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## ANTIOXIDANT ACTIVITY OF RICE BRAN PASTA

Nithya, D.J<sup>\*1</sup>, Bosco, K.A.S<sup>2</sup>, Jagan Mohan, R<sup>3</sup> and Alagusundaram, K<sup>4</sup>

Address(es): D. J. Nithya

<sup>1</sup>Research Associate, <sup>2</sup>Senior Research Fellow <sup>3</sup>Associate Professor and Head, Department of Food Product Development, Indian Institute of Crop Processing Technology, Thanjavur-614003, Tamil Nadu, India.

<sup>4</sup>Director, Indian Institute of Crop Processing Technology, Thanjavur-614003, Tamil Nadu, India.

\*Corresponding author: [neethu\\_jacinth@yahoo.co.in](mailto:neethu_jacinth@yahoo.co.in)

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### ABSTRACT

Rice bran is a concentrated source of phytochemicals such as oryzanols, tocopherols and tocotrienols which have high antioxidant properties. The study was carried out to incorporate rice bran (5%-30%) at different levels in rice pasta. The antioxidant of pasta developed by incorporating rice bran was studied by DPPH assay and reducing power as per the standard method. Increase in the incorporation of rice bran was found to have significant effect ( $P < 0.0007$ ) on the percentage radical scavenging and reducing power. Pasta made from 100% rice flour showed low percentage inhibition and reducing power when compared with rice bran incorporated pasta. From the study, the potential antioxidant property of rice bran can be observed.

**Keywords:** Rice bran, antioxidants, Pasta, DPPH, Reducing power



## INTRODUCTION

Rice milling by-products in the form of rice bran have unique functional and nutritional properties, however their utilization in various aspects has been minimal or rather conspicuously negligible. Much literature have supported the rich nutrient composition of rice bran especially in its vitamins, protein and fibre content. There are several studies showing the marked antioxidant activity of cereal products (Andlauer & Furst, 1998; Borrelli et al., 2004; Miller et al., 2000). This activity is mainly due to phenol compounds that can be either the same contained in fruits and vegetables, or unique of the specific cereal (Adom et al., 2003; Ho, 1992). Phenolic compounds have been widely investigated as potential models for the development of new primary antioxidants, which can prevent or delay in vitro and/or in vivo oxidation processes (Barreira et al., 2008; Siquet et al., 2006). Several studies exhibited good correlation between the total phenolic content and the antioxidant activity of plant extracts (Butsat et al., 2009). Rice-Evans et al., (1997) reported that phenolic compounds play a crucial role in determining the antioxidant properties. Recent interest in these substances has been stimulated by the potential health benefits arising from the anti-oxidative activity, free radical scavenging capacity, coronary heart disease prevention, and anticancer activity, whilst some flavonoids exhibit potential for anti-human immunodeficiency virus functions (Yao et al., 2004). It is clear that antioxidant are concentrated in the bran fraction, however, it is not clear at which extent both free and carbohydrate-bound compound are measured by a given assay (Zhou et al., 2004). This finding indicates the fundamental contribution of bran and germ to the whole cereal antioxidant activity and it is explained by the fact that phenol compounds are associated with the outer layers, particularly the aleurone layer (McKeehen et al., 1999; Mueller-Harvey et al., 1986; Regnier & Macheix, 1996). Despite this evidence this parameter was scarcely considered in the characterisation of durum rice by-products, although it is conceivable that the antioxidant compounds present in the pericarp and aleurone layers are recovered in the by-product fractions (Dexter & Wood, 1996; Martiane-Tomea et al., 2004).

The present study had two main goals. Firstly development of extruded product using by-products obtained from rice milling industries and at the same time the research investigated the potential antioxidant properties of the developed product.

## MATERIAL AND METHODS

Rice brokens and rice bran of the ADT49 variety were collected from local rice mill in Thanjavur, Tamil Nadu, India. Bran and milled rice flours produced

from the same batch of parboiled paddy rice were used in this study. Both rice and rice bran were sieved in order to obtain flour with less than 200  $\mu$ m particle size.

### Formulation of Rice Bran pasta

Flours from parboiled rice, rice bran and water were blended in order to produce a mixture with a final water content of 45%. After mixing, (12-15 min) conventional extrusion was carried out in a continuous press for Macaroni-shaped pasta production (la monferina, Asti, Italy). A jacket with cold water kept dough temperature at about 45-50 °C at an extrusion pressure of 10-11 MPa.

### Proximate composition analysis

The moisture and ash contents of the rice-rice bran flours were determined according to official standard methods AACC (2000) 44-15A and AACC (2001) 08-12. Protein estimation was carried out according to the AOAC (2000) method 990.03. A value 5.95 was used as the conversion factor. Fat content was determined as per the procedure by Sadasivam (1996) 2.1. The total fibre content was determined according to Sadasivam (1996) 1.13. All these determinations were made in triplicates.

### Cooking quality

Cooking loss was evaluated by determining the amount of solids lost into cooking water (AACC, 2000) 16-50. One gram of pasta sample was cooked in boiling water for cooking time (pasta:water ratio =1:10) with no salt added. The optimum cooking time of rice-bran pasta was evaluated as the time required for disappearance of the dry central core when gently squeezed between two glass plates (D'Egidio et al., 1990).

### Chemicals and reagents

The 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu's reagent, Gallic acid. All other solvents purchased from fisher scientific were of the highest available purity.

**Extraction and determination of total phenolics content and antioxidant activity**

The samples were extracted using the method adopted from **Abu Bakar et al., (2009)**. Briefly, 1g samples was extracted for 2 h with 10 ml of 80% methanol at 28°C on an orbital shaker (digisun 2008) set at 180 rpm. The mixture was centrifuged (Rota4R-V/Fm) at 1400 g for 20 min and the supernatant was decanted into a 30 ml vial. The pellet was re-extracted under identical conditions. The supernatant was combined and used for the total antioxidant activity and total phenolics.

**Determination of total phenolics content**

The total phenolics content was determined using the Folin–Ciocalteu reagent as followed by **Abu Bakar et al., (2009)**. Briefly, 0.3ml of extract was mixed with 2.25 ml of Folin–Ciocalteu reagent diluted (1:10) in distilled water and allowed to stand at room temperature for 5 min; 2.25ml of sodium carbonate (60g/l) solution was added to the mixture. After 90 min at 28°C, the absorbance was measured at 725 nm using a spectrophotometer(Shimadzu UV-1800 UV spectrophotometer). The total phenolic content of the extracts was calculated and expressed as gallic acid equivalents per gram of dry weight (mgGAE/g) based on the gallic acid standard curve.

**DPPH free radical scavenging activity**

The hydrogen atom or electron-donation ability of the corresponding extracts and some pure compounds was measured from the bleaching of a purple-coloured methanol solution of DPPH (**Gulluce et al., 2007**). The antioxidant activity of the extracts, on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, was determined by the method described by **Braca et al., (2001)**. Methanolic extract (0.1 ml) was added to 3 ml of a 0.001 M DPPH in methanol. The absorbance at 517 nm was determined after 30 min, and the percent inhibition of activity was calculated as:

$$[(Ao - Ae) \div Ao] \times 100$$

(Ao = absorbance without extract; Ae = absorbance with extract).

**Reducing power**

The reducing power of the sample was determined by the method described by **Oyaizu (1986)**. An aliquot of each sample(500µl) was mixed with 500 µl sodium phosphate buffer (0.2 M, pH 6.6) and 500 µl of 1% potassium ferricyanide, followed by incubation at 50 °C for 20 min. After the addition of 500 µl of 10% TCA, the mixture was centrifuged at 12,000g for 10 min and the supernatant (1.0 mL) was incubated in the presence of 1.0 mL of distilled water and 200µl of 0.1% ferric chloride for 10 min. The absorbance was read at 700 nm. The result was expressed as a percentage of the activity shown by 0.01 mg/mL BHT.

**Statistical analysis**

The data was subjected to analysis of variance (ANOVA) to determine if there were statistically significant (P < 0.05) differences among the samples. All values are presented ±SD, all determinations were carried out in triplicates.

**RESULTS AND DISCUSSION**

**Rice- rice bran flour composition**

In agreement with the literature (**Champagne et al., 2004**), the incorporation of rice bran induced a pronounced increase in protein, lipid, ash, and fibre, that are concentrated in the germ and bran layers (Tab 1).

**Table 1** Chemical characteristics of rice bran pasta

Rice flour %	Rice bran %	Moisture %	Protein g/100g	Crude Fat g/100g	Ash g/100g	Crude fibre g/100g
95	5	9.6 ± 0.4	6.2±0.2	1.3±0.02	0.9±0.02	0.3±0.02
90	10	8.5 ± 0.2	7.0±0.1	2.2±0.06	1.1±0.06	0.7±0.08
85	15	9.3 ± 0.1	8.3±0.2	3.2±0.09	1.7±0.08	1.1±0.02
80	20	10.2 ± 0.3	10.2±0.2	4.8±0.1	2.4±0.04	1.7±0.03
100	-	9.8 ± 0.2	5.3±0.3	0.1±0.02	0.6±0.02	-

**Cooking quality**

The cooking parameter of rice bran pasta samples was affected minimally by the composition of raw materials (Tab 2). Rice pasta (100%) exhibited higher water absorption and lower cooking loss, compared to the pasta sample incorporated with rice bran. In gluten-free pasta, solid loss during cooking is mostly due to solubilisation of loosely bound gelatinized starch from the surface

of the product. This phenomenon mainly depends on the degree of starch gelatinization and the strength of the retrograded starch network surrounding the gelatinized starch (**Resmini and Pagani, 1983**). The increase in cooking loss observed in pasta from rice bran incorporation was likely due to its higher fibre content, responsible for weakening of the starch network.

**Table 2** Cooking quality of rice bran pasta

Rice flour %	Rice bran %	Water absorption %	Gruel Loss %	Cooking time Min.
95	5	78.5 ± 0.5	3.2 ± 0.2	5
90	10	68.3 ± 1.0	6.1 ± 0.3	6
85	15	64.5 ± 0.3	6.9 ± 0.2	6
80	20	57.3 ± 0.3	8.3 ± 0.5	6
100	-	51.8 ± 0.4	11.5 ± 0.5	7

**Total phenolic content(TPC)**

The TPC values of the pasta samples were found increasing with increase in rice bran composition. Phenolic compounds have gained much attention, due to their antioxidant activities and free radical-scavenging abilities, which potentially have beneficial implications for human health (**Govindarajan et al., 2007**). The total phenolic content (TPC) was determined in comparison with standard Gallic acid and the results expressed in terms of mg GAE/g (Tab 3).

**Table 3** Antioxidant properties of rice bran pasta

Rice flour %	Rice bran %	% RS	Reducing Absorbance (mg/g)	TPC (GA eq)
95	5	3.21 ± 0.1	0.192	2.5 ± 0.1
90	10	19.8 ± 2.0	0.561	11.7 ± 0.3
85	15	18.9 ± 3.0	0.836	16.8 ± 0.5
80	20	45.0 ± 2.0	1.140	21.6 ± 0.5
100	-	58.7 ± 3.0	1.658	25.85 ± 1.0

**Antioxidant activity**

The antioxidant activity of pasta samples was analysed by DPPH radical scavenging activity and reducing power property and is reported in Table 3. Interestingly, rice bran incorporated pasta have a marked antioxidant activity in comparison with rice pasta. The highest antioxidant activity was observed for 20% incorporated bran pasta. Pasta made from 100% rice showed a negligible antioxidant activity. This finding confirms the hypothesis that the aleurone layer is rich of methanol soluble antioxidant compound. The main antioxidant compounds present in cereals are phenolic acids covalently bound to the cell wall together with carotenoids, tocopherols, tochtotrienols (**Andlauer & Furst, 1998; Ho, 1992; Mueller-Harvey et al., 1986; Regnier & Macheix, 1996**). A high antioxidant activity were also observed in selected cereal grain and attributed to phenolic compounds (**Zielinski & Kozłowska, 2000**).

**CONCLUSION**

The incorporation of rice bran improved the nutritional and antioxidative property of the pasta, but the gruel solid loss was increased with bran incorporation. However no significant variation in cooking time was obtained in the pasta samples.

Further studies are underway to relate the influence of glass transition of the rice bran based pasta on the product quality, its storage property and to optimize both the pasta-making process and formulation in order to reduce the solid loss of the cooked product and also the role of fibre in its interactions with starch which seems to warrant investigation in view of the ever increasing popularity of antioxidant rich, high-fibre pasta and of the associated technological problems.

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