Indian Journal of Natural Products and Resources
Vol. 12(1), March 2021, pp 153-157

Short Communication

Colour based nutraceutical potential of some traditional rice (*Oryza sativa* L. ssp. *indica*) varieties of India

Sandipan Ray[1], Debal Deb[2] and Mousumi Poddar Sarkar[3*]

[1]Department of Botany, University of Calcutta, 35 Ballygunge Circular road, Kolkatta 700019, West Bengal, India

[2]Centre for Interdisciplinary Studies, 9 Old Calcutta Road, Barrackpore, Kolkata 700123, West Bengal, India

[3]Department of Life Sciences, Presidency University, College Street, Kolkata 700073, West Bengal, India

Received 01 November 2019; Revised 19 November 2020

Consumption of unpolished grain, rather than polished grain has become a modern trend and consumers are mainly putting their thoughts and effort to consume products with more antioxidant capacity. Rice is the main staple food and apart from being considered as the main source of energy, it contains many nutraceutical properties because of its enriched secondary metabolites. This study is an effort to bring back the indigenous traditional rice landraces that almost disappeared from the farm fields after the advent of the Green Revolution in India. This article focuses on colour-based nutritional properties of six coloured and four non-coloured indigenous rice varieties based on antioxidant potential, total phenol and flavonoid content along with secondary metabolites profiling by high performance liquid chromatography. The biochemical uniqueness of these varieties has been explored that opens the gate for the conservation of more indigenous rice varieties for food security, as a cheap source of nutritional food and to construct a better niche for public health in developing country like India.

Keywords: Antioxidant, Coloured rice, High performance liquid chromatography, Secondary metabolites.

IPC code; Int. cl. (2015.01)- A61K 36/00, A61P 39/00

Introduction

South Asia is a great repository of genetic diversity of rice with more than 100,000 folk landraces of the *indica* group[1]. Different parameters are taken into consideration for the selection of different rice varieties for their breeding purposes, for various gastronomic preferences and culinary practices in different food cultures from time immemorial[2]. As a staple food of more than half of the people in the world, it supplies the nutritional values in our daily diet. With the increasing commercialization of rice crop, we are gradually compromising the nutritional properties of rice by milling out the pericarp and aleurone layer, commonly known as bran. Most of the important nutritional compounds are bound to this bran layer[3,4], which is the main site of micro-and macro-nutrients and a range of important phytochemicals, such as oryzanols, tocopherols, phenolic compounds, flavonoids, and anthocyanin that impart an overall nutritional property of rice[5,6]. Nowadays, consumers are increasingly showing their preferences for consumer products with more antioxidant activities and a trend has started toward the consumption of unpolished whole grain rice[7,8].

India is home to thousands of extant rice landraces (*Oryza sativa* L. ssp. *indica*), with various natural hues of black, purple, red, brown, and white grains. The colour content varies with the composition of secondary metabolites. Rice contains many unique polyphenolic compounds present both as bound with the cell wall polysaccharide and in free form. Normally, grains with red and black pericarp colours have a higher concentration of phenolic compounds compared to those with a light brown pericarp colour[9]. About 38%(ref 10) to 60%(ref 11) of the total polyphenol content is present in grains of light brown rice. In contrast, the red and black pericarp of coloured grains contains around 81% of the polyohenols[11]. The natural hues of rice bran colour have a profound effect in determining the nutritional content of grains. The free phenolics, mostly concentrated in the pericarp, are usually eliminated with the bran during milling[3,4]. Thus, the functional dietary potential and the protective effect of the rice grain in the prevention of age-related degenerative diseases become reduced during processing[12]. The present article highlights the antioxidant potential of coloured and non-coloured indigenous rice varieties which are still growing in different remote marginal farms in India[2].

Materials and Methods

Samples procurement

Ten rice landraces (six coloured and four non-coloured) were selected based on the agronomic and morphological characters (Fig. 1). Samples were collected from the germplasm bank of Basudha farm (www.cintdis.org/basudha), located in the Rayagada

*Corresponding author
Email: mousumipsarkar1@gmail.com, mps.dbs@presiuniv.ac.in

district of Odisha (19°33'06" N, 83°23'28.14" E), where each of these landraces is conserved *in situ* and characterised (Table 1). We cultivated these landraces in the Kharif season (June to December) in the University Agricultural Experimental Farm (22°22'03.5" N and 88°26'07.2" E). Rice varieties were grown in separate plots at a density of 16 hills/m^2 in the tropical climate of the lower Gangetic plain of West Bengal by regular watering but without application of chemical fertilizers, pesticides and herbicides.

Evaluation of antioxidant potential

Rice grains were dehulled with a traditional small hand-made rice pounder followed by winnowing the whole grains with bran and were crushed into a fine powder. Rice powder was soaked overnight with methanolic water (7:3 v/v) and kept in refrigeration. Total phenolic content (TPC) of the methanolic extract of the samples was estimated by the Folin-Ciocalteu method[14], measuring spectrophotometrically at 765 nm (Jasco V-630, USA) and expressed as mg of gallic acid (Sigma-Aldrich Chemistry, USA) equivalent (GAE) per 100 g dry weight. Total flavonoid content (TFC) was estimated by adding methanolic extract, 10% aluminium chloride, 5% sodium nitrite and water in a ratio of 1:1:1:7[(ref 15)]. Optical Density was measured spectrophotometrically at 510 nm (Jasco V-630, USA) after 25 minutes incubation and the result was expressed as mg of quercetin (ChromaDex, USA) equivalent (QE) per 100 g dry weight of rice grain. Antioxidant potential (AP) of the samples was measured by the radical scavenging property of 2-diphenyl-1-picrylhydrazyl (DPPH)[16]. Aqueous methanolic (3:7 v/v) DPPH (0.16 mM) was added with the sample aliquot in 3:1 ratio and the degree of decolourisation was measured at 517 nm in a UV-spectrophotometer (Jasco V-630, USA) after 30 minutes of incubation in the dark. The IC50 value of the sample was calculated against the degree of decolourization in respect of ascorbic acid (Sigma, USA) and the AP was expressed as the inverse of the IC50 value. The experiment was repeated thrice in all cases.

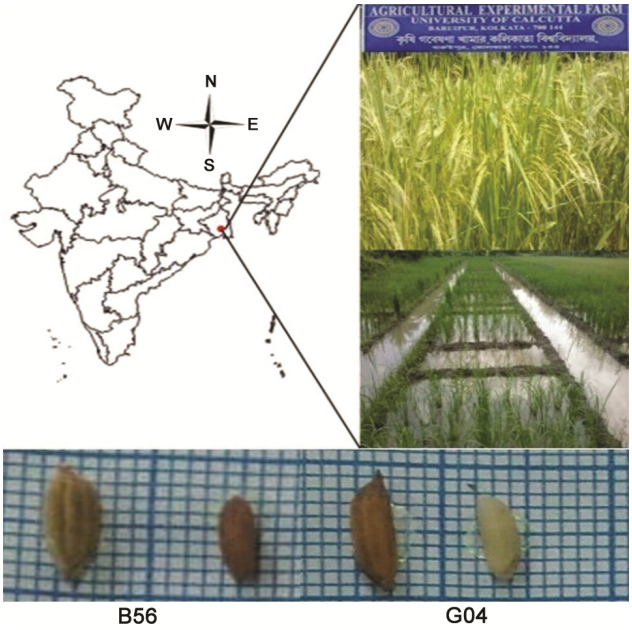

Fig. 1 — Map of India showing the location of the experimental farm (22°22'03.5"N and 88°26'07.2"E) for the cultivation of these indigenous landraces and types of some representative samples.

Analysis of secondary metabolites

Rice powder (whole grain) was treated with 1:2:1 chloroform, methanol and water and kept for 72 hours under refrigeration for the removal of lipid, following which the chloroform phase was discarded. The methanolic phase was concentrated by speed vac (Thermo SPD 2010) and subjected to high pressure

Table 1 — List of rice varieties studied with specific morphological characteristics of grain and aroma quality

S. No.	Vrihi code	Variety name	Place of origin	Grain colour	Kernel length (mm)	Kernel width (mm)	100-grain weight (g)	Kernel colour	Aroma[13]
1	A07	*Asitkalma*	West Bengal, India	Straw	6.90	2.10	2.49	White	0
2	B09	*Baiddhusuri*	West Bengal, India	Black furrows	6.70	3.10	2.88	Light brown	0
3	B56	*Boro*	Netrakona, Bangladesh	Brown furrows	5.60	2.53	2.47	Dark brown	0
4	B72	*Burma black*	Manipur, India	Black furrows	6.58	2.45	2.75	Black	1
5	G04	*Garam masala*	Maharashtra, India	Tawny brown	5.50	2.30	1.54	White	1
6	K69	*Kalomota*	West Bengal, India	Black furrows	5.83	2.92	2.59	Speckled brown	0
s7	L01	*Lugdhi-sal*	West Bengal, India	Gold furrows	6.33	2.53	2.36	White	0
8	P23	*Palberi*	West Bengal, India	Straw	6.00	2.60	2.58	Light brown	0
9	SH01	*Shishaphal*	West Bengal, India	Straw	5.26	2.53	1.90	Light brown	0
10	TT03	*Tulsimanjari*	West Bengal, India	Purple spots on straw	4.90	2.10	1.11	White	2

liquid chromatography (HPLC) (Agilent, USA) for the analysis of phenolic acids and flavonoids following the modified method of Dey *et al.*, (2015)[ref 17]. HPLC was attached with Zorbax SB-C18 column (4.6x150 mm, 3.5µ, Agilent USA) and equipped with a photo-diode array detector. Gradient of two mobile phases i.e., methanol (A) and water with 0.02% aqueous H_3PO_4 (B) were set at 25% A + 75% B for 5 minutes> 30% A + 70% B for 10 minutes> 45% A + 55% B for 30 minutes> and 80% A + 20% B for 60 minutes. The injection volume was 20 µL. The flow rate was kept at 0.4 mL/min and analytes were scanned at 280 nm wavelength. The peaks were identified by comparing the relative retention time with proper peak integration, co-chromatography with standard and calibration against absorption spectra obtained from the authentic compounds (Sigma-Aldrich Chemistry), USA; Chroma Dex, USA). Analytes were estimated using an external method following standard validation guideline and finally, the amounts of the analytes were expressed as µg/g of the dry weight of the sample.

Statistical evaluation

The correlations between TPC, TFC, and IC50 value were plotted on 3D planes and elucidated by Pearson's product-moment correlation test following PAST3 software.

Results and Discussion

Antioxidant potential and secondary metabolites of rice

AP was expressed in terms of the quantitative assessment of secondary metabolites for scavenging reactive oxygen species. The 3D interpolation based on the TPC, TFC, and IC50 value for DPPH reduction projected the interrelationship among these three variables (Fig. 2). AP was found to be the highest with antioxidant capacity (AOC) of 1.46 mg/mL in B56 and lowest AP with AOC of 58.91 mg/mL in G04. A high correlation was observed between TPC and IC50 of DPPH with the coefficient value of –0.671. Similarly, a high correlation with Pearson product coefficient of -0.9009 for TFC and IC50 was also obtained (Fig. 2). A strong positive relationship (r =0.856) was also obtained between TPC and TFC. Therefore, it can be concluded that antioxidant potentiality is highly influenced by the presence of TPC and TFC. All the correlation values are highly significant at P <0.05. It was revealed from the above interpretation that all the coloured rice landraces (B09, B72, B56, K69, P23, SH01) had higher AP than the non-coloured one.

A total of 14 different secondary metabolites, namely gallic acid (GA), protocatechuic acid (PRCA), 4-hydroxybenzoic acid (4HBA), vanillic acid (VA), caffeic acid (CA), syringic acid (SY), p-coumaric Acid (PCA), ferulic acid (FA), rutin (RU), o-cresol (OCRE), p-cresol (PCRE), myricetin (MY), 3,4-xylenol (3,4XYL), and quercetin (Q) were identified and quantified from different cultivars (Fig. 3). GA is present in almost all the studied samples, but more prominently in a higher amount in all non-coloured rice grains whereas coloured grain had a higher abundance of PRCA and RU. CA and FA are ubiquitously present in all samples with a more or less equal amount. It is found that 4HBA and PCA are prevalent in non-coloured samples than the coloured ones. One striking feature is that SY is only

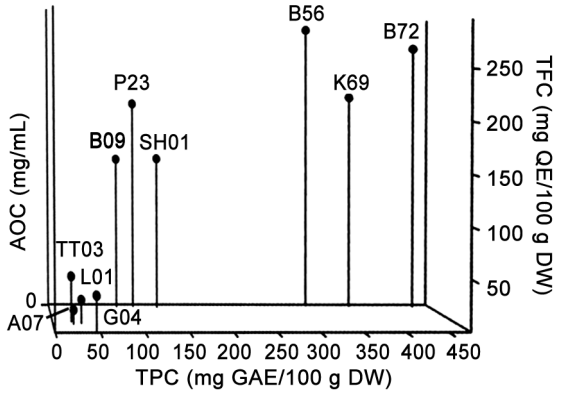

Fig. 2 — 3D scatter plot showing the correlation between total phenolic content (TPC), total flavonoid content (TFC) and IC_{50} value for calculating antioxidant potentiality. The number indicated on ball and stick represents the sample number of rice as per Table 1.

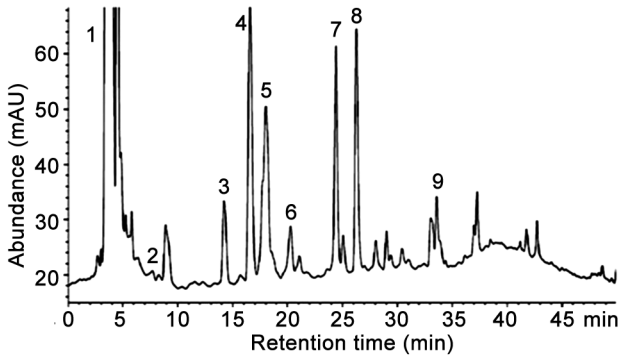

Fig. 3 — HPLC Chromatogram of secondary metabolites of the sample A07. Peak numbers indicate secondary metabolites, 1 GA, 2- PRCA, 3- 4HBA, 4- VA, 5- CA, 6- SY, 7- PCA, 8- FA, 9- PCRE. The X-axis represents retention time in minutes and the Y-axis represents the abundance of particular compounds in the milli absorbance unit (mAU).

present in non-coloured samples. The quantitative profile of secondary metabolites examined in these ten landraces indicated their "uniqueness" for nutritional benefits (Fig. 4). Wanyo *et al.*[18] found that GA, PRCA, *para*-hydroxybenzoic acid, VA, chlorogenic acid, CA, SY, PCA, FA and sinapic acid are present in 105 rice varieties of northeastern Thailand.

For overproduction of grains by developing high-yielding varieties and by modern grain processing technology, we are gradually compromising with the nutritional quality of rice. The mechanized husking and polishing process of rice grains remove nutritionally rich pericarp, testa and aleurone layer, commonly known as bran[3,4]. Rice bran is reported to contain a diverse array of phenolic acids and varied classes of flavonoids[19]. The pigmented rice contains a more diverse form and higher quantity of phenolic acids and FAis the principal component of bran conferring black colour of the grain[20]. The AOC in rice is directly influenced by the quality and quantity of flavonoids, reported by several authors[21,22]. The six coloured varieties contain higher proportions of AOC, TPC, and TFC (Fig. 2). Among the whole flavonoid class, anthocyanin is the major compound responsible for the pigmentation of rice grain[23]. Shikimic acid pathway in combination with the acetate pathway is responsible for the biosynthesis of many flavonoids, flavono-lignans, and isoflavonoids[23] which ensure the colour variation of the rice grains.

Phenolic compounds confer variability in taste, flavour, and health-promoting properties in cereals, vegetables, and fruits[24]. It has been reported earlier that phenolic substances, besides their known antioxidant capacity, serve a protective role in the human body against many chronic metabolic disorders and chronic diseases such as cardiovascular disease and hyperlipidemia by upregulating the expression of nuclear factor-erythroid 2

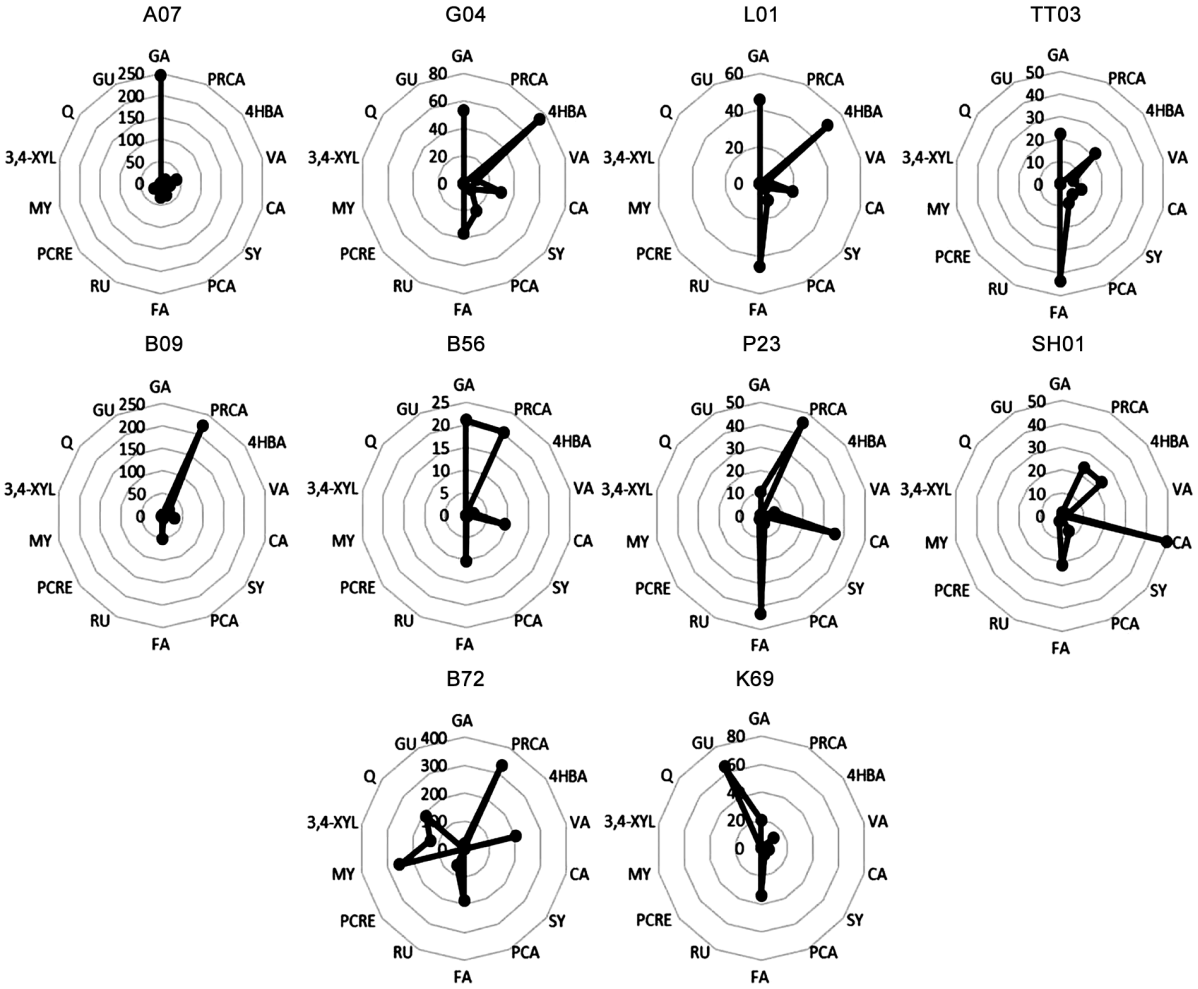

Fig. 4 —Radar chart generated based on the concentration of secondary metabolites representing a unique profile of 10 different landraces. The numeric values in the radar chart represent the amount of each secondary metabolites expressed in µg/g of dry weight of the sample.

(Nrf2)[25], peroxisome proliferator-activated receptors (PPARs), and activating AMPk/SIRT1 (50 adenosine monophosphate-activated protein kinase/sirtuin 1) signalling cascade[26].

The authors tried to interpret the 'uniqueness' of these ten landraces in terms of the amounts of phenolic acid, polyphenols, and flavonoids (Fig. 4). Coloured rice showed a high amount of AP in all cases. Therefore, the direct co-relation between AP and coloured grain is indicative of the additional nutritive value of the grains.

Conclusion

The present findings may hopefully promote an extensive and comprehensive exploration of the nutritional potential of more indigenous landraces, which have remained largely neglected in mainstream rice research. These findings may encourage further conservation of these rare and evanescent landraces to provide nutritional security for people, especially in the rice-eating part of the Globe.

Acknowledgement

The authors acknowledge the financial assistance from DST-FIST programme (SR/FST/LSI-459/2010 dated 10.03.2011) at the Department of Botany, University of Calcutta, Govt. of India for providing HPLC instruments. SR is grateful to the University Grant Commission, Government of India for the JRF grant (UGC/341/Jr. Fellow (upgradation), dt. 05.05.2015). The authors also thank Debdulal Bhattacharjee of Basudha for helping in maintaining and procuring the rice samples.

Conflict of interest

Authors have no conflict of interest.

Reference

1 Richharia R H and Govindasamy S, *Rices of India*, (Academy of Development Science, Karjat), 1990.

2 Deb D, Folk rice varieties, traditional knowledge and nutritional security in South Asia, in *Agroecology, Ecosystems, and Sustainability in the Tropics,* edited by G. Poyyamoli, (Studera Press, New Delhi), 2017, 117-134.

3 Lai P, Li KY, Lu S and Chen H H, Phytochemicals and antioxidant properties of solvent extracts from Japonica rice bran, *Food Chem,* 2009, **117**, 538-544.

4 Walter M and Marchesan E, Phenolic compounds and antioxidant activity of rice, *Braz Arch Biol Technol*, 2011, **54**, 371-377.

5 Hernandez N, Rodriguez-Alegría M E, Gonzalez F and Lopez-Munguia A, Enzymatic treatment of rice bran to improve processing, *J Am Oil Chem' Soc*, 2000, **77**, 177-180.

6 Jiang Y and Wang T, Phytosterols in cereal by-products, *J Am Oil Chem' Soc*, 2005, **82**, 439-444.

7 https://wholegrainscouncil.org/newsroom/whole-grain-statistics.

8 Jones J M and Sheats D B, Consumer trends in grain consumption, *Reference Module in Food Science*, edited by Smithers G & Knoerzer K (Elsevier, Amsterdam, The Netherlands), 2016.

9 Tian S, Nakamura K and Kayahara H, Analysis of phenolic compounds in white rice, brown rice, and germinated brown rice, *J Agric Food Chem*, 2004, **52**, 808–4813.

10 Adom K K and Liu R H, Antioxidant activity of grains, *J Agric Food Chem*, 2002, **50**, 6182–6187.

11 Mira N V M, de Massaretto I L, Pascual C S C I and Lanfer-Marquez U M, Comparative study of phenolic compounds in different Brazilian rice (*Oryza sativa* L.) genotypes, *J Food Compos Anal*, 2009, **22**, 405–409.

12 Roy P, Orikasa T, Okadome H, Nakamura N and Shiina T, Processing conditions, rice properties, health and environment, *Int J Environ Res Public Health*, 2011, **8**(6), 1957-976.

13 Sood B C and Siddiq E A, A rapid technique for scent determination in rice, *Indian J Genet Plant Breed*, 1978, **38**, 268–271.

14 Harborne J B, *Phytochemical methods*, 2nd edn, (Chapman and Hall, London), 1973.

15 Goufo P and Trindade H, Rice antioxidants: Phenolic acids, flavonoids, anthocyanins, proanthocyanidins, tocopherols, tocotrienols, γ-oryzanol, and phytic acid, *J Food Sci Nutr*, 2014, **2**(2), 75-104.

16 Brand-Williams W, Cuvelier M E and Berset C L W T, Use of free radical method to evaluate antioxidant activity, *LWT-Food Sci Technol*, 1995, **28**(1), 25-30.

17 Dey P, Ray S, Sarkar M P and Chaudhuri T K, Chemical characterization and assessment of antioxidant potentiality of *Streptocaulonsylvestre* Wight, an endangered plant of sub-Himalayan plains of West Bengal and Sikkim, *BMC Complement Altern Med*, 2015, **15**(1),107.

18 Wanyo P, Meeso N and Siriamornpun S, Effect of different treatments on the antioxidant properties and phenolic compounds of rice bran and rice husk, *Food Chem*, 2014, **157**, 457–463.

19 Huang Y P and Lai H M, Bioactive compounds and antioxidative activity of coloured rice bran, *J Food Drug Anal*, 2016, **24**(3), 564-574.

20 Deng G-F, Xu XR, Zhang Y, Li D, Gan R-Y, *et al.,* Phenolic compounds and bioactivities of pigmented rice, *Crit Rev Food Sci Nutr*, 2013, **53**(3), 296-306.

21 Goffman F D and Bergman C J, Rice kernel phenolic content and its relationship with antiradical efficiency, *J Sci Food Agric*, 2004, **84**, 1235–1240.

22 Zhang M W, Guo B J, Zhang R F, Chi J W, We1 Z C, *et al.*, Separation, purification and identification of antioxidant compositions in black rice, *Agric Sci China*, 2006, **5**(6), 431-440.

23 Dewick P M, *Medicinal Natural Products: A Biosynthetic Approach*, III edn, (John Wiley & Sons, New Jersey), 2009.

24 Tomás-Barberán F A and Espín J C, Phenolic compounds and related enzymes as determinates of quality in fruits and vegetables, *J Sci Food Agric*, 2001, **81**, 853-876.

25 Chiva-Blanch G and Visioli F, Polyphenols and health: Moving beyond antioxidants, *J Berry Res*, 2012, **2**, 63-71.

26 Giampieri F, Alvarez-Suarez J M and Battino M, Strawberry and human health: Effects beyond antioxidant activity, *J Agric Food Chem*, 2014, **62**(18), 3867-3876.