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Anthocyanin, Lutein, Polyphenol Contents and Antioxidant Activity of Black, Red and White Pigmented Rice Varieties

Saravanan Ponnappan[1], Arun Thangavel[1] & Omprakash Sahu[1*]

[1] Faculty of Chemical and Food Engineering, Bahir Dar Institute of Technology, Bahir Dar University, Ethiopia

[*] Omprakash Sahu, E-mail: ops0121@gmail.com

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Abstract

Colour rice varieties are rich in antioxidants and functional based properties such as anthocyanin, lutein and phenolic compounds. In this experiment, two of red pigmented (TPS-1and TKM-9), one white (glutinous rice) and black pigmented rice varieties are cultivated from India were analysed to determine their antioxidants and nutrition based functional properties. Based on the result, the anthocyanin content was very high on black rice than other variety contents up to 244.45 mg/100 g. Polyphenol compound were varied significantly within the compared varieties. Highest polyphenol compound content (463.05 mg/100 g) was found in the black rice and also showed rich antioxidant properties. Obviously, black rice rich source of lutein compound was also higher than other varieties where under the experimental condition. DPPH (Determination of 2, 20-diphenyl-1picrylhydrazyl radical scavenging ability) scavenging capacity starting from 69.46% to 76.4% ranged to level of remain DPPH.

Keywords

antioxidants, anthocyanin, polyphenol compound, DPPH

1. Introduction

Rice is the essential cereal crop in developing world countries. Rice is utilizing as a staple food for one half of the population in developing countries (Bhattacharjee et al., 2002). But most of the developing countries living populations are widely eating white rice, even though they are cultivating enormous rice variety which have been containing various pigments such as black, red and purple color kernels. That kernels contains rice are rich in inevitable antioxidants such as phenolic compounds and lutein (Perera & Yen, 2007).

From the report of previous study have been conducted by Lee et al. (2008), anthocyanin playing major role of cholesterol reduction and also inhibitory effects on in vitro allergic reaction in human body

(Abdel-Aal et al., 2006; Yang et al., 2008). Peonindin-3-glucoside and cyanindin-3-glucoside are all the major anthocyanin compounds which are present in the extracts of black rice, also these compounds are influencing an inhibitory effect of invasion of cells on different cancer (Chen et al., 2006). Lamberts and Delcour (2008) studied that carotenoids decreases the formation of cancer cells and other heart disease. Enormous *in vitro* and *in vivo* studies have revealed that LDL modification through the help of oxidation mainly plays onset of exacerbates and atherosclerosis on clinical manifestation (Siddiq, 2004). Pigmented rice are studied as a major source of antioxidants and other vital functional properties especially lutein and phenolic compounds (Frei & Becker, 2005). Normally, the health benefits of pigmented rice varieties are very high but the production and supply are very less for the demand of population needs (Yawadio et al., 2007). Zeazanthin and lutein are the essential non provitamin-A which were present in the eye protection against both infection and disease (Rose, 1999; Tan et al., 2005). With significant health benefits of therapeutic values in rice varieties have been known since from heritage scriptures and experience from the peoples (Siddiq, 2004; Johnson, 2002).

2. Methods

2.1 Sample Collection

To study the sample experimental pigmented rice varieties (Figure 1) were selected and purchased from various available markets in India. The paddy of black rice (kavuni rice) was obtained from Dry Land Agriculture Research Station, Kanadukathan, Chettinad, India. Red rice paddy varieties TPS-1 were obtained from Regional Rice Research Station, Thirupathisaram and TKM-9 obtained from Rice Research Station, Thirurkuppam, Thiruvallur. Glutinous white rice which was sourced from Mumbai was purchased from Remuki Departmental stores, Madurai, India. Finally, the macerated and grounded ricesample flours were prepared in laboratory. Under the following methods were analysed appropriate compounds from different pigmented rice varieties.

2.2 Anthocyanins

Potassium chloride buffer (0.03 mol/l) and sodium acetate buffer were added with 20 µl rice sample extracts from pigmented rice varieties. Mixed well and also allow those compounds for 15 min to absorb measurements on spectrophotometer at 500 nm and 700 nm (Nicoue et al., 2007). Distilled water used for blank purpose. The concentration of anthocyanin content (mg/l) from the extracted samples was calculated with standard formula and also result has expressed as cyanin-3-glucoside equivalents.

$$\text{Anthocyanin content} = (A \times MW \times DF / \varepsilon \times 1) \times 1000$$

Where,

A = (Aλ700) pH 1 - (Aλ700) pH 4.5;

MW = Mol. wt. of Cyanidin-3-glucoside;

DF = Factor of dilution;

Ɛ= Extinction coefficient (L x cm - 1 x mol-1) = 26,900 for Cyanidin-3-glucose;

where L (length) = 1.

2.3 Polyphenols Compounds

In test tubes, the extraction of methanol was taken and also prepare the volume up to 3 ml with distillation water. Then, 0.5 ml Folin-ciocalteau reagent was mixed with methanol solution. About 20 per cent sodium carbonate was added under the volume of 2 ml after three min approximately and mixed well again. Absorbent measurement range on spectrophotometer wavelength range at 650 nm against the blank using with VIS spectrophotometer. A set of standard solutions of gallic acid prepared using with distilled water (10 µg-100 µg per ml) was treated in the same manner as described earlier and read against a blank. Polyphenol compounds was expressed in mg and also it was equivalent to gallic acid per 100g on FWB basis.

2.4 Lutein Content of Selected Pigmented Rice Varieties

Chemicals: Acetonitrile, hexane, methanol, ethanol and dichloromethane were of HPLC grade standard.

Carotenoid extraction: Macerated colour rice samples flour were mixed with sodium sulfate (5 g) and 2 mm-Rtocopherol in methanol solution. Ice-cold acetone was used to prepared colorless carotenoids content extracted solution (volume: 400 ml). Prepared crude extract was mixed and shaken with 100ml hexane. Three or four times the extraction was repeated finally make the known volume up to 250 ml. The mixed crude hexane was dried with anhydrous sodium sulfate (20 g) and filtered through filter paper (whatman No.1). An aliquot filtered known extracted volume (100 ml) solution was dried under the nitrogen stream and the remaining residue of the hexane solution was redissolved in 1 ml acetonitrile chemical. Finally, the prepared samples were analysed by HPLC.

HPLC Analysis: SGE C-18 (ODS) column was used to separate carotenoids through HPLC instrument. About 0.1 per cent ammonium acetate with methanol was used as mobile phase for the carotenoids separation. 20 µl measured samples were injected in ODS column on HPLC equipment and also isocratic condition was continued at 1 ml/min flow rate. λmax values of the compound were determined by the time of retention and standard chromatograms assessed through the help of SPD-10 AVD detector. In Column result where the quantified peak area were related to the standard reference.

2.5 Anti-Oxidant Activity

2.5.1 Diphenyl-Picryl-Hydrazyl (DPPH) Assay

DPPH is a purple coloured stable free radical and will form yellow colour when it was reduced as Diphenyl-Picryl-Hydrazine complex. The sample extracts electron donation ability was calculated from purple colour beaching of DPPH methanol solution. Scavenging antioxidant potential indicates by discoloration. The DPPH assay was performed by Goupy et al. (1999). 2 ml of 60 µM 2, 2-diphenyl-1-picryl-hydrazyl in methanol (initial absorbance of DPPH was 0.62 ± 0.02.) was added to one ml of various concentrations of sample extract. Prepared 1 min vortexed mixture and kept in room temperature under the dark condition for 30 minutes. Decreased absorbance was measured in spectrophotometer under the wavelength of 517 nm with used methanol as a blank portion. Ascorbic

acid was applied as positive control point. Calibration curve was diagrammed indicated for using absorbance versus concentration of ascorbic acid and the results were noted that as a mg (vit-C) equivalent per 100 g of sample on FWB basis.

Figure 1. Selected Pigmented Rice Varieties for Study

3. Results

Carotenoids are essential chemical compounds of coloured rice varieties. Selected rice flour samples were very low levels of β-carotene content were eluted by HPLC. But the coloured rice varieties were good source of lutein shown in Figure 2. The lutein content found to be maximum in TPS-1 (1075 ng/100 g) followed by black rice (280 ng/100 g), glutinous white rice (240 ng/100 g) and TKM-9 (215 ng/100 g) respectively. The different significant ratio ($P < 0.05$) was noticed in terms of lutein content between the pigmented rice varieties and white rice. The data indicates the presence of maximum level of anthocyanin mainly in black rice (244.45 mg/100 g) while the other pigmented rice varieties such as TPS-1 and TKM-9 had anthocyanin levels of 1.39 and 1.25 mg/100 g respectively, with lowest levels recorded in glutinous white rice (0.32 mg/100 g).

Table 1. Lutein, Anthocyanin, Polyphenols Content and Antioxidant Activity of Selected Pigmented Rice Varieties

Varieties	Lutein (ng/100 g)	Anthocyanin (mg/100 g)	Polyphenols (mg/100 g)	Antioxidant activity DPPH (%)
Glutinous white Rice	240 ± 2.08	0.32 ± 0.01	88.21 ± 0.04	69.46 ± 0.05
Black rice	280 ± 1.52	244.45 ± 0.02	463.05 ± 0.07	86.12 ± 0.05

TPS-1	1075 ±4.50	1.39 ±0.01	340.13 ±0.08	84.42 ±0.05
TKM-9	215 ±1.52	1.25 ±0.01	208.51 ±0.06	79.79 ±0.03
SEd	1.2247	0.0053	0.0156	0.0091
CD (0.05)	2.9970**	0.0129**	0.0381**	0.0223**

The polyphenol content of pigmented rice varieties was noticed to be minimum in glutinous white rice (88.21 mg/100 g) compared to higher levels recorded in black rice (463.05 mg/100 g), followed by red rice varieties TPS-1 (340.13 mg/100 g) and TKM-9 (208.51 mg/100 g).

The data pertaining to antioxidant activity of the pigmented rice varieties is presented in Table.1. The stable DPPH radical is continuously used to test hydrogen donating antioxidants in enormous plant species. It can be inferred, that antioxidant activity of selected pigmented rice varieties was maximum in black rice at 15.81 per cent in terms of DPPH value followed by TPS-1 (13.42%), TKM-9 (12.68%) and lowest in glutinous rice (10.48%). Significant difference in terms of total antioxidant activity was noted between the selected varieties.

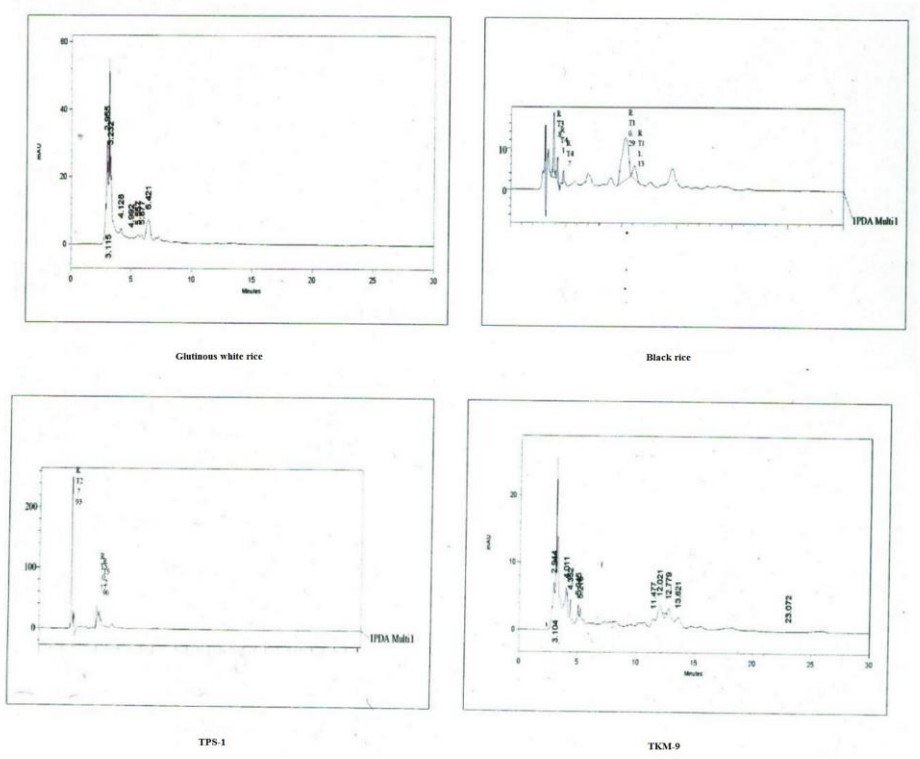

Figure 2. Lutein Content of Selected Pigmented Rice Varieties

4. Discussion

Anthocyanin, lutein and polyphenol compounds were essential antioxidants which were majorly present in pigmented rice varieties. The data results shows presence the level of anthocyanin, polyphenol and other vital antioxidant activities were higher in black rice variety. But the lutein content

was maximum in TPS-1 red rice variety. Sompong et al. (2011) reported that cyanidin 3-glucoside and peonoidin 3-glucoside as the dominant anthocyanins in black rice varieties with contents ranging from 19.4 to 140.8 mg/100 g DM and 11.1-12.8 mg/100 g DM respectively. Red rice varieties to have polyphenol compounds in the range of 79.2 and 691.4 mg FA equivalent/100 g with a mean TPC of 364.8 mg FA equivalent/100 g. The black rice had a higher mean polyphenol compounds of 492.8 mg FA equivalent/100 g than the red ones. Also he was evaluated the DPPH value of three black rice varieties to range from 16.04 to 30.25 per cent and that of 10 red rice varieties to range from 12.99 to 76.38 per cent. No significant difference between the black and red rice varieties were reported.

In Conclusion, this experimental study has shown that the pigmented rice varieties were good source of antioxidants and other vital functional properties such as lutein, anthocyanin, and polyphenol compounds. The lutein content found to be maximum in TPS-1 (1075 ng/100 g) followed by black rice (280 ng/100 g), glutinous white rice (240 ng/100 g) and TKM-9 (215 ng/100 g) respectively. The different significant ratio ($P < 0.05$) was noticed in terms of lutein content between the pigmented rice varieties and white rice. Maximum level of anthocyanin mainly in black rice (244.45 mg/100 g) while the other pigmented rice varieties such as TPS-1 and TKM-9 had anthocyanin levels of 1.39 and 1.25 mg/100 g respectively, with lowest levels recorded in glutinous white rice (0.32 mg/100 g). The polyphenol content of pigmented rice varieties was noticed to be minimum in glutinous white rice (88.21 mg/100 g) compared to higher levels recorded in black rice (463.05 mg/100 g), followed by red rice varieties TPS-1 (340.13 mg/100 g) and TKM-9 (208.51 mg/100 g). Antioxidant activity of selected pigmented rice varieties was maximum in black rice at 15.81 per cent in terms of DPPH value followed by TPS-1 (13.42%), TKM-9 (12.68%) and lowest in glutinous rice (10.48%).

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