

Physico-chemical Properties and Antioxidant Activities of Dietary Fiber Derived from Defatted Rice Bran

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Abstract: The objective of the study is to study the Physico-chemical properties and antioxidant activities of total, soluble and insoluble dietary fibers from defatted rice bran. Enzymatic-gravimetric method was used to obtain Total, Soluble and Insoluble fraction of dietary fiber from defatted rice bran. The fractions were used to study their structure (SEM), physical properties (color, density, porosity and oil or fat adsorption capacity), hydration properties (water holding, water binding and swelling emulsifying capacity), and antioxidant capacity (DPPH-scavenging activity, metal chelating and reducing power capacity). The physical properties except FBC of SDF were significantly higher than IDF and TDF; however hydration properties were lower than IDF and TDF ($p < 0.05$). Compared to SDF, IDF showed the highest CEC, while its GDRI value was lowest ($p < 0.05$). All dietary fiber fractions at high concentration (5% or 50 mg/mL) showed a high antioxidant activity.

Key words: Antioxidant activity, defatted rice bran, dietary fiber, physico-chemical properties

INTRODUCTION

Dietary fiber is the edible portion of plants or analogous carbohydrates that are resistant to digestion and adsorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fiber includes polysaccharides, oligosaccharides, and associated plant substances. Dietary fibers promote beneficial physiological effects including laxation, and blood cholesterol and glucose attenuation (AACC, 2001). Some of these materials may in fact be partially digested in the lower gastrointestinal tract by the microbial flora of the colon. According to solubility, dietary fiber may be classified as Soluble Dietary Fiber (SDF) or Insoluble Dietary Fiber (IDF), and the soluble and insoluble are the two fractions of Total Dietary Fiber (TDF), since both types are associated with particular metabolic and physiological effects in human (Roehrig, 1988).

The role of dietary fiber in health and nutrition has stimulated a wide range of research activities. Accumulating evidence favours the view that increased intake of Dietary Fiber (DF) has beneficial effects against chronic diseases (cardiovascular diseases, diverticulosis, diabete and colon cancer), in humans (Anderson *et al.*, 2009; Abdul-Hamid and Luan, 2000; Champ and Guillon, 2000; Schneeman, 1998; Cara *et al.*, 1992; Chen and Anderson, 1986; Cummings, 1985; Dukehart *et al.*, 1989; Wrick *et al.*, 1983 *et al.*, Spiller, 1980; Reddy,

1982; Tarpila *et al.*, 1978) with recommendations for consumption ranging from 30 to 45 g/day (Schweizer and Wursch, 1991; Spiller, 1986; Stephen, 1981; Burkitt and Trowell, 1977).

The health benefit and physiological properties of dietary fiber are difficult to predict on the basis of their structures alone, however, they are predictable on the basis of physico-chemical properties such as water holding capacity, swelling, oil or fat capacity, viscosity, cation exchange capacity, bile acid binding, particle size etc. So the physiological effects of fiber are dependent on a complex mixture of structural, chemical and physical properties (Blackwood *et al.*, 2000).

The purpose of this study was to develop a comparative study of Physico-chemical properties and antioxidant activity of soluble, insoluble and total dietary fiber derived from defatted rice bran.

MATERIALS AND METHODS

This study was conducted in the State Key Laboratory of Food Science and Technology of Jiangnan University, 1800 Lihu Road, Wuxi 214122, PR China in 2011. Defatted rice bran was obtained from Xuzhou oil Company (Xuzhou, Jiangsu Province, China). The major content of defatted rice bran including moisture, protein, fat, ash, carbohydrate, TDF, and phytic acid were 8.72, 16.20, 2.8, 10.74, 21.62, 32.98, 6.94%, respectively.

Extraction of dietary fiber: Insoluble, soluble and total dietary fiber were extracted according to the method described by Mirko *et al.* (2003) and using the modified AOAC enzymatic-gravimetric method of Prosky *et al.* (1988).

The defatted rice bran was cooked with termamyl (heat stable α -amylase 20.000 U/g -Novozymes Biological Engineering - Beijing - China) at 100°C for 1 h to give gelatinisation, hydrolysis and depolymerisation of starch after that it was digested with alcalase (Alcalase 2.4 L (2.4 AU/g) Novozymes Biological Engineering Beijing-China) at 60°C for 1 h to solubilise and depolymerise proteins and enzymatic treatment was complete with incubation with amyloglucosidase (100.000 U/g Novozymes Biological Engineering Beijing - China) at 60°C for 1 h to hydrolyse starch fragments to glucose.

After these treatments to the suspension: were added four volumes of 80% ethanol (preheated at 60°C) to precipitate Total Dietary Fiber (TDF) and remove depolymerised protein and glucose, the precipitate was allowed to form at room temperature for 60 min, followed by centrifugation, and the residue was washed twice with 78% ethanol, 95% ethanol and acetone respectively, and was dried in a vacuum oven at 60°C. For IDF and SDF the suspension was centrifuged and the supernatant was kept for isolation of SDF while the residue was washed twice with hot water (70°C), 95% ethanol and acetone and finally dried in vacuum oven at 60°C to give IDF. Water washing were combined with the supernatant for preparation of SDF. SDF was precipitated in four volumes 80% ethanol (60°C). After centrifugation, the residue was washed twice with 78% ethanol, 95% ethanol and acetone respectively, and was dried in a vacuum oven at 60°C. TDF, IDF and SDF were corrected for residual protein and ash content.

Analysis of physical properties:

Scanning Electron Microscopy (SEM): Examination by SEM was carried out. Samples were gold coated and scanned by using an Electro Scan model Quanta 2000 environmental Scanning microscope (Fei Company Netherlands).

Determination of color: Color of dietary fiber was determined using a color-guide meter (Model 45/0, BYK-Gardner, Germany). Three values of L, a, and b were measured, where L = 100 (white), L = 0 (black), +a = red, -a = green, +b = yellow and -b = blue.

Density: Direct density (pd) and bulk density (pb) were determined according to the methods described by Parrott and Thrall (1978). The results were expressed as gram per milliliter (g/mL).

Porosity: fiber porosity was determined by using the calculation of specific volume that could be used as an

index of the capillary structure differences (Chen *et al.*, 1984; Cadden, 1987).

Analysis of hydration properties:

Water Holding Capacity (WHC): Water holding capacity was determined according to the methods described by Robertson *et al.* (2000) and Robertson and Eastwood (1981a) with some modifications. Sample (1 g) was accurately weighed in the graduated test tube. 30 mL of deionized water containing 0.02% sodium azide were added and it was hydrated for 18 h. After that the supernatant was removed by allowing the wet sample to drain on a fine-meshed wire screen. The hydrated sample was carefully removed, weighed and dried to constant weight (± 0.05 mg) in forced-air oven at 110 . It was also determined using a solution of NaCl (0.15 mol/L) as described by Guillon *et al.* (1992) and Auffret *et al.* (1994). WHC was expressed as the amount of water retained per gram dry sample (g/g dry weight).

WHC (g/g) =

$$\frac{(\text{Hydrated residue weight} - \text{Dry residue weight})}{\text{Dry residue weight}}$$

Water Binding (Retention) Capacity (WBC): Water binding capacity was determined according to the methods of Robertson *et al.* (2000), Thibault *et al.* (1992), Oakenfull (1993) and Robertson and Eastwood (1981a) with some modifications. Sample (1 g) was accurately weighed in graduated centrifuged tube; 30 mL of deionized or distilled water containing 0.02% sodium azide were added and let to stand at room temperature for 18 h. After this time samples were centrifuged (3000×g) for 20 min. the supernatant was removed by passing through a sintered glass crucible under applied vacuum. The hydrated residue was recorded and sample was dried at 105 for 24 h to obtain dry weight. It was measured using a solution of NaCl (0.15 mol/L). WBC was expressed as the amount of water retained per gram dry sample.

WBC (g/g) =

$$\frac{(\text{Residue hydrated weight after centrifugation} - \text{Residue dry weight})}{\text{Residue dry weight}}$$

Swelling Capacity (SC): swelling capacity was determined according Robertson *et al.* (2000) 0.2 g dry sample was accurately weighed and placed in a graduated test tube, 10 mL of water containing 0.02% sodium azide was added after thoroughly mixing, tubes were let to stand for 18 h at room temperature. Then the bed volume was recorded and swelling capacity was calculated as ml per gram of dry sample for 18 h, after that the final volume attained by fiber was measured:

SC (ml/g) =
Volume occupied by sample / Original sample weight

Fat Binding Capacity (FBC): Fat binding capacity was measured using method adapted from Lin *et al.* (1974). The sample (5 g) was added to 20 mL of soybean oil in 50 mL centrifuge tube. The content was then stirred for 30 sec every 5 min and after 30 min the tube were centrifuged at 1600×g for 25 min. The free oil was then decanted and absorbed oil was determined by difference and expressed as ml (oil)/gram sample.

FBC (mL/g) =
(Precipitation weight - Dry weight) / Dry Weight

Emulsifying Capacity (EC): Emulsifying capacity was measured according to the method of Yasumatsu *et al.* (1972). 20 mL of 7% aqueous dispersion of the fiber was mixed with 20 mL of soybean oil and blended in a warring blender for 5 min at high speed. An aliquot was then centrifuged at 3000×g for 5 min. The percentage of total mixture that remained emulsified after centrifugation was expressed as stability index. The stability index of a good emulsion is greater than 94% while that of a poor emulsion is only 50% according Wang and Kinsella (1976) and Yasumatsu *et al.* (1972).

Antioxidant properties:

2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) radical scavenging activity: The DPPH radical scavenging activity was measured according to the method of Yen and Wu (1999), with some modifications. Dietary fiber was dissolved in distilled water to obtain different concentrations (w/v): 1% (10 mg/mL), 2% (20 mg/mL), 3% (30mg/ml), 4% (40 mg/mL), and 5% (50 mg/mL). To 4ml of each sample solutions, 1.0 mL of 0.2 mM DPPH was added and mixed vigorously. After incubating for 30 min, the absorbances of the resulting solutions were measured at 517 nm using spectrophotometer. The control was conducted in the same manner, except that distilled water was used instead of sample. DPPH radical-scavenging activity was calculated according to the following equation (Yen and Wu, 1999):

DPPH radical-scavenging activity (%) =
[1 - (A517 of sample/A517 of control)] × 100

Metal-chelating activity: the chelating activity on Fe²⁺ was determined, using the method of Decker and Welch (1990). One milliliter of different concentrations (w/v) sample solutions (1, 2, 3, 4 and 5%) were mixed with 3, 7 mL of distilled water, then the mixtures were reacted with 0.1 mL of 2 mM FeCl₂ and 0.2 mL of 5 mM 3-(2-pyridyl)-5, 6-bis(4-phenyl-sulfonic acid)-1, 2, 4-triazine (ferrozine) for 20 min at room temperature. The

absorbances were read at 562 nm. The control was prepared in the same manner except that distilled water was used instead of the samples. Chelating activities were then calculated as follows (Decker and Welch, 1990).

Chelating activity (%) =
[1 - (A562 of sample/A562 of control)] × 100

Reducing power: Reducing power was determined by the method of Oyaizu (1986) with some modifications. The sample solutions of different concentrations (w/v) 1, 2, 3, 4 and 5% (0.5 mL) were mixed with 2.5 mL of 0.2M phosphate buffer (pH6.6) and 2.5 mL of potassium ferricyanide. The mixtures were incubated at 50 C for 20 min. an aliquot (2.5 mL) of 10% trichloroacetic acid was added to the mixture, followed by centrifugation at 3000 rpm for 10 min. The upper layer of solution (2.5 mL) was mixed with 2.5 mL of distilled water and 2.5 mL of 0.1% ferric chloride and the absorbances were read at 700 nm. Increased absorbance of the reaction mixture indicates increasing reducing power.

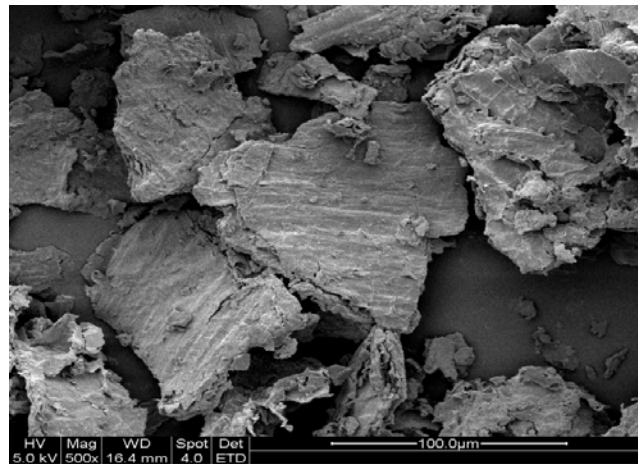
Statistical analysis: All experiments were carried out in triplicate. For statistical analysis, Statistical Package for the Social Science (SPSS, version 17.0) was used. The results were subjected to analysis of variance (ANOVA), followed by Turkey multiple-range test for mean comparison at the level of 0.05.

RESULTS AND DISCUSSION

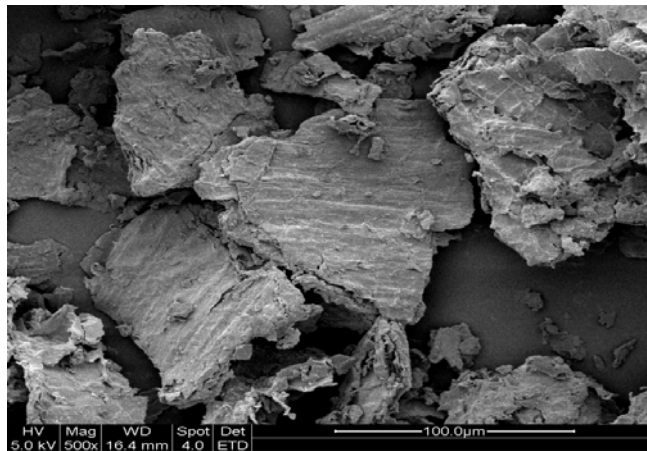
Scanning Electron Microscopy (SEM) analysis: SEM has been shown to be powerful tools for the study of the physicochemical properties of Dietary Fiber (DF). The results of scanning electron microscopy (SEM) are showed in Fig. 1a, b, c. The SEM analysis are shown not significant different between total dietary fiber and insoluble fiber, however, there are significantly different between soluble fiber and total dietary, insoluble dietary fiber (Fig. 1a, b, c).

Colour determination: The results of color analysis of TDF, IDF and SDF are showed in the Table 1. The values are expressed as mean±Standard Deviation. There is no significant difference between means (within the same property) designated by the same letter at p<0.05.

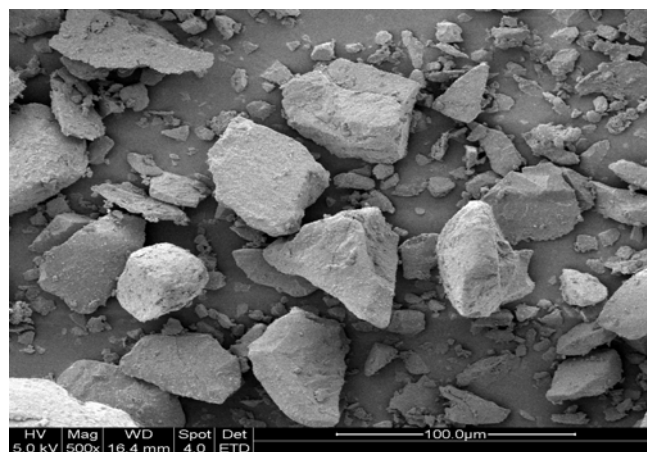
As can be seen in the Table 1, the color varies with the different fractions. TDF and IDF are less white (L = 66.98 and 65.57, respectively) than SDF (L = 70.89) but all three fraction are darker than standard and this is more pronounced with TDF and IDF than SDF (-13.39; -11.72 against -7.79, respectively). Red color is less visible (average a = 4.50) for the three fractions which are more red than standard (+ a), while the intensity of the yellow color is higher for TDF and IDF (b = 17.82 and 17.31,



(a)



(b)



(c)

Fig. 1: SEM of TDF, IDF and SDF: (a) SEM of TDF, (b) SEM of IDF, (c) SEM of SDF

Table 1: Analysis of colour of TDF, IDF and SDF

Fiber	L	a	b	L	a	b	E
TDF	65.56±0.74f	4.80±0.02c	17.82±0.04e	-13.39±0.76a	1.84±0.04b	1.64±0.05b	13.48±0.68d
IDF	66.97±0.45f	4.29±0.05c	17.31±0.13e	-11.72±0.45a	1.35±0.05b	1.13±0.12b	11.77±0.44d
SDF	70.89±0.2g	4.40±0.04d	11.44±0.04f	-7.79±0.19a	1.46±0.03c	- 4.73±0.04b	9.24±0.19e

The values are expressed as mean±Standard Deviation. There is no significant difference between means (within the same property) designated by the same letter at $p < 0.05$

Table 2: Analysis of density and porosity of TDF, IDF and SDF

Fiber fractions	Direct density (pd) mg/mL	Bulk density (pb) mg/mL	Porosity (cm ³ /g)
TDF	426.47±0.92b	468.40±0.54b	0.676±0.005b
IDF	345.17±0.76a	395.47±0.60a	0.429±0.01a
SDF	1197.47±0.64c	1005.81±0.52c	1.932±0.02c

The values are expressed as mean±Standard Deviation. There is no significant difference between means (within the same property) designated by the same letter at $p < 0.05$

respectively) than SDF (b = 11.44) so TDF and IDF are yellower than standard (+ b) while SDF is bluer than standard (- b).

Analysis of density and porosity: As shown in the Table 2 the values of the direct determination of density (pd) and bulk density (pb) were significantly different for TDF, IDF and SDF. The highest values were obtained for SDF followed by TDF (1197.47 and 426.47 mg/mL for pd, and 1005.81 and 468.40 mg/mL for pb, respectively) the smallest values obtained were for IDF (345.17 mg/mL for pd and 395.47 mg/mL for pb).

Table 2 also depicts the porosity. The porosity of SDF was significantly different ($p < 0.05$) from that of TDF and IDF. These values of porosity showed that SDF has more porous surface than TDF and IDF (1.932, 0.676 and 0.429, respectively). The porosity of SDF, TDF and IDF was reduced by hydrolysis, because the enzyme disrupts the chains of polysaccharides linked by β (1-4) glycosidic bond between two glucose molecules, causing a reduction of particle size. A similar effect was described by Cadden (1987) in wheat bran.

The values are expressed as mean±Standard Deviation. There is no significant difference between means (within the same property) designated by the same letter at $p < 0.05$

Analysis of hydration properties: Table 3 shows the Water Holding Capacity (WHC), water Binding Capacity (WBC), Swelling Capacity (SWC), Emulsifying Capacity (EC) and fat binding capacity (FBC) of TDF, IDF and SDF from defatted rice bran. The WHC, WBC and SWC were determined using deionized water and a solution of NaCl (0.15 mol/L).

According Table 3, the highest WHC value (3.84 g/g) correspond to TDF in distilled water, while the lowest value (1.18 g/g) was obtained for SDF in NaCl. There is no significant difference between the values of TDF and IDF in water and in NaCl, but their WHC decreased from when determined in water to when determined in NaCl. We observed the same trend with the WBC and SWC ($p < 0.05$).

Hydration properties (WBC; SWC) are determined by the content in water soluble fiber components of foods (Sosulski and Cadden, 1982) and their values should have a similar evolution. In addition to the chemical composition of fiber, some physical properties (structure, particle size, porosity and density) are important to understand the behavior of TDF; IDF; and SDF during hydration (Robertson and Eastwood, 1981a; Auffret *et al.*, 1994). In this case the fact that TDF and IDF showed the higher WBC, SWC than SDF (soluble fraction) could indicate that the structural characteristics plays more important role in the kinetics of water uptake than the chemical composition. However, some components of IDF, such as hemicelluloses and lignin, have water affinity (Holloway and Greig, 1984).

Dietary fibers interact with water through two mechanisms mainly: (1) water held in capillary structures as a result of surface tension strength and (2) water interacting with molecular components through hydrogen bonding or dipole forms (Chen *et al.*, 1984). SWC and WHC of fibers represent the volume of hydrated fibers or the amount of water held by fibers, measured under gravitational force, while WBC represents the amount of water held by fibers measured under centrifugal forces (Auffret *et al.*, 1994).

Thus the higher values of WHC, SWC for IDF and TDF confirm that the physical properties of fibers related to fiber structure have an important effect on the insoluble fractions hydration, while the lower value of WBC for SDF, which should be higher than TDF and IDF (because in the measurement of WBC the fraction of water that interact with molecular components will be determined, removing the water bound weakly to fiber, therefore chemical composition is the main factor that determines water uptake for WBC) may be explained by some modifications in soluble hemicellulose responsible for hydrophobic property, and also enzymatic hydrolysis can invert the WBC of SDF and IDF. The components present in all fractions of dietary fibers are responsible for part of the fiber's hydrophobic characteristics, and a similar reduction of WBC has been observed in other sources of fiber (Heller *et al.*, 1977; Auffret *et al.*, 1994).

On the other hand WHC, SWC, and WBC of TDF, IDF and SDF decreased in value with the higher ionic strengths of the solubilization medium. A similar trend was observed by Parrott and Thrall (1978), Bertin *et al.* (1988), Fleury and Lahaye (1991), Auffret *et al.* (1994) and López *et al.* (1996). These authors explained that the

Table 3: Hydration properties of TDF, IDF and SDF

Fiber	WHC (g/g)		WBC (g/g)		SWC (mL/g)		EC (%)	FBC (mL/g)
	Water	NaCl	Water	NaCl	Water	NaCl		
TDF	3.84±0.05c	2.78±0.20b	4.53±0.07cd	4.09±0.05cd	3.06±0.07b	2.05±0.11a	14.48±0.5e	4.66±0.39d
IDF	3.27±0.28cd	2.28±0.09b	4.16±0.03c	4.01±0.08cd	1.39±0.12a	0.99±0.1a	10.47±0.55e	2.55±0.10bc
SDF	2.11±0.20d	1.18±0.08bc	1.29±0.04e	1.18±0.02be	0.65±0.05ab	0.5±0.05a	15.58±0.69f	1.37±0.04c

The values are expressed as mean±standard deviation. There is no significant difference between means (within the same property) designated by the same letter at $p<0.05$; WHC: Water Holding Capacity; WBC: Water Binding Capacity; SWC: Swelling Capacity; EC: Emulsifying Capacity; FBC: Fat Binding Capacity

presence of charged polysaccharides (uronic acids) in a medium with ions in solution could produce a screening of their charges, thus a reduction of the electrostatic repulsion between polysaccharides, and thus decrease their affinity for water.

The values of emulsifying capacity in Table 3 showed dietary fiber prepared from rice bran is not a good emulsifier due to stability index less than 50%, but TDF exhibited significantly greater emulsifying capacity than IDF and SDF (14.48, 2.55 and 1.37%, respectively; $p<0.05$). A similar result was obtained by Abdul-Hamid and Luan (2000).

Luh (1980) reported that emulsion activity of rice bran was 50%, while Prakash and Ramanathan, (1995) showed that emulsifying capacity of protein concentrates from rice bran, ranged from 52 to 57%; therefore the lower emulsifying capacity of TDF, IDF and SDF may be due to the lower protein level in the fiber fractions. It is important to emphasize that although dietary fibers have low EC, it can still be used to stabilize food emulsion systems.

FBC of TDF and IDF are greater than that of SDF (4.66, 2.55 and 1.37 mL/g respectively $p<0.05$) because the oil absorption is related to the nature of the surface and the density or thickness of particles, so those particles with the greatest surface area theoretically present a greater capacity to absorb and bind components of an oil nature (Amado, 1994). It was found that lignin-rich samples had higher FBC. So IDF and TDF had higher FBC levels than SDF due to their percentage of particles with large size, and for the lignin to be found in their chemical composition.

Antioxidant properties:

2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) radical scavenging activity: Scavenging activity of all fraction of rice bran dietary fiber increased with the increase in concentration (Fig. 2a). At 5%, the scavenging activities were more than 60 and 80% for SDF and IDF respectively. Scavenging activities of TDF and IDF were similar, and higher than SDF.

These results revealed that rice bran dietary fiber is free radical inhibitor or scavenger, acting possibly as primary antioxidants. They might react with the propagator of the autoxidation chain of fat (peroxy

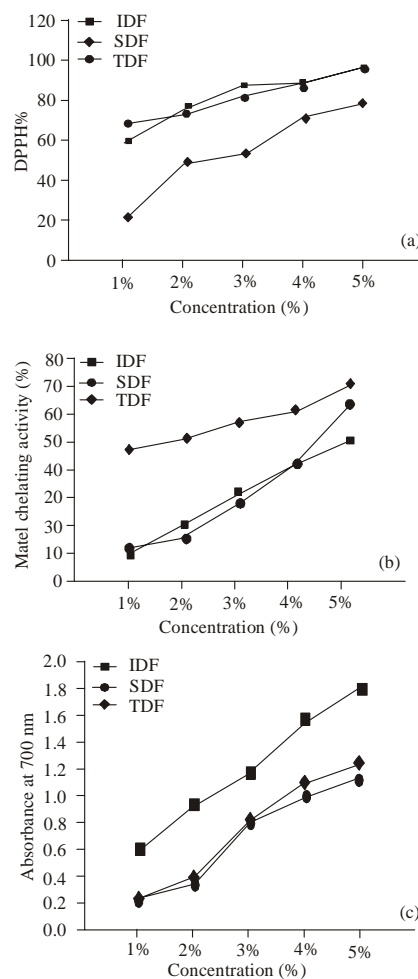


Fig. 2: Antioxidant activity of DF, (a) DPPH radical Scavenging of TDF, IDF and SDF; (b) Metal chelating activity of TDF, IDF and SDF; (c) Reducing Power activity of TDF, IDF and SDF

radicals) thereby terminating the chain reaction (Gordon, 1990; Frankel, 1991; Shahidi and Wanasundara, 1992). The antioxidant activity of natural antioxidant has been shown to be involved in termination of free radical reactions and reducing power (Shimada *et al.*, 1992; Tanaka *et al.*, 1988).

Metal-chelating activity: Chelating effect of all fraction of rice bran dietary fiber increased with the

increase in concentration (Fig. 2b). At 5% the chelating effect of SDF (~60%) was higher than IDF (~45%), while TDF showed the greater value more than 60%. It is contemplated that higher chelating effect would be observed with the concentration of 5%, because ferrous ions are the most effective pro-oxidants in the food system (Yamaguchi *et al.*, 1988). The higher chelating effects of SDF and TDF would be beneficial.

Reducing power: Reducing power of rice bran dietary fiber increased with the increase in concentration (Fig. 2c). SDF and TDF showed similar trend, but at 5%, reducing power of TDF was slightly higher than SDF while IDF showed the highest level reducing power at all concentration. This result shows that IDF has high scavenging activity, as well as high reducing power, thus IDF is a good electron donor to free radicals by providing electronic stability of the material to break into a free radical chain reaction (Pin *et al.*, 1999).

As well as plant non-starch polysaccharides, rice bran dietary fiber possess antioxidant properties and may be exploited as potential novel antioxidants. Several polysaccharide fractions from rice bran offer protection against the superoxide radical, hydroxyl free radical, anti-lipid peroxidation and exhibit good potential for reducing power and chelating ferrous ions (Zha *et al.*, 2009). This suggests possibilities for the use of rice bran dietary fibres with high antioxidant activities as ingredients that allow the stabilization of fatty foodstuffs, thereby improving their oxidative stability and prolonging their shelf life

CONCLUSION

According the results of this study, we can conclude that the defatted rice bran dietary fiber fractions (TDF, IDF and SDF) have important physiological and functional properties. IDF showed the higher cation exchange and bile salt adsorption capacity, however GDRI and cholesterol lowering effect were greater for SDF. They also showed an antioxidant activity. These properties are influenced by their Physico-chemical properties. Therefore, defatted rice bran dietary fiber can be used in food system as a natural additive possessing health, technological and antioxidant properties.

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