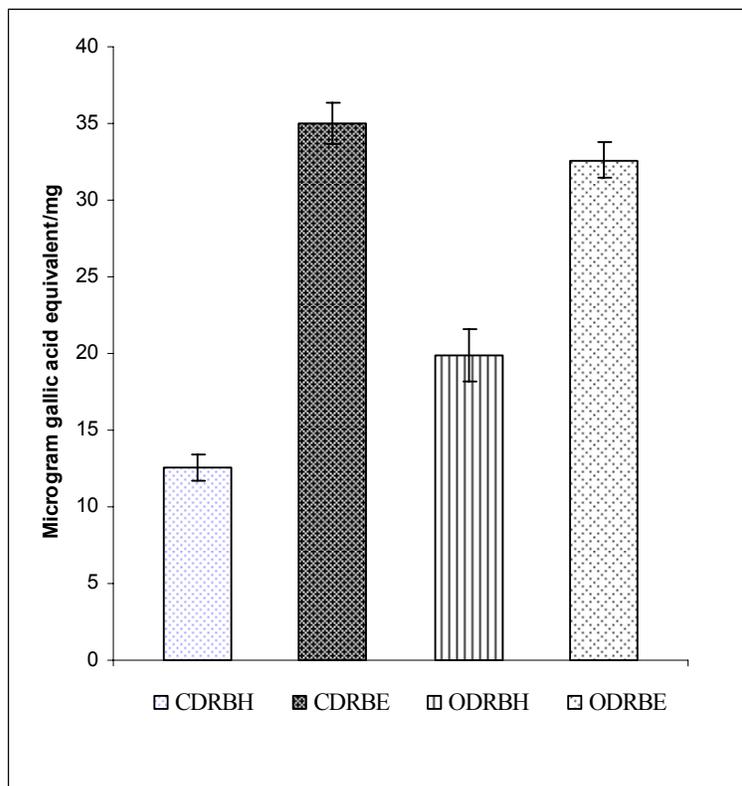


Figure 3. Total antioxidant capacity of defatted rice bran extracted by hexane and enzyme.

Conclusions

The growing system indicated significant effect on some chemical compositions such as protein and fat in both rice bran and defatted rice bran. The extraction methods also showed significant effect on the chemical compositions and antioxidant activity of defatted rice bran. However, this study has been investigated with only one cultivar, one location and one crop year; more information is necessary to confirm the results. Therefore, the second and third crop years are now in progress by the same research group.

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