YAYASAN PEMBINA PENDIDIKAN / PERGURUAN VETERAN SUKOHARJO
UNIVERSITAS VETERAN BANGUN NUSANTARA SUKOHARJO
PERPUSTAKAAN PUSAT
Alamat : JL. Letjend. Sujono Humardani No. 1 Kampus Jombor Telp. 593156

SURAT KETERANGAN
No. 31 /Perpus.Univet.Btr/VII/2018

Perpustakaan Pusat Universitas Veteran Bangun Nusantara Sukoharjo menerangkan bahwa berdasarkan hasil uji kemiripan (similiraty) dengan sofware Turnitin artikel dengan,

Judul Artikel	:	Antioxidant Activity and in vitro Inhibition human plasma LDL oxidation of defatted rice bran var. *Menthikwangi* extract
Nama Jurnal	:	**International Food Research Journal** *Journal homepage: http://www.ifrj.upm.edu.my*
Tahun/Vol/No	:	**24(4): 1651-1659 (August 2017)**
Alamat Jurnal	:	Faculty of Food Science and Technology Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
URL	:	**http://www.ifrj.upm.edu.my/ifrj-2017-24-issue-4.html**
Hasil Uji Turnitin	:	**Similarity index 99 %**

Meskipun hasil test dengan uji turnitin menunjukkan samilarity index 99 % namun artikel tersebut dinyatakan TIDAK termasuk plagiasi karena,

1. Turnitin menguji artikel dengan judul yang sama dengan nama penulis yang sama yang ada pada terbitan jurnal tersebut.
2. Jurnal tersebut telah mempublish artikel tersebut jauh hari sebelum uji turnitin dilakukan.
3. Jurnal tersebut menggunakan OJS (open journal system).

Demikian keterangan diberikan untuk dipergunakan sebagaimana mestinya.

Sukoharjo, 27 juli 2018.
Kepala Perpustakaan

Herry Mulyono, S.Pd., M.Psi
NIP. 19570209 198508 1 309

Antioxidant activity and in vitro inhibition human plasma LDL oxidation of defatted rice bran var. Menthikwangi extract

by Sri Hartati

Submission date: 09-Apr-2018 08:56AM (UTC+0700)
Submission ID: 943229118
File name: 41.pdf (355.69K)
Word count: 6354
Character count: 33861

International Food Research Journal 24(4): 1651-1659 (August 2017)
Journal homepage: http://www.ifrj.upm.edu.my

Antioxidant activity and in vitro inhibition human plasma LDL oxidation of defatted rice bran var. *Menthikwangi* extract

[1,2]Hartati, S., [2]Suparmo, [2]Santoso, U. and [2*]Marsono, Y.

[1]Study Program of Agricultural Product Technology, Faculty of Agricultural, Veteran Bangun Nusantara Sukoharjo University, Jl. Letjend S. Humardani No. 1 Sukoharjo 57521
[2]Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, GadjahMada University, Jl. Flora, Bulaksumur, Yogyakarta 55281

Article history

Received: 23 May 2016
Received in revised form: 21 July 2016
Accepted: 12 August 2016

Keywords

Defatted Rice Bran
Antioxidant
Ferulic acid
Human plasma LDL
In vitro

Abstract

Defatted Rice Bran (DRB), a by-product of the rice milling process, which has been separated from its lipid content, is a source of natural antioxidant mainly hydrophilic antioxidant such as ρ-coumaric and ferulic acid. The objective of this research was to evaluate the antioxidant potential of DRB var. Menthikwangi extracts. Defatted rice bran samples were prepared by removing lipid content with hexane solvent. Three different DRB extracts were prepared, including: (1) METE (methanol extract) was obtained from extraction of DRB with methanol, (2) RESE (residue extract) was obtained from extraction of the hydrolyzate of METE residue, (3) DRBE (DRB extract) was obtained from extraction of hydrolyzed DRB using ethyl acetate. Results showed that total phenolic content (TPC) of METE, RESE, and DRBE extracts were 39.54±2.92, 305.83±18.40, 285.00±23.38 mg ferulic acid (FA) equivalents/g DRB extract, respectively. Antioxidant activities (expressed as IC-50 of a free radical scavenger of DPPH) of FA, DRBE, RESE, and METE were 33.37±1.10, 143.37±12.40, 177.41±12.40, and 19,525±28.28 ppm, respectively. The ρ-coumaric and ferulic acid content of DRBE and RESE (based on DRB extract) were 4.17±0.10 and 4.91±0.15%, and 3.80±0.09% and 4.31±0.09%, respectively, but not detected in the extract of METE. In vitro inhibitory of LDL oxidation (IC-50 value) of ferulic acid (FA) was 8.8327µg/ml, while for ρ-coumaric (CUM) was 25.8346 µg/ml. IC-50 values of METE, DRBE and RESE were 77.0561 µg/ml, 25.7528 µg/ml and 29.7190 µg/ml respectively. Defatted rice bran extracts especially DRBE and RESE showed potentiality as antioxidant.

Introduction

Phenolic acids are a member of polyphenols group obtained from plant secondary metabolites. Phenolic acids are known for their strong antioxidant capacity, especially to fight against free radicals and reactive oxygen species (ROS), the main causes of human chronic diseases such as cancer, cardiovascular diseases, and diabetes (Andreasen *et al.*, 2001; Yu *et al.*, 2002; Yu *et al.*, 2003). Phenolic acids are mostly located in the outer layer of cereal grain. Rice bran is a good source of phenolic acids, but most of the products were used only as animal feed. In fact, from various research results rice bran were known to have potential health benefits such as antihypercholesterol (Sharma *et al.*, 1987; Marsono *et al.*, 1993; Revilla *et al.*, 2009), antidiabetic (Mc-Peak *et al.*, 2001; Qureshi *et al.*, 2002; Jung *et al.*, 2007), anticolon cancer (Li *et al.*, 2011), antiproliferative (Damayanthi, 2002; Rao *et al.*, 2010), and antioxidants (Damayanthi *et al.*, 2004; Devi and Arumoghan, 2007; Chotimarkom *et al.*, 2008; Rivilla *et al.*, 2009; Damayanthi *et al.*, 2010). The health benefits of rice bran were due to bioactive compounds such as polyphenols, tocopherols, tocotrienol, oryzanol, pangamic acid (Kahlon *et al.*, 1994; Chen and Bergman, 2005), phenolic antioxidant (Sompong *et al.*, 2011) as well as phenolic acids (Devi and Arumughan, 2007a, 2007b; Laokuldilok *et al.*, 2011). Previous studies of bioactive compounds from rice bran have been focused mainly on the lipophilic compounds such as tocopherol, tocotrienol, and oryzanol, which have shown antioxidant activity. However, there is not too many information on the hydrophilic compounds such as phenolic acids.

The utilization of rice bran as food component faced some technical problems because the bran is very sensitive to hydrolytic and oxidative rancidity (Astawan and Febrinda, 2010). Degradation of bran occurs shortly after brown rice milling process due to the interaction between rice bran oil with lipase and lipoxygenase enzymes that are naturally present in rice bran. Defatting of rice bran is an alternative solution to overcome the problem. Defatted rice bran

*Corresponding author.
Email: yustimar49@yahoo.co.id

studied by Hartati *et al.* (2014) has a fat content of 3.67± 0.16% db. The product (defatted rice bran, DRB) was hypothesized as a potential antioxidant due to its phenolic content.

Devi and Arumughan (2007b) who studied the phytochemical composition of defatted rice bran, suggested that the total phenolic content in the crude extract of DRB metanolic was 5.3% wt. Constituents of that extract were dominated by the polar phase of phenolic acids of 15,820 ppm. The DRB extract has a DPPH scavenging activity as well as superoxide scavenging activity. The antioxidant activity is in consequence of the majority of total phenolic content (TPC) and ferulic acid contained in the extract. Yamanaka *et al.* (2010) studied DRB and then added to methyl oleate (ML) under various conditions and evaluated the suppressive effect on lipid oxidation of ML. These studies revealed that the ML oxidation occurs more slowly in the presence of DRB.

Phenolic acids can be classified into 2 groups; i.e. benzoic acid derivative and cinnamic acid derivative. Phenolic acids of rice bran were a member of a hydroxycinnamic acid derivative group (Kim *et al.*, 2006). The presence of CH=CH–COOH in hydroxycinnamic recognized as a key group, which significantly has higher antioxidant activity compared to COOH in hydroxybenzoic acids (Rice-Evans *et al.*, 1996; White and Xing, 1997). Hydroxycinnamic acids consist of ρ-coumaric acid, caffeic acid, ferulic acid and sinapic acid. Although the phenolic acids of rice bran had been studied by some researchers, but information about the composition of phenolic acids in DRB, mainly local varieties (Menthikwangi) from Indonesia, is still limited. The objective of this research was to study the extraction of DRB which phenolic acid rich fraction and to evaluate the total phenolic content (TPC), radical scavenging activity (DPPH test), ferulic and ρ-coumaric acids content of the extracts as well as the in vitro inhibition ability of human plasma LDL oxidation.

Materials and Methods

Materials

Rice bran var Menthikwangi was obtained from a local farmer in Boyolali, Central Java, Indonesia. Chemicals used were hexane, methanol, acetic ethyl, ρ-coumaric acid (Sigma), ferulic acid (Sigma), 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin–Ciocalteu reagent, LDL (low density lipoprotein) human plasma L8292-IVL (Sigma), ethylenediaminetetra acetic acid (EDTA), thiobarbituric acid (TBA), CuSO4, trichloroacetic acid (TCA), hydrochloric acid (HCl) and natrium hydroxide (NaOH).

Sampel preparation

Defatted rice bran samples were prepared according to (Hartati *et al.*, 2014). Fresh rice bran samples were collected directly from the milling machine in the airtight polyethylene bags, stored in a cooler box (± 4ºC) and carried out to the laboratory. Rice bran was defatted by extracting its lipid content with hexane using maceration method, and then re-extraction using the same solvent. Fresh rice bran was added to hexane (1:4 w/v), being macerated for 3 h and stirred in a water bath at 60ºC, followed by filtration. Re-extraction was performed in the same method as previous work. Residual solvent in DRB were removed using N_2 gaseous.

DRB extraction procedure

The extraction of DRB conducted produce three kind of extracts, i.e. methanol extract (METE), residual extract (RESE) and DRB extract (DRBE). The extraction method of METE adopted from Jang and Xu (2009). The extraction method of RESE and DRBE adopted from Qiu *et al.* (2010). Defatted rice bran residue from methanol extraction and DRB samples (10 g) were collected in a bottle, and further being hydrolyzed with 4 M NaOH (120 ml), blown with N_2 to remove the solvent and immediately closed tightly. Each sample were shaken in a water bath for 4 hours, before acidified with 6 N HCl to a pH of 1.5 to 2 gradually and added to 70 mL ethyl acetate with gentle shaking. Ethyl acetate formed in upper layer was separated by a separatory funnel. The addition of ethyl acetate was repeated twice. Ethyl acetate filtrate was being collected for further evaporation with the Rotary evaporator (IKA Werke RV06 ML) at a temperature of 35ºC.

Determination of total phenolic content (TPC)

The total phenolic content (TPC) of the DRB extract was determined using the Folin-Ciocalteu reagent (Sompong *et al.*, 2011). One hundred and twenty microlitres (120 µl) of the extract was added to 600 µl freshly diluted of Folin-Ciocalteu reagent (1:10 immediately). Nine hundred and sixty microlitres (960 µl) of sodium carbonate solution (0.7 M) was added to the mixture after 2 min. The absorbance of resulting blue color was measured at λ 760 nm against a blank after 5 min of reaction at 50ºC. Ferulic acid (FA) was used as the standard and the TPC was expressed as mg FA equivalents per g of DRB extract.

Determination of DPPH radical scavenging activity

DPPH radical-scavenging activity of DRB extracts was evaluated according to Sompong *et al.* (2011) with slight modification. One point five mililitres (1.5 ml) of DPPH (2,2-diphenyl-1-picrylhydrazyl) 0.06 mM was added to 300 µL DRB extract sample with various concentrations, shaken using vortex then incubated in a dark room for 40 min at room temperature. DPPH solution was prepared by dissolving DPPH with methanol HPLC-grade. The absorbance was measured at the wavelength of 515 nm using spectrophotometer (Spectronic 200 Thermo Scientific). Absorbance difference between sample and control (blank sample) indicated radical scavenging activity. The radical scavenging activity was expressed as DPPH scavenging percentage and calculated as follows:

$$Radical\ Scavenging\ Activity(\%) = \frac{[Control\ Absorbance - Sample\ Absorbance]}{Control\ Absorbance} x\ 100$$

Antioxidant activity of the extract was expressed as an IC-50 value. It denotes the concentration of the sample, which was required to scavenge 50% of DPPH free radicals. An IC-50 value was being calculated using linear regression between sample's concentration (as X-axis) and RSA (%) (as Y-axis).

Identification of phenolic acid composition of DRB extracts

The phenolic acid composition was identified to determine the individual components of phenolic acids in the DRB extract, especially the ferulic acid (FA) and ρ-coumaric acid. Composition of DRB extract were identified using high performance liquid chromatography (Waters Alliance e2695, YMC Packed Column ODS 150 x 4.6 mm, 5 µm) with a stationary phase and a mobile phase (0-4 min, methanol:acetic acid 2% (5:95), (4-13 min, methanol:acetic acid 2% (35:65 to 38:62), and using a PDA detector at wave length of 200-410 nm with a run time 13 min. The calibration curve of ρ-coumaric acid and ferulic using extract content series of 50.4 mg/ml; 100.7 mg/ml; 201.4 mg/ml; 402.8 mg/ml; and 805.6 mg/ml, obtained linear regression equations of Y=69500X+18515 and Y=67865X-25483, respectively.

Determination of in vitro inhibition of human plasma LDL oxidation

Determination of in vitro inhibition of LDL oxidation was adopted from Warnakulesuriya *et al.* (2014). Briefly, the suspension of LDL from human plasma (IVL L8292 Sigma-Aldrich) was added to the sample with various concentrations (ppm) before it was oxidized. Oxidative stress on LDL plasma was set up by the addition of CuSO4 as an initiator. Termination of the oxidation reaction used ethylene diamine tetra acetic acid (EDTA). The level of LDL oxidation was measured by TBARS (Thiobarbituric Acid Reactive Substances) assay. This test measured the malondialdehyde (MDA) as a result of secondary products of lipid peroxidation. Malondialdehyde (MDA) of the isolates was determined using TBARS-LDL (Xu *et al.*, 2007). Inhibition of LDL oxidation ratio was calculated as follows:

$$\%\ Inhibitory of\ LDL\ oxidation = \frac{Ac - As}{Ac} x\ 100$$

where Ac is the absorbance of the LDL control and As is the absorbance of the sample. Inhibition of LDL oxidation ability was expressed as an IC-50 value. The IC-50 value is the concentration of the extract demonstrated the ability to inhibit 50% of the oxidation of LDL, which is calculated by a linear regression between the concentration (as the X-axis) and the inhibition of LDL oxidation (%) as the Y-axis.

Preparation of LDL suspension

Low density lipoprotein (LDL) vial (Sigma) was added with 5 ml of PBS (phosphate buffered saline) pH 7.4 and was slightly shaken overnight using a waterbath shaker. This suspension was used as a stock suspension and stored at 8ºC. Each time the tests were performed the suspension sonicated 15 min before it was taken for testing. The suspension diluted with PBS solvent (pH 7.4) as needed before further the test.

LDL-TBARS (LDL-thiobarbituric acid reactive substances) assay

The LDL oxidation was determined spectrophotometrically by measuring the amount of TBARS adopted from Xu *et al.* (2007) with slight modification. Briefly, 320 µL of an LDL suspension (containing 100 µg protein /ml) in PBS (pH 7.4) was incubated with 40 µL Cu2+ 200µM in the presence or absence (control) of 40 µL of diluted DRB extracts (at various concentrations) and other samples. The oxidation was performed in a screw capped 10 mL test tube at 37ºC in a shaking water bath for overnight in the dark. Oxidation reaction was stopped by adding 20 µL EDTA, 1 mM. Two milliliter of 15% (w/v) trichloroacetic acid (TCA) and 0.67% (w/v) thiobarbituric acid (TBA) in 0.1 N HCl were added to the post-incubation mixture. The mixture was heated at 95ºC for 1 h and cooled. After centrifugation at 3000 rpm for 15 min to remove precipitated proteins,

Table 1. Yield extract, total phenolic content (TPC) and antioxidant activity (IC-50 radical scavenging activity) DRB var. Menthikwangi

No.	Samples	Yield (% DRB)	(mg equivalent FA/g extract)
1	METE	3.79±0.18[a]	39.54±2.92[a]
2	RESE	0.59±0.16[b]	305.83±18.40 [b]
3	DRBE	0.65±0.17[b]	285.00 ±23.38 [b]

Mean within a column with different letters are significantly different (P<0.05) according to DMRT

the absorbance of the supernatant was measured at λ 532 nm.

Statistical analysis

All the experiments were performed in triplicate, and experimental data were analyzed using analysis of variance and expressed as a mean value±standard deviation. A Duncan's multiple ranges tests were conducted to assess significant differences among experimental mean values ($p<0.05$). All statistical computations and analyses were conducted using SPSS version 16.0 for Windows.

Results and Discussion

Yield extract, total phenolic content (TPC) and anti-oxidant activity of DRB extracts

Yields extracts from three DRB extracts obtained in this study (METE, RESE and DRBE) were 3.79 ± 0.18; 0:59 ± 0.16 and 0.65 ± 0.17% DRB respectively (Table 1). Defatted rice bran extract obtained by extracting DRB with methanol (METE) has the highest extract yields while RESE and DRBE yield extracts were not significantly different. These results are similar to Devi and Arumugan (2007a) that reported the extraction of DRB with methanol has a yield of 4.00%. Methanol was the most efficient solvent among other solvents used to extract TPC, oryzanol and ferulic acid from DRB (Devi and Arumugan, 2007a).

Methanol extract (METE) has the lowest TPC content (39.54±2.92 mg FA equivalents per g of extract DRB) compared to other DRB extracts (Table 1). Total phenolic contents of RESE and DRBE extracts were 305.83±18.40 and 285.00±23.38 mg FA equivalents per g of extract DRB. Although METE extracts showed the highest yield but its TPC content apparently showing the lowest among the three extracts of DRB. High yield in METE extracts may be due to methanol which can dissolve the components that are not part of the TPC. This results differed from

Table 2. Antioxidant activities of three DRB extracts and ferulic acid

No.	Samples	IC-50 (ppm)
1	METE	19,525.00±28.28[a]
2	RESE	177.41±12.40[b]
3	DRBE	143.37±12.40[c]
4	FA (Ferulic acid)	33.37±1.10[d]

Mean within a column with different letters are significantly different (P<0.05) according to DMRT

the previous study by Mariod *et al.* (2010) which has extracted two kinds of DRB, e.g. SRB (stabilized defatted rice bran) and USRB (unstabilized defatted rice bran). The content of total phenolic components of methanol extracts of SRB (519.6 mg / 100 g GAE) was higher than the USRB extracts (480.1 mg / 100 g GAE). These results were higher than the previous studies (Devi and Arumughan, 2007), which reported 5.3% total phenolic in defatted rice bran. Chatha *et al.* (2006) reported that different TPC from rice bran extracts varied from 250-397 mg/100 g using 80% methanol as the most efficient solvent in phenolic antioxidants extraction from rice bran compare to other solvents. IC-50 values according to the scavenging activity of free radicals DPPH were 0.69 mg/ml, 0.79 mg/ml, and 0.17 mg/ml in SRB extract, USRB extract and ascorbic acid (standard), respectively (Mariod *et al.*, 2010). The differences in the TPC of this study and other researches may be contributed by environmental factors, varieties of rice and milling process (Xu *et al.*, 2001; Nam *et al.*, 2005).

Antioxidant activities of DRB extract

In this research, DRB extract was being assessed its antioxidant activity as a free-radical scavenger (DPPH Test) and expressed in IC-50 value. An IC-50 value was defined as extract concentration to show radical scavenging activity (RSA) of 50%. The highest antioxidant activity indicated by the lowest IC-50.

Antioxidant activities of the three DRB extracts and ferulic acid as control were shown in Table 2. Methanol extract have the lowest antioxidant activity. Residual extract (RESE) and DRBE extracts seem to have similar antioxidant activity, however, the result is lower than the antioxidant activity of pure ferulic acid (FA). The antioxidant activity of FA, DRBE, RESE and METE as expressed in IC-50 value was 33.37±1.10 ppm, 143.37±12.40 ppm, 177.41±12,40, and 19,525±28.28 ppm, respectively.

There is strong negative correlation between TPC and IC-50 value (r= -0,99; P<0,05). This means that

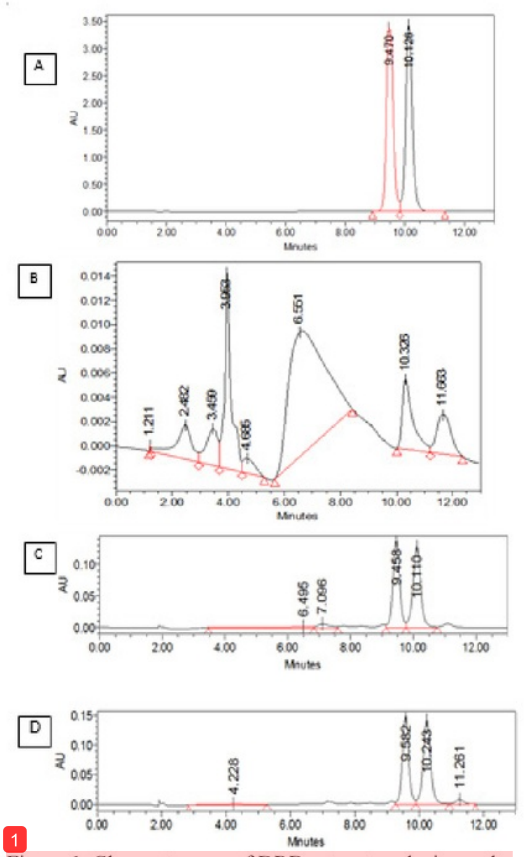

Figure 1. Chromatogram of DRB extract analysis results (A. Standard of ferulic and ρ-coumaric acid; B. METE; C. RESE; D.DRBE)

the higher TPC of extract exhibited the lower IC-50 value and vice versa. Thus, the higher TPC has the higher antioxidant activity. Some researchers have reported that commercial defatted rice bran contains phytochemicals such as oryzanols, tocols (tocopherol) and ferulic acid that are related to possible health benefits as an antioxidants which promote a high capacity for free radical scavenging and lipid peroxidation (Devi and Arumughan, 2007a; Devi and Arumughan, 2007b; Devi *et al.*, 2007, 2008). Defatted rice bran also contains significant amounts of protein, carbohydrate, dietary fiber, and phenolic substances, which are beneficial as health promoting and functional substances in foods (Saunders, 1985; Zhou *et al.*, 2004). The substances have good properties such as radical scavenging, anti-oxidative and emulsifying activities (Hamid-Abdul and Luan, 2000; Iqbal *et al.*, 2005).

These results revealed that rice bran dietary fiber is a free radical inhibitor or scavenger, acting possibly as primary antioxidants. They might react with the propagator of auto-oxidation chain of fat (peroxy-radicals) by terminating the chain reaction (Gordon, 1990; Frankel, 1991; Wanasundara and Shahidi, 1992). The antioxidant activity of natural antioxidant has been shown to be involved in the termination of free radical reactions and reducing power (Tanaka *et al.*, 1988; Shimada *et al.*, 1992). Daou and Zhang (2011) revealed that all dietary fiber fractions at high concentration (5% or 50 mg/mL) showed a high antioxidant activity. Scavenging activity of all fraction of rice bran dietary fiber increased with the increase in concentration.

Identification of DRB phenolic acid extract constituents

Identification of phenolic acid extract obtained from DRB was analyzed using HPLC with ferulic acid and ρ-coumaric acid as a standard. Histogram of analyzed extracts shown in Figure 1. The ferulic acid content of DRB extract of METE was lowest than RESE and DRBE samples, moreover ρ-coumaric acid content of METE samples was not detected. The ferulic acid and ρ-coumaric acid content of RESE and DRBE were 4.31±0.09 g/100 g and 3.80±0.09 g/100 g extract, 4.91±0,15g/100 g of extract and 4.17± 0.10 g/100 g extract, respectively.

This result was similar to the previous studies that phenolic substances of rice bran extract are dominated by ferulic acid, ρ-coumaric, p-hydroxybenzoic acid and vanillic acid, while caffeic acid, gentisic acid, protocatechic acid and syringic acid were trace compounds (Zhou *et al.*, 2004; Tian *et al.*, 2005; Okai and Higashi-Okai, 2006). Among phenolic substance in rice bran, ferulic acid was recognized for its significant quantity and strong free radical scavenging activity (Zhou *et al.*, 2004; Tian *et al.*, 2005; Okai and Higashi-Okai, 2006).

Phenolic acid is the dominant phenolic acid in all rice bran samples studied by Laokuldilok *et al.* (2011). Total phenolic acids found ranged from 1.53 to 3.29 mg/g in all samples, slightly lower than those phenolic acids have been reported in wheat bran (3.36-3.97 mg/g). Laokuldilok *et al.* (2011) stated that the main antioxidant found in normal rice bran (not pigmented) were γ-oryzanol and phenolic acids (62.9% and 35.9% of the total antioxidant) while α-tocopherol only 1.2% of total antioxidants. Phenolic acids are also known to have a strong antioxidant capacity and can delay the rate of chain oxidation reaction of free radicals in easily oxidized materials (Tarnawski *et al.*, 2006). The scavenging activity of free radicals DPPH of ferulic acid and α-tocopherol are greater than BHT (Nenadis and Tsimidou, 2002).

Phenolic acids of rice bran included in hydrocycinnamic acids derivatives group (Kim *et*

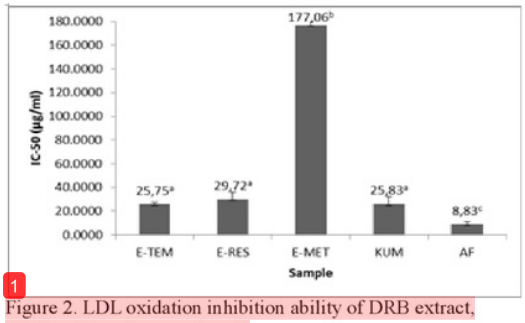

Figure 2. LDL oxidation inhibition ability of DRB extract, ferulic acid and coumaric acid

al., 2006). Andreasen *et al.* (2001) stated that there are mainly two groups of phenolic acids in cereal bran: benzoic and cinnamic acid derivatives. Ferulic acid and other hydroxycinnamic acids (caffeic and ρ-coumaric acid derivates) have been found to have good antioxidant activities. The presence of the CH=CH-COOH group in the hydroxycinnamic acids is conscidered to be key for the significantly higher antioxidative efficiency than the COOH in the hydroxybenzoic acids (White and Xing, 1997). Defatted rice bran extract shows a rich fraction of ferulic and ρ-coumaric acid.

In vitro Inhibition of LDL isolate oxidation testing of DRB extract

In vitro inhibition of LDL isolate oxidation testing (using pure isolates Sigma L8292-IVL) of DRB extract was carried out following procedure performed by Xu *et al.* (2007). The principle of this test is based on measurement of lipid peroxidation level of reaction between malondialdehyde (MDA) and TBA at high temperatures and in acidic conditions (Jetawattana, 2005). Low density lipoprotein (LDL) oxidation was performed using CuSO4 as an initiator. Malondialdehyde (MDA) reaction with TBA will result in MDA-TBA complex consisting of 2 mol of TBA and 1 mol of MDA with pink color. This complex can be detected by spectrophotometry at a wave length of 521-552 nm. The antioxidant activity of the DRB extract is expressed as the inhibitory ability of MDA formation(%). The ability of inhibition expressed in IC-50, the concentration of the extract demonstrated the ability to inhibit the formation of MDA by 50%. The smaller the concentration of the extract or sample required to inhibiting MDA formation, the higher antioxidant activity.

Thiobarbituric acid reactive substances (TBARS) method is commonly used in the study of lipid oxidation in general and LDL oxidation (Xu *et al.*, 2007; Yoshida and Kisugi, 2010). It has been reported that among all in vitro antioxidant activity test, physiopathology measurement of antioxidant activity in inhibiting the oxidation of LDL seems to be more informative than other methods used to test the antioxidant activity in food to prevent the occurrence of atherosclerosis (Katsube *et al.*, 2005).

The highest antioxidant activity of tested samples in inhibiting MDA formation in LDL oxidation using in vitro test was found in ferulic acid (FA) samples, which shown on its IC-50 value of 8.83 µg/ml (Figure 2). Meanwhile, the lowest antioxidant activity was METE (177.06 mg/ml). IC-50 values of DRBE, RESE, and ρ-coumaric acid did not differ significantly and were 25.75, 29.72 and 25.83 µg/ml, respectively.

Ferulic acid shows therapeutic influence, which is widely attributed to its potential on antioxidant capacity. Chain-breaking activity may have a role in contributing to the protective effect of ferulic acid in oxidative damage (oxidative injury) in humans and studies in vivo (Itagaki *et al.*, 2009). Castelluccio *et al.* (1996) showed that ferulic acid is more effective against LDL oxidation than the hidrophilic antioxidant, ascorbic acid. While Andreasen *et al.* (2001) suggested that the antioxidant activity of hydroxycinamate monomers extracts of wheat decreased in the following order: caffeic acid>sinapic acid>ferulic acid>ρ-coumaric acid.

Phenolic acid is a phenolic component, which has three distinguish forms (specific) that may contribute to its free radical scavenging capability. The presence of electrons donors group to a benzene ring (3 methoxys and more importantly 4 hydroxyls) of ferulic acid contribute to its properties in chain termination reactions of free radicals. The next function is carboxyl acid groups on adjacent ferulic acid with unsaturated C–C double bonds, which can provide additional offensive side to free radicals, preventing these radicals from attacking the membrane. In addition, the carboxyl acid group also serves as an anchor of ferulic acid to bind into the lipid bilayer, therefore, providing protection against lipid peroxidation (Kanaski *et al.*, 2002).

Defatted rice bran extracts both RESE and DRBE has high antioxidant activity towards DPPH radicals (Table 2) are supported in this study. Residual extract and DRBE contained both ferulic acid and ρ-coumaric acid (Fig. 1) which will influenced antioxidant activity toward DPPH radicals. Meanwhile, METE did not contain both of them so that the inhibition of LDL oxidation requires the higher concentration of METE than RESE and DRBE. This study showed that the extract RESE and DRBE have high antioxidant activity due to the dominant ferulic acid component. In accordance with the opinion of

some researchers stating that phenolic substances of rice bran extract are dominated by ferulic acid, ρ-coumaric, p-hydroxybenzoic acid and vanillic acid, while caffeic acid, gentisic acid, protocatechic acid and syringic acid were trace compounds (Zhou *et al.*, 2004; Tian *et al.*, 2005; Okai and Higashi-Okai, 2006).

Conclusion

Except METE extract, RESE, and DRBE extracts have high total phenolic content. The antioxidant activity as DPPH free radicals scavenger of RESE and DRBE extracts were also high, although their activities were lower than ferulic acid (FA). The IC-50 value of the antioxidant activity of FA, DRBE, RESE, and METE were 33.35±1.10; 143.09±9.70; 177.66±12.40; 19,525±28.28 ppm, respectively. Bioactive component of DRBE and RESE extracts were ρ-coumaric acid and ferulic acid. In vitro inhibition of LDL oxidation (IC-50 value) of ferulic acid and ρ-coumaric were 8.83 µg/ml and 25.83 µg/ml, respectively, whereas METE, DRBE, and RESE extracts were 177.06 µg/ml, 25.75 µg/ml, and 29.72 µg/ml, respectively. Defatted rice bran extracts especially DRBE and RESE have a potential as an antioxidant.

Acknowledgements

The first authors gratefully acknowledge to the Directorate General of Higher Education Ministry of Research, Technology, and Higher Education, Republic of Indonesia for awarding the Doctoral Dissertation Grant in 2015 under which the present study was carried out.

References

Andreasen, M. F., Landbo, A. K., Christensen, L. P., Hansen, A. and Meyer, A. S. 2001. Antioxidant effects of phenolic rye (Secale cereal L.) Extracts, monomeric hydroxycinnamates, and ferulic acid dehydrodimers on human low-density lipoproteins. Journal of Agricultural and Food Chemistry 49: 4090–4096.

Astawan, M. and Febrinda, A.E. 2010. Potential bran and rice bran as ingredient food and functional food products. Pangan 19 (1): 14-21.

Castelluccio, C., Bolwell, G.P., Gerrish, C. and Rice-Evans, C. 1996. Differential distribution of ferulic acid to the major plasma constituents in relation to its potential as an antioxidant. Biochemical Journal 316: 691-694.

Chatha S.A.S., Anwar, F., Manzoor, M. and Bajwa, J. 2006. Evaluation of the antioxidant activity of rice bran extracts using different antioxidant assays. Grasasy Aceites 57(3): 328-335.

Chotimarkorn, C., Benjakul, S. and Silalai, N. 2008. Antioxidant components and properties of five long-grained rice bran extracts from commercial available cultivars in Thailand. Food Chemistry 111: 636–641.

Chen, M.H. and Bergman, C.J. 2005. A rapid procedure for analysing rice bran tocopherol, tokotrienol and γ-oryzanol contents. Journal of Food Compositionand Analysis 18: 139–151.

Damayanthi, E. 2002. Characteristics of stabilized rice (Oryza sativa) bran and in vitro antioxidant and cancer cell line antiproliferation activities of rice bran oil and its fractions. Bogor, Indonesia: Bogor Agricultural Institute, PhD Dissertation.

Damayanthi, E., Muchtadi D., Zakaria F.R., Syarief H., Wijaya H. and Damardjati D.S. 2004. In vitro antioxidant activity of stabilized rice bran and its fraction. Jurnal Teknologi dan Industri Pangan 15 (1): 11-19.

Damayanthi, E. and Listyorini, D.I. 2006. Utilizing defatted rice bran flour in making of simulated chips. Jurnal Gizi dan Pangan 1 (2): 34-44.

Damayanthi, E., Kustiyah, L., Khalid, M. and Farizal, H. 2010. Antioxidant activity rice bran higher than tomato juice and the decreasing of total antioxidant activity serum after high antioxidant beverage intervention. Journal of Nutrition and Food 5 (3): 205–210.

Devi, R.R. and Arumughan, C. 2007a. Phytochemical characterization of defatted rice bran and optimization of a process for their extraction and enrichment. Bioresource Technology 98: 3037–3043.

Devi, R. R. and Arumughan, C. 2007b. Antiradical efficacy of phytochemical extracts from defatted rice bran. Food and Chemical Toxicology 45: 2014–2021.

Daou, C. and Zhang, H. 2011. Physico-chemical properties and antioxidant activities of dietary fiber derived from defatted rice bran. Advance Journal of Food Science and Technology 3(5): 339-347.

Frankel, E.N. 1991. Recent advances in lipid oxidation. Journal of Science and Food Agriculture 54: 495-511.

Gordon, M. H., 1990. The mechanism of antioxidant action in vitro, In. Hudson, B. J. F. (Ed.), Food Antioxidants. p.1-18. New York: Elsevier Applied Science.

Hamid-Abdul, A. and Luan, Y. S. 2000. Functional properties of dietary fibre prepared from defatted rice bran. Food Chemistry 68:15– 19.

Hartati S., Marsono Y., Suparmo and Santoso U., 2014. Antioxidant Activity of Hydrophilic Extract Prepared from Defatted Rice Bran var. Menthikwangi. Proceeding of PATPI International conference, p. 27. Jakarta: JEC Kemayoran.

Itagaki, S., Kurokawa, T., Nakata, C., Saito, Y., Oikawa, S., Kobayashi, M., Hirano, T. And Iseki, K. 2009. In vitro and in vivo antioxidant properties of ferulic acid: A comparative study with other natural oxidation inhibitors. Food Chemistry 114: 466–471.

Iqbal, S., Bhanger, M. I. and Anwar, F. 2005. Antioxidant properties and components of some commercially

available varieties of rice bran in Pakistan. Food Chemistry 93: 265–272.

Jang, S. and Xu, Z. 2009. Lipophilic and hydrophilic antioxidants and their antioxidant activities in purple rice bran. Journal of Agricultural and Food Chemistry 57: 858–862.

Jetawattana, S. 2005. Malondialdehyde (MDA), a lipid oxidation product. Free Radicals in Biology and Medicine. Spring 77(222): 1-10.

Jung, E. H., Kim, S. R., Hwang, I. K. and Ha, T.Y. 2007. Hypoglycemic effects of a phenolic acid fraction of rice bran and ferulic acid in C57BL/ksj-db/db mice. Journal of Agricultural and Food Chemistry 55: 9800–9804.

Laokuldilok, T., Shoemaker, C. F., Jongkaewwattana, S. and Tulyathan V. 2011. Antioxidants and antioxidant activity of several pigmented rice brans. Journal of Agricultural and Food Chemistry 59: 193–199.

Li, S. C., Chou, T. C. and Shih, C.K. 2011. Effects of brown rice, rice bran, and polished rice on colon carcinogenesis in rats. Food Research International 44: 209–216.

Kahlon, T. S., Chow, F. I. and Sayre, R. N. 1994. Cholesterol lowering properties of rice bran. Journal of Cereal Food Word 39(2): 99-102.

Kanaski, J., Aksenova, M., Stoyanova, A. and Butterfield, D. A. 2002. Ferulic acid antioxidant protection against hydroxyl and peroxyl radical oxidation in synaptosomal and neuronal cell culture systems in vitro: Structure activitystudies. The Journal of Nutritional Biochemistry 13: 273–281.

Katsube, T., Iwamaka, N. Y., Yamazaki, Y., Shiwaku, K. and Yamane, Y. 2005. Antioxidant flavonol glycosides in mulberry (morus alba l.) leaves isolated based on LDL antioxidant activity. Food Chemistry 97: 25-31.

Kim, K. H., Tsao, R., Yang, R. and Cui, S. W. 2006. Phenolic acid profiles and antioxidant activities of wheat bran extracts and the effect of hydrolysis conditions. Food Chemistry 95: 466-473.

Marsono, Y., Illman, R. J., Clarke, J. M., Trimble, R. P. and Topping, D.L. 1993. Plasma Lipids and large bowel volatile fatty acids in pigs fed on white rice, brown rice and rice bran. British Journal of Nutrition 70: 503-513.

Mariod, A. A., Adamu H. A., Ismailab, M. and Ismailab, N. 2010. Antioxidative effects of stabilized and unstabilized defatted rice bran methanolic extracts on the stability of rice bran oil under accelerated conditions. Grasasy Aceites 61(4): 409-415.

Mc-Peak, P., Rukmini, C. and Reddy, S. C. 2001. Supportive therapy for diabetes, hyperglycemia and hypoglycemia. US 6.303.586 B1. Washington: United States Patent.

Nam, S.H., Choi, S.P., Kang, M.Y., Kozukue, N. and Friedman, M. 2005. Antioxidative, antimutagenic, and anticarcinogenic activities of rice bran extracts in chemical and cell assays. Journal of Agricultural and Food Chemistry 53: 816-822.

Nenadis, N. and Tsimidou, M. 2002. Observation on the estimation of scavenging activity of phenolic compounds using rapid 1,1-diphenyl-2-picrylhydrazyl (DPPH) tests. Journal of the American Oil Chemists' Society 79:1191–1195.

Okai, Y. and Higashi-Okai, K. 2006. Radical-scavenging activity of hot water extract of Japanese rice bran-association with phenolic acids. Journal University of Occupational and Environmental Health 28(1):1-12.

Rao, A. S.V.C., Reddy S. G., Babu, P.P. and Reddy A.R. 2010. The antioxidant and antiproliferative activities of methanolic extracts from Njavara rice bran. BMC Complementary and Alternative Medicine 10 (4): 1-9.

Revilla, E., Maria, C. S., Miramontes, E., Bautista, J., Martínez, A. G., Cremades, O., Cert, R. and Parrado J. 2009. Nutraceutical composition, antioxidant activity and hypocholesterolemic effect of a water-soluble enzymatic extract from rice bran. Food Research International 42: 387–393.

Rice-Evans, C. A., Miller, N. J. and Paganga, G. 1997. Antioxidant properties of phenolic compounds. Trends in Plant Science 2(4): 152-159.

Robbins, R. J. 2003. Phenolic Acids in foods: An overview of analytical methodology. Journal of Agriculture and Food Chemistry 51(10): 2866-2887.

Saunders, R. M. 1985. Rice bran: Composition and potential food uses. Food Reviews International 1(3): 465-495.

Shahidi, F. and Wanasundara,P. K. J. 1992. Phenolic antioxidants. Critical Critical Reviews in Food Science and Nutrition 32: 67-103.

Sharma, P., Mishra, S., Ajmera, P. and Mathur, S. 2005. Oxidative stress in metabolic syndrome. Indian Journal of Clinical Biochemistry 20(1): 145-149.

Shimada, K., Fujikawa, K., Yahara, K. and Nakamura, T. 1992. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. Journal of Agricultural and Food Chemistry 40: 945-948.

Shin, Z. L., Chang, Y. S., Kang, W. S. and Jung, S. U. 1992. Antioxidant extracted from the defatted rice bran. US 5.175.012.A. Washington: United States Patent.

Sompong, R., Siebenhandl-Ehn, S., Linsberger-Martin, G. and Berghofer E. 2011. Physicochemical and antioxidative properties of red and black rice varieties from Thailand, China and Sri lanka. Food Chemistry 124: 132–140.

Tanaka, M., Kuie, C. W., Nagashima, Y. and Taguchi, T. 1988. Application of antioxidative Maillard reaction products from histidine and glucose to sadine products. Nippon Suisan Gakkaishi 54: 1409-1414.

Tarnawski, M., Depta, K., Grejciun, D. and Szelepin, B. 2006. HPLC determination of phenolic acids and antioxidant activity in concentrated peat extract - a natural immunomodulator. Journal of Pharmaceutical and Biomedical Analysis 41: 182–189.

Tian, S., Nakamura, K., Cui, T. and Kayahara, H. 2005. High-performance liquid chromatographic determination of phenolic compounds in rice. Journal

of Chromatography 1063: 121–128.
Qiu, Y., Liu, Q. and Beta, T. 2010. Antioxidant properties of commercial wild rice and analysis of soluble and insoluble phenolic acids. Food Chemistry 121: 140-147.
Qureshi, A. A., Sami, S. A. and Khan, F. A. 2002. Effects of stabilized rice bran, its soluble and fiber fractions on blood glucose levels and serum lipid parameters in humans with diabetes mellitus Types I and II. Journal of Nutritional Biochemistry 13 : 175–187.
Wanasundara, U. N. and Shahidi, F. 1994. Canola extracts as an alternative natural antioxidant for canola oil. Journal of the American Oil Chemists' Society 71: 817-822.
Warnakulasuriya, S. N., Ziaullah and Rupasinghe, H.P.V. 2014. Long chain fatty acid acylated derivatives of quercetin-3-o-glucoside as antioxidants to prevent lipid oxidation. Biomolecules 4: 980-993.
White, P. J. and Xing, Y. 1997. Antioxidants from cereals and legumes. In F. Shahidi (Eds). Natural antioxidants, chemistry, health effects, and application, p. 25-63. Champaign IL: AOCC press
Xu, B. J., Yuan, S. H. and Chang, S. K. C. 2007. Comparative studies on the antioxidant activities of nine common food legumes against copper induced LDL oxidation in vitro. Journal Food and Science 72: S522–527.
Xu, Z., Hua, N. and Godber, J.S. 2001. Antioxidant activity of tocopherols, tocotrienols, and - oryzanol components from rice bran against cholesterol oxidation accelerated by 2,2'- azobis (2-methylpropionamidine) dihydrocholoride. Journal of Agricultural and Food Chemistry 49: 2077-2081.
Yamanaka, K., Watanabe, Y., Yoshimoto, K., Uda, Y. and Nomura M. 2010. Suppressive effect of defatted rice bran on the oxidation of methyl linoleate. Food Chemistry 123: 1132–1135.
Yoshida, H., and Kisugi, R. 2010. Mechanisms of LDL oxidation. Clinica Chimica Acta 411 (23-24): 1875-1882.
Yu, L., Haley, S., Perret, J., Harris, M., Wilson, J. and Qian, M. 2002. Free radical scavenging properties of wheat extracts. Journal of Agricultural and Food Chemistry 50: 1619–1624.
Yu, L., Perret, J., Harris, M., Wilson, J. and Haley, S. 2003. Antioxidant properties of bran extracts from "Akron" wheat grown at different locations. Journal of Agricultural and Food Chemistry 51: 1566-1570.
Zhou, Z., Robards, K., Helliwell, S. and Blanchard, C. 2004. The distribution of phenolic acids in rice. Food Chemistry 87: 401–406.

Antioxidant activity and in vitro inhibition human plasma LDL oxidation of defatted rice bran var. Menthikwangi extract

ORIGINALITY REPORT

99%	99%	31%	9%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	www.ifrj.upm.edu.my Internet Source	99%

Exclude quotes	Off	Exclude matches	Off
Exclude bibliography	On		