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Effect of different processing technologies on phenolic acids, flavonoids and other antioxidants content in pigmented rice

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ABSTRACT

Rice is consumed in the form of different products. The present study reported the extractable and free form of phenolic acids and flavonoid profile from different processed products (popped, beaten, puffed and boiled rice) of four black rice cultivars (*Chakhao, Kalobhat, Mamihunger. Manipuri Black*) along with a white rice variety (*Swarna sub-1*) as reference. In addition, vitamin-E, γ-oryzanol, anthocyanin, phytic acids and antioxidant activity were also examined. The HPLC-PDA analysis of phenol extracts detected 14 phenolic compounds comprising of 4 hydroxybenzoic acid, 5 hydroxycinnamic acid and 5 flavonoids in most of the products with their higher content in black rice based products than that of reference cultivar. Among the different products, popped rice ranked first in terms of mean phenolic compounds (467.74 μg/g) of which 54% occurring in free form, vitamin-E, γ-oryzanol and antioxidant activity. Boiled rice had the least amount of all these components. Based on PCA, popped rice of MN was identified as a promising product that could contribute to potential health benefits.

Introduction

Rice forms an important staple crop and provides energy to majority of the world population due to its high starch content. In addition, the embryo fraction and bran layer in rice grain containing hypoallergenic protein anchors many functional molecules including phenolic compounds (Iqbal et al., 2005) vitamin-E (Orthoefer, 1996) and γ-oryzanols (Golder & Juliano, 2004). These components of rice grain with their antioxidant function are hypothesized to arrest several oxygen-linked chronic diseases and thus contribute positively to human health. The retention of these bioactive molecules in rice grain is governed by the post-harvest processing methods such as hulling, parboiling and milling etc. Various traditional products of rice viz. boiled, puffed, popped and beaten rice are very popular in eastern India and consumed in lunch, dinner and as breakfast. The adherence of bran in differently processed rice products is therefore very important for human health. The tradition of consuming differently processed rice products derived from conventional white rice may likely to cause deficiencies of essential vitamins, minerals and other nutritional and functional compounds (Bouis, 2003). In the recent past, pigmented rice cultivars such as brown, black and red rice have gained considerable recognition primarily because of their nutritional properties. Many pigmented rice cultivars with different medicinal properties are being explored in India (Siva et al., 2010). The bran layers of pigmented rice are correlated well with the accumulation of significant amount of phenolic compounds including anthocyanins, phenolic acids and flavonoids (Sumczynski et al., 2016; Shao et al., 2018; Sompong et al., 2011; Wu et al., 2013), which are sequestered in the vacuole. The black grain colour is caused mainly by the deposition of anthocyanins (Goufo and Trindade, 2014). Phenolic compounds in rice grains are usually present in soluble free, soluble bound and insoluble bound forms (Park et al., 2012) with a natural abundance of insoluble bound form (Zhang et al., 2010) that result from incorporation of a major portion of free phenolic acids viz. ferulic acid and p-coumaric acid to cell wall polysaccharides (arabinoxylans) and lignin (Zhou et al., 2004). In addition to γ-oryzazol and vitamin-E, the black rice contains ferulic, protocatechuic and *trans-p*-coumaric acid as major free phenolic acid, while ferulic acid, vanillic acid and quercetin are present in bound form (Sumczynski et al., 2016; Shao et al., 2018). In terms of usability of different forms of phenolic compounds, the soluble form with their ready absorption in the stomach and small intestine, can prevent free

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radical mediated oxidation of biological macromolecules (Shao and Bao, 2015), whereas the bound forms, although undergo poor absorption in the intestine, are made available only after digestion by the various enzymes (Saura-Calixto et al., 2007).

In view of the nutritional significance of pigmented rice, we prepared some popular black-rice based products viz. popped, puffed, beaten, boiled and milled rice along with conventional white rice. The physicochemical, sensory properties and consumers preferences of these products were reported earlier (Pal et al., 2019). We extended our study to include the evaluation of these products on the contents of individual free and bound phenolic compounds together with other antioxidant components such as anthocyanin, vitamin-E and γ-oryzanol. The antioxidant activity of different products using ABTS and DPPH radical were also examined. Finally, Principal component analysis was made to establish possible relationships between the different chemical variables examined in this study as well as to identify the nutritionally superior product, taking cultivars into account.

2. Materials and methods

2.1. Preparation of products

The four black rice cultivars viz. *Kalobhat (KB), Chakha (CH), Mamihunger(MA), Manipuri Black (MN),* along with white rice variety *Swarna sub-1(SW)* (Fig. 1) as control were chosen for the preparation of various rice products such as milled, popped, puffed, beaten and boiled according to the protocol described earlier by Pal et al. (2019). Briefly, the preparation methods of these products are described below. (a)*Raw polished rice*- The rough grains were dehusked and subsequently polished through Satake rice huller and miller machine (Satake Corporation, Japan) to obtain raw polished grain (b*) Popped rice*-The rough rice was roasted in an iron pan containing heated sand (>177 °C) for 40–50 s with continuous stirring. The entire partially or fully detached husk was removed manually from the popped kernel to obtain fresh edible popped rice. (c) *Puffed rice*-The double parboiled polished rice grain was roasted with 10–12% brine solution for 1–2 min with constant stirring and then transferred to a iron pan containing preheated (approx. 220 °C) fine sand. Finally, constant stirring (for 15–20 s) of grains in sand container produced puffed rice, which was separated from sand by sieving. (d) *Beaten rice*-Rough rice was cleaned and graded to remove impurities and then it was soaked in water overnight followed by hot water (approximately 60 °C) treatment for about 45 min. After drying, the grain was roasted for 2–3 min to make flakes with the rice flaking machine. (e) *Boiled rice*-It was made through boiling of the parboiled polished rice and water in a container for 18–20 min followed by decanting of excess water. For the entire biochemical assay, the samples were taken as a fresh material with three replications and data were presented as dry weight basis.

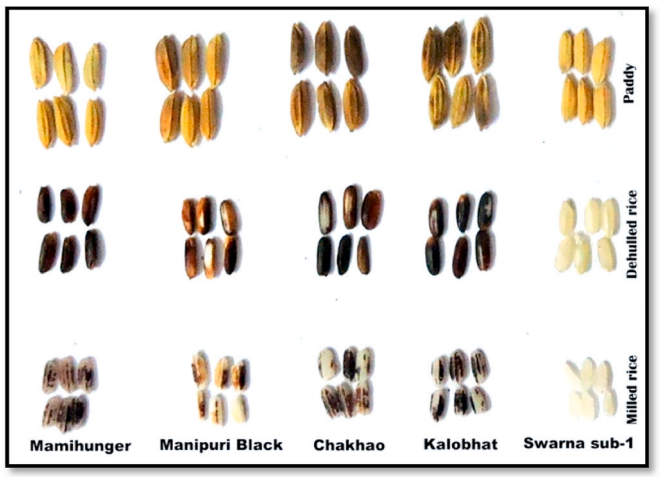

Fig. 1. Dehulled and milled rice of some black rice and white rice.

2.2. Chemicals and reagents

2,2′-azino-bis-(3-ethylbenzonthiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and analytical standard of phenolic acids viz. caffeic acid (CAF), chlorogenic acid (CHL), cinnamic acid (CIN), ferulic acid (FER), gallic acid (GAL), *p*-coumeric acid (PCO), *p*-hydroxy benzoic acid (PBA), protocatechuic acid (PRO), vanillic acid (VAN) and the flavonoids such as kaempferol (KAM), catechin (CAT), apigenin (API), luteolin (LUT), myricetin (MYR) and rutin (RUT)were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Other essential chemicals were purchased from Merck, India Pvt. Ltd. The HPLC grade methanol, acetic acid and acetonitrile were used for the extraction, purification and HPLC analysis were purchased from Merck, India Pvt. Ltd.

2.3. Determination of free phenolic (FP) and extractable phenolic (EP) compounds

Analysis of FP and EP compounds in different rice products were made based on method adopted by Vinson et al. (1998) with some modification. For the extraction of FP and EP compounds, 1.0 g sample of each of the rice products was mixed with 10 ml of 50% aqueous methanol and acidified (1.0N HCl) 50% aqueous methanol (PH > 2.5) respectively and heated at 80 °C for 3h. Each sample was extracted thrice. The supernatant obtained after centrifugation at 15000*g* for 15 min was filtered through syringe filter (0.22 mm nylon syringe filters; Phenomenex, Torrance, CA, USA). Finally, the analysis was made by a HPLC (Agilent 1220) coupled with C-18 RP column (125/4 mm with the 5-mm particle size), photo diode array detector (WL 280 nm). Filtered extracts were injected in the HPLC system. The separation of phenolic compounds was performed with a gradient consisting of solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile) at a flow of 1 ml/min with the following gradient program as follows: 15% B linear from 0 to 12 min; 50% B linear from 12 to 35 min; 85% B linear from 35 to 45 min; 15% A linear from 45 to 50 and additional 10 min equilibration time. The column temperature was 35 °C. The sample (20 μL) was injected. Chromatographic peaks were identified based on comparison of retention time with that of authentic analytical standard and quantification was made by chromatographic chemistation software using a linear regression for the relationship of peak area versus phenolic compound concentration.

2.4. Determination of antioxidant activity

Total antioxidant activity was measured using DPPH and ABTS radicals separately as per methods described by Zhu et al. (2011) and Sanghamitra et al. (2018) respectively with some modification. In DPPH assay, 1g finely powdered rice products was extracted in a mortar and pestle with 10 ml ethanol followed by centrifugation at 10000*g* for 20 min. The supernatant was collected and volume was made to 10 ml with ethanol. The reaction mixture containing 1 ml of extract, 2 ml ethanol DPPH (10 μM i.e.2mg/50 ml) solution was kept at room temperature for 30 min. Subsequently, the absorbance was read at 517 nm against solvent blank. The antioxidant in sample was measured through percentage inhibition of absorbance. Each extract was measured in triplicate.

The ABTS + reagent was prepared with 7 mM ABTS+ and 2.45 mM potassium persulfate in double distilled water and was kept as it is for 16 h in dark at normal room temperature (30 °C). This reaction mixture was again dissolved in the mixture of ethanol: water (50:50, v/v) to adjust the absorbance to 0.700 ± 0.020 at 734 nm.6 ml ABTS + reagent was added to 10 mg of finely powdered rice flour (100 mesh size) and vortexed for 1.5 min to perform the surface specific reaction and then

centrifuged at 9200g for 2 min. The absorbance was measured at 734 nm after 30 min. The antioxidant capacity was expressed as micromole of ascorbic acid equivalent (AAE) g^{-1} of sample.

The FRAP radical scavenging assay was conducted according to the method of Zhu et al. (2011) with little modification. Briefly, 1.5 g powdered sample was mixed with 15 ml ethanol-water (70:30) and was kept overnight at room temperature after shaking. The supernatant was collected following centrifugation (5000 rpm 10 min). The whole process was repeated thrice. The combined supernatant was evaporated off and subsequently volume was made to 10 ml with ethanol-water (70:30). 0.5 ml extract +1.25 ml Phosphate buffer saline solution (PBS; 0.2M pH6.6) + 1.25 ml 1% potassium ferricyanide were mixed. The solution was incubated at 50 °C for 20 min in water bath. After cooling, 1.25 ml 10% TCA was added. The resulting solution was centrifuged at 5000 rpm for 10 min. 1.25 ml aliquot +1.25 ml distilled water +0.25 ml ferric chloride (0.1%) were mixed and kept it for 10 min at room temperature. The absorbance of the solution was read at 700 nm. Increase in absorbance is associated with increase in reducing power.

2.5. Anthocyanin content of grains and their products

Anthocyanin content in each of the products was estimated according to the method described by Pal et al., (2019). Briefly, 1 g of rice sample was homogenized with 5 ml acidified organic solvent (95% methanol: 1.5 N HCl 85:15, pH = 1) and kept overnight followed by centrifugation for 10 min at 10000 rpm. The final volume of supernatant was made up to 10 ml, which was used to measure the absorbance at 535 nm. Total anthocyanin content of the samples were calculated as mg per 100 g of sample using the multiplication factor = 16.73 X Absorbance or Anthocyanin (mg/100g)=(abs x Molecular wt x 1000)/(molar absorptivity (e) x path length in cm) = abs x 450 × 1000/26900 × 1.

2.6. Estimation of γ-oryzanol and Vitamin-E

Extraction of γ-oryzanol and Vitamin-E was performed according to Chen and Bergman (2005) with some modification. Briefly, 0.5 g of respective rice products were mixed with 5 ml of HPLC-grade isopropanol in a falcon tube, vortexed for 2 min at 25 °C. The supernatant was obtained after centrifugation at 4500*g* for 10 min. The remaining residue was extracted twice each time with 5 ml of isopropanol followed by centrifugation. The combined supernatant fractions were evaporated to dryness using a vacuum rotary evaporator and dissolved in 5 ml of HPLC-grade isopropanol. The extract was filtered through a 0.45 μm membrane filter and 20 μL aliquot was transferred directly into a HPLC vial for the analysis of γ-oryzanol and tocopherol. Separation was made by an analytical Shimadzu HPLC system equipped with LC-20AT pump, SPD-M10AVP photodiode array detector and scanning fluorescence detector (Shimadzu, Kyoto, Japan) using the method described by Pascual et al. (2011) with some modification. Samples were injected onto a 250 mm × 4.60 mm (5μ) (C18) Phenomenex Column. Elution was performed at 25 °C and at a flow rate of 1 mL/min. The composition of the mobile phase was 35% acetonitrile, 55% methanol and 10% isopropanol and operated in low pressure gradient mode. Total run time was 35 min including column equilibration. The γ-oryzanol standard, monitored with PDA detector at 325 nm that separated 4 main peaks between 13 and 18 min. Under the experimental condition employed, four peaks were not individually identified and the quantification of total γ-oryzanol was based on the sum of the peak area of those peaks. Similarly, the standard Vitamin-E was injected with desired concentration at an excitation wavelength of 298 nm and an emission wavelength of 328 nm. 4 well-defined peaks were detected. Vitamin- E content was calculated accordingly taking 4 peak area all together.

2.7. Phytic acid assay

Sample extraction: Rice grains (2 g) were ground in a centrifugal grinding mixer and passed through a 0.5 mm sieve to obtained uniform particle size. One gram of ground powder was thoroughly mixed with 20 ml of 0.66 M HCl in falcon tubes and shaken at 220 rpm for 16 h in a shaker and centrifuged at 13,000 rpm for 10 min. The crude extract measuring 0.5 ml was mixed with 1.5 ml of 0.75 M NaOH solution for neutralization. The neutralized sample extract was used for the enzymatic dephosphorylation reaction procedure by phytase and alkaline phosphatase enzyme which gives total phosphorus. PA was measured by an assay procedure specific for the measurement of phosphorus released as available phosphorus from PA, myo-inositol (phosphate)n, and monophosphate esters by phytase and alkaline phosphatase using the PA (phytate)/total phosphorus kit from Megazyme (Megazyme, Ireland) (Kumar et al., 2017).

2.8. Statistical analysis

Data analyses were performed with SAS version 9.1 software (SAS Institute, Cary, NC, U.S.A.). Differences among different rice samples were found by using split plot design and ANOVA, followed by Tukey's multiple comparison tests. Statistical significance was defined at a level of $P < 0.05$. Principal component analysis (PCA) was performed using all the phenolic compounds obtained from EP extract and their associated antioxidant activity, anthocyanin, vitamin-E and γ-oryzanol in different rice products. The PCA analysis has been done using the International Rice Research Institute (IRRI) software "Statistical Tool for Agricultural Research" (STAR)Version: 2.0.1.

3. Results and discussion

3.1. Phenolic compounds in different rice products

The HPLC analysis of FP and EP extracts of different rice products (Fig. 2 & Table 1) on comparison with the retention time of different standard phenolic compounds demonstrated the qualitative and quantitative presence of several phenolic compounds consisting of hydroxybenzoic acids (GAL, PBA, PRO and VAN), hydroxycinnamic acids (CIN, PCO, FER, CAF and CHL) and flavonoids (API, MYR, LUT, KAM and CAT). The level of almost all extractable and free phenolic compounds showed significant differences across different rice products. GAL was detected as predominant acid in all the products, with its abundance in popped rice. PRO ranked distinct second. However, it could be detected in none of the SW products. VAN was observed as next major acid but not detected in SW milled and puffed rice. Similar to GAL, PBA was detectable in all the products. Among the different products, boiled rice had the least quantity of all these acids. The levels of PRO and VAN recorded in different rice products compared well with the report of Sompong et al. (2011) and Shao et al. (2018) respectively. Among the different rice products, the mean extractable hydroxybenzoic acids content was higher in popped rice (75.78 μg/g DW) and least in boiled rice (17.50 μg/g DW). The other products had more or less similar amount around 48 μg/g DW. The stability of some phenolic compounds is adversely affected by heat leading up to 50% reduction in their contents at the time of boiling. After heat treatment, the distribution of phenolic acids was changed due to the breaking of esterified bond and glycosylated bond. Heating temperature and duration of heat generally destroy the structure of phenolic acids, which reduces their level in different processed products, albeit free phenolic acids increased. Almost all hydroxybenzoic acids except PBA, were present primarily in free form. Moreover, popped rice had higher mean free acid (83%) followed by boiled rice (75%), beaten rice (72%), puffed rice (69%) and milled rice (64%). The content of free acids present in milled rice and beaten rice was compared well with the report of Adom & Liu (2002). Popped (MA and KB) rice had comparable and relatively higher

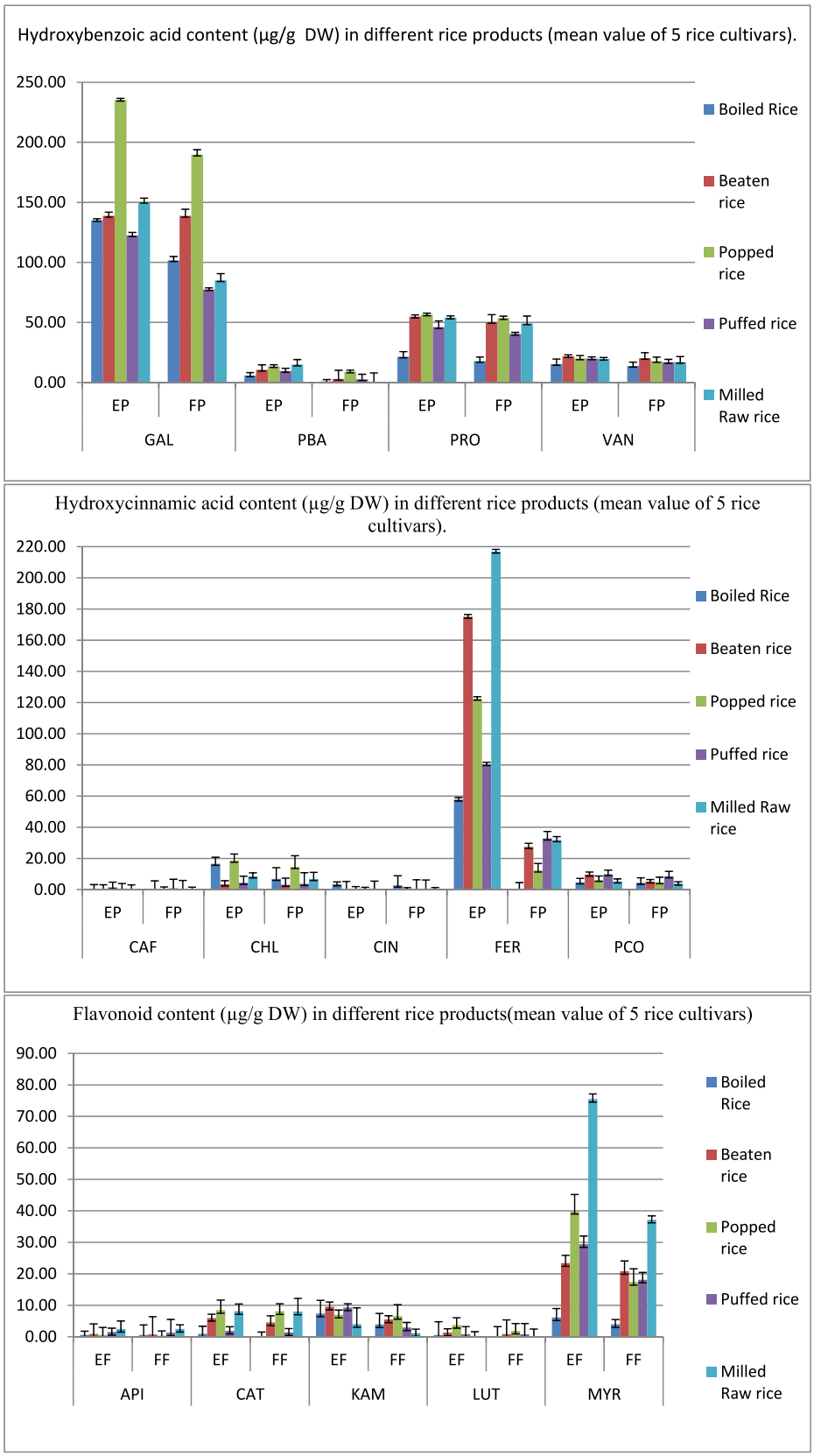

Fig. 2. Distribution of different phenolic acids and flavonoids according to different rice products.
*GAL: Gallic acid, PBA: *p*-hydroxy benzoic acid, PRO: protocatechuic acid, VAN: Vanillic acid; CAF: Cafeic acid, CHL: chlorogenic acid, CIN: Cinnamic acid, FER: Ferulic acid, PCO: *P*-coumeric acid; API: Apigenin, CAT: Catechin, KAM: Kaempferol, LUT: Luteolin, MYR: Myricetin. EP: Extractable Phenol, FP: Free phenol, EF: Extractable Flavonoids, FF: Free Flavonoids

extractable hydroxybenzoic acids, 69% and 87% of which were present as free acid in MA and KB popped rice respectively.

The results relating to extractable and free hydroxycinnamic acids (Fig. 2 & Table 1) in different rice products indicated that FER, PCO and CHL were detectable in all the products with few exceptions such as PCO in MA-boiled rice, CHL in SW-boiled and CH-milled rice. The study also disclosed complete absence of CAF in boiled rice and CIN in beaten and puffed rice of different cultivars. Moreover, CAF was not detectable in

Table 1
Distribution of extractable phenolic acids and flavonoids content (μg/g DW) according to different rice cultivars in different rice products.

	Hydroxybenzoic acids				Hydroxycinnamic acid			Flavonoids							
Boiled Rice Cultivars	GAL	PBA	PRO	VAN	CAF	CHL	CIN	FER	PCO	API	KAM	CAT	LUT	MYR	TOTAL
CH	45.50^{D}	1.93^{E}	8.33^{C}	7.03^{B}	0.00	6.53^{A}	0.30^{D}	0.00^{E}	0.95^{C}	3.05^{A}	9.93^{A}	0.00^{E}	0.00^{E}	3.38^{D}	86.90^{E}
KB	52.83^{C}	2.10^{D}	11.88^{A}	8.15^{A}	0.00	3.88^{B}	0.23^{E}	0.00^{D}	0.98^{A}	0.00^{D}	5.43^{D}	0.00^{D}	0.00^{D}	3.00^{E}	88.45^{D}
MA	57.68^{B}	2.95^{A}	9.18^{B}	5.37^{D}	0.00	0.95^{D}	0.33^{C}	24.88^{A}	0.00^{E}	0.00^{C}	8.75^{B}	0.00^{C}	0.00^{C}	6.45^{C}	116.53^{A}
MN	68.80^{A}	2.20^{C}	8.18^{D}	5.70^{C}	0.00	2.00^{C}	1.33^{A}	0.00^{C}	0.95^{B}	0.00^{B}	7.78^{C}	4.75^{A}	0.00^{B}	7.95^{B}	109.63^{B}
SW	45.45^{E}	2.45^{B}	0.00^{E}	4.45^{E}	0.00	0.00^{E}	0.58^{B}	21.43^{B}	0.90^{D}	0.00^{E}	5.07^{E}	0.00^{B}	2.93^{A}	10.08^{A}	93.33^{C}
Mean	54.05	2.33	7.51	6.14	0.00	2.67	0.55	9.26	0.76	0.61	7.39	0.95	0.59	6.17	98.97
p-Value	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Beaten Rice															
CH	126.67^{C}	8.28^{C}	45.70^{C}	19.34^{C}	0.00^{E}	2.84^{D}	0.00	149.47^{B}	8.25^{B}	1.04^{B}	19.24^{A}	9.29^{A}	0.75^{C}	29.55^{B}	420.42^{C}
KB	132.23^{B}	9.84^{B}	80.03^{B}	37.91^{A}	0.73^{A}	3.73^{A}	0.00	143.37^{D}	6.26^{E}	0.95^{C}	6.87^{D}	6.22^{C}	3.42^{A}	31.00^{A}	462.55^{B}
MA	109.91^{E}	16.45^{A}	40.99^{D}	14.76^{D}	0.57^{C}	3.03^{C}	0.00	146.72^{C}	7.74^{D}	0.57^{E}	8.64^{B}	3.10^{D}	0.00^{E}	20.59^{D}	373.05^{D}
MN	156.05^{A}	8.09^{D}	85.50^{A}	27.22^{B}	0.68^{B}	3.41^{B}	0.00	178.92^{A}	10.63^{A}	1.36^{A}	5.63^{E}	8.53^{B}	0.61^{D}	24.18^{C}	510.83^{A}
SW	113.11^{D}	5.58^{E}	-0.00^{E}	1.24^{E}	0.52^{D}	1.36^{E}	0.00	122.40^{E}	8.00^{C}	0.59^{D}	7.41^{C}	2.77^{E}	1.87^{B}	11.62^{E}	276.47^{E}
Mean	127.6	9.65	50.44	20.09	0.50	2.87	0.00	148.18	8.18	0.9	9.56	5.98	1.33	23.39	408.67
p-Value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Popped Rice															
CH	216.57^{C}	17.64^{A}	65.13^{B}	27.63^{B}	0.74^{C}	7.04^{D}	0.49^{A}	128.57^{C}	7.33^{A}	0.65^{B}	6.76^{D}	13.38^{A}	7.82^{A}	27.42^{D}	527.17^{D}
KB	227.86^{B}	14.92^{C}	98.48^{A}	31.48^{A}	3.33^{A}	15.31^{B}	0.43^{C}	132.73^{B}	6.45^{B}	0.64^{C}	10.95^{A}	9.26^{C}	2.06^{C}	36.08^{C}	589.99^{B}
MA	278.23^{A}	16.71^{B}	59.88^{C}	19.36^{C}	2.85^{B}	44.82^{A}	0.43^{B}	94.88^{D}	5.12^{C}	0.58^{D}	6.85^{C}	12.42^{B}	6.96^{B}	45.17^{B}	594.26^{A}
MN	212.63^{D}	10.46^{D}	41.58^{D}	14.72^{D}	0.00^{E}	7.95^{C}	0.17^{E}	163.55^{A}	3.80^{E}	0.68^{A}	7.16^{B}	6.83^{D}	1.60^{D}	79.97^{A}	551.09^{C}
SW	159.25^{E}	3.08^{E}	0.00^{E}	-0.00^{E}	0.00^{D}	6.26^{E}	0.27^{D}	15.74^{E}	4.45^{D}	0.23^{E}	2.93^{E}	0.00^{E}	0.00^{E}	9.13^{E}	201.35^{E}
Mean	218.91	12.56	53.01	18.64	1.38	16.28	0.36	107.09	5.43	0.56	6.93	8.38	3.69	39.55	492.77
p-Value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Puffed Rice															
CH	100.92^{E}	4.45^{E}	54.43^{C}	26.45^{B}	0.00^{E}	2.53^{E}	0.00	70.29^{C}	6.71^{D}	1.30^{B}	3.96^{E}	7.14^{A}	0.93^{C}	35.23^{B}	314.34^{C}
KB	125.00^{B}	6.71^{C}	96.57^{A}	31.97^{A}	0.97^{A}	3.03^{D}	0.00	127.28^{A}	14.11^{A}	0.44^{E}	4.26^{C}	-0.00^{D}	1.92^{A}	43.59^{A}	455.85^{A}
MA	151.73^{A}	19.49^{A}	55.48^{B}	16.66^{D}	0.81^{B}	5.99^{A}	0.00	76.08^{B}	14.04^{B}	4.19^{A}	6.98^{B}	-0.00^{C}	0.00^{E}	28.68^{D}	380.14^{B}
MN	101.73^{D}	5.34^{D}	14.77^{D}	18.81^{C}	0.78^{C}	4.26^{C}	0.00	62.91^{D}	4.60^{E}	0.87^{C}	26.56^{A}	2.27^{B}	0.00^{D}	31.87^{C}	274.74^{D}
SW	108.62^{C}	9.97^{B}	-0.00^{E}	1.92^{E}	0.58^{D}	4.64^{B}	0.00	38.37^{E}	6.74^{C}	0.80^{D}	4.20^{D}	-0.00^{E}	1.18^{B}	6.09^{E}	183.12^{E}
Mean	117.6	9.19	44.25	19.16	0.63	4.09	0.00	74.98	9.24	1.52	9.19	1.88	0.81	29.09	321.64
p-Value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	.	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Milled Rice															
CH	138.64^{B}	10.70^{D}	46.22^{D}	21.70^{B}	0.95^{A}	-0.00^{E}	0.15^{E}	175.27^{B}	2.52^{E}	1.58^{D}	6.57^{A}	14.10^{A}	0.00^{E}	51.26^{D}	469.67^{B}
KB	187.18^{A}	14.28^{C}	75.11^{A}	20.59^{C}	0.56^{C}	25.64^{A}	0.25^{A}	293.30^{A}	8.09^{A}	3.56^{B}	2.01^{D}	11.00^{C}	0.00^{D}	168.86^{A}	810.43^{A}
MA	130.72^{C}	17.69^{B}	69.23^{B}	20.05^{D}	0.00^{E}	4.03^{B}	0.19^{B}	131.78^{D}	4.17^{B}	4.64^{A}	5.61^{B}	13.27^{B}	0.00^{C}	57.52^{C}	458.91^{C}
MN	120.42^{D}	18.44^{A}	47.10^{C}	24.27^{A}	0.00^{D}	2.00^{C}	0.15^{D}	147.47^{C}	2.95^{C}	2.43^{C}	1.57^{E}	0.00^{E}	0.00^{B}	69.26^{B}	436.07^{D}
SW	84.49^{E}	4.24^{E}	0.00^{E}	0.00^{E}	0.64^{B}	1.27^{D}	0.15^{C}	91.89^{E}	2.67^{D}	0.40^{E}	4.25^{C}	2.44^{D}	1.07^{A}	30.83^{E}	224.33^{E}
Mean	132.29	13.07	47.53	17.32	0.43	6.59	0.18	167.94	4.08	2.52	4.00	8.16	0.21	75.55	479.88
p-Value	<.0001		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

*API: Apigenin, CAF: Cafeic acid, KAM: Kaempferol, CAT: Catechin, CHL: chlorogenic acid, CIN: Cinnamic acid, FER: Ferulic acid, GAL:Gallic acid, LUT: Luteolin, MYR: Myricetin; PBA: *p*-hydroxy benzoic acid, PCO: *P*-coumeric acid; PRO: protocatechuic acid, VAN: Vanillic acid; Superscript of the data reflects the ranking.

many of the products such as milled rice (MA and MN), puffed rice (CH), popped rice (MN and SW). The level of individual extractable acids viz. CHL, CIN, PCO and CAF detected in different products were comparable to earlier reports (Sompong et al., 2011; Niu et al., 2013 and Sumczynskiet al., 2016; Shao et al., 2018). The mean extractable hydroxycinnamic acids content in different products increased in the order: boiled rice < puffed rice < popped rice < beaten rice < milled rice indicating the loss of these acids during processing, which was more pronounced with boiled rice. Unlike hydroxybenzoic acid, hydroxycinnamic acids were present predominantly in bound form in most of the products except popped rice. Among these acids, FER and CIN were present only in bound form in boiled and puffed rice respectively. Moreover, some cultivar specific products were devoid of some free hydroxycinnamic acids. KB milled rice followed by MN-puffed rice had higher total extractable hydroxycinnamic acids, which were present primarily in bound form (80%). Fig. 2 indicated that FER formed the most prevalent hydroxycinnamic acid in pigmented rice. In contrast to the distribution of PCO in the rice kernel, FER is usually linked to cell wall constituents, arabinoxylans and distributed uniformly throughout the whole grain (Zhou et al., 2004). Thus, it appears that FER is hardly lost during the grain processing.

The results relating to extractable and free flavonoid compounds (Fig. 2 &Table 1) indicated that MYR followed by KAM formed the predominant flavonoids in different products. Mean extractable MYR content was higher in Milled rice (75.55 μg/gDW) followed by popped rice (39.55 μg/g DW). Mean extractable KAM content was higher in beaten (9.56 μg/g DW) followed by puffed rice (9.19 μg/gDW). CAT formed the next major flavonoid with its abundance in popped rice (8.38 μg/g DW) followed by milled rice (8.16 μg/g DW). Mean extractable API content was higher in milled rice (2.52 μg/g DW). However, LUT was not detectable in black rice based boiled and milled rice. API and CAT were detected only in CH and MN boiled rice, respectively. In addition, CAT and LUT were absent in many of the cultivar specific different products. In contrast to our result, Papadoyannis et al. (2012) reported that major flavonoids in the milled black rice grown in Greece include API (14.86 μg/g) followed by epicatechin (14.14 μg/g), CAT (4.9 μg/g), quercetin (4.23 μg/g) and MYR (4.00 μg/g). This discrepancy in results could be ascribed to differences in genotype and environment. Mean extractable flavonoid content decreased in the order: milled rice > popped > puffed > beaten > boiled rice with the predominance of mean free flavonoids in beaten rice (79%), which ranged from 54 to 57%. in other products. Moreover, extractable flavonoid content was highest in KB milled rice followed by MA milled rice. Considering these three classes of phenolic compounds, our result demonstrated that popped rice registered highest mean phenolic compounds (492.77 μg/g DW) followed by milled rice (479.88 μg/g DW). Boiled rice had about 5-fold lower mean phenolic compounds than that of milled rice, indicating a substantial loss of phenolic compounds during its preparation (Zaupa et al., 2015).

3.2. Antioxidant activity and related compounds in different rice products

The anthocyanin, vitamin-E (tocopherol and tocotrianol), γ-Oryzanol, phytic acid content and antioxidant activity of EP extract of different rice products are summarized in Table 2. The results indicated that all these chemical parameters varied significantly ($p < 0.0001$) in most of the products.

Table 2
Different antioxidant activity and related compounds of different rice products derived from four black rice and one white rice cultivars.

Product	Cultivars	ABTS (ppm AAE/mg)	DPPH (% inhibition)	FRAP (mg AAE/g)	Vitamin E (μg/g)	γ-Oryzanol (mg/g)	Phytic acid (%)	Anthocyanin (mg/100g)
Boiled rice	CH	1.67^{P}±0.02	70.77^{E}±1.2	0.35^{J} ± 0.02	31.80^{K} ± 2.51	0.21^{JK}±0.02	0.14^{N} ± 0.01	1.31^{R} ± 0.53
	KB	1.05^{T} ± 0.04	81.49^{C} ± 2.12	0.38^{IJ}±0.03	32.45^{K} ± 3.21	0.25^{IJ}±0.01	0.25^{LM}±0.02	3.93^{M} ± 0.24
	MA	1.22^{S} ± 0.03	84.74^{B} ± 1.52	0.42^{H} ± 0.04	36.75^{J} ± 1.28	0.32^{H} ± 0.03	0.13^{N} ± 0.03	0.84^{ST}±0.21
	MN	0.80^{U} ± 0.02	72.07^{D}±1.23	0.35^{J} ± 0.07	29.40^{K} ± 2.04	0.20^{KL}±0.04	0.30^{JKL}±0.06	3.35^{N} ± 0.98
	SW	0.78^{V} ± 0.08	50.32^{H} ± 2.01	0.22^{M} ± 0.00	16.00^{LM}±2.13	0.12^{M} ± 0.02	0.18^{MN} ± 0.08	0.21^{V} ± 0.01
Mean		1.10	71.88	0.34	29.28	0.22	0.20	1.93
Beaten rice	CH	2.78^{H} ± 0.07	21.73^{N} ± 2.14	0.47^{G}±0.02	46.35^{DEF}±3.21	0.48^{F} ± 0.01	0.62^{I}±0.01	33.09^{C} ± 1.24
	KB	2.77^{I}±0.12	12.08^{P}±1.32	0.48^{FG}±0.01	45.20^{EFG}±4.21	0.42^{G}±0.05	0.74^{GH} ± 0.04	31.67^{E}±1.25
	MA	3.21^{C} ± 0.10	66.47^{F} ± 2.54	0.51^{F} ± 0.02	47.70^{DE}±1.25	0.84^{A} ± 0.01	1.63^{A} ± 0.02	16.72^{I}±1.39
	MN	3.20^{D}±0.08	22.32^{N} ± 1.35	0.48^{FG}±0.07	41.25^{GHI}±2.31	0.63^{D}±0.02	0.75^{GH} ± 0.01	22.78^{G}±1.34
	SW	1.72^{O}±0.05	11.59^{P}±1.25	0.25^{L}±0.01	13.60^{M} ± 2.54	0.26^{I}±0.03	1.68^{A} ± 0.06	2.74^{O}±0.74
Mean		2.74	26.84	0.44	38.82	0.53	1.08	21.4
Popped Rice	CH	3.20^{E}±0.10	52.32^{G}±1.56	0.57^{E}±0.00	50.25^{CD}±4.32	0.84^{A} ± 0.02	0.81^{FG}±0.04	16.01^{J} ± 0.98
	KB	3.21^{B} ± 0.15	42.38^{J} ± 1.25	0.65^{D}±0.05	56.75^{B} ± 3.51	0.75^{B} ± 0.04	0.66^{HI}±0.03	31.93^{D}±1.35
	MA	3.20^{Dv}±0.02	88.74^{A} ± 1.34	0.76^{B} ± 0.03	62.00^{A} ± 4.21	0.88^{A} ± 0.06	0.40^{J} ± 0.01	4.30^{L}±0.05
	MN	2.98^{G}±0.06	32.45^{L}±1.20	0.63^{D}±0.04	51.90^{C} ± 2.34	0.75^{B} ± 0.01	0.62^{I}±0.02	5.83^{K} ± 0.01
	SW	2.20^{K} ± 0.08	27.24^{M} ± 2.01	0.25^{LM}±0.03	17.35^{LM}±1.30	0.21^{JKL}±0.02	0.92^{E}±0.03	1.63^{Q}±0.03
Mean		2.96	48.63	0.57	47.65	0.69	0.68	11.94
Puffed Rice	CH	1.95^{L}±0.1.0	47.91^{I}±3.02	0.37^{IJ}±0.01	36.60^{J} ± 1.54	0.61^{D}±0.09	0.26^{KLM}±0.02	1.00^{S} ± 0.07
	KB	2.58^{J} ± 0.09	50.12^{H} ± 2.12	0.40^{HI}±0.03	37.65^{IJ}±1.54	0.64^{D}±0.01	1.04^{D}±0.06	17.62^{H} ± 0.12
	MA	1.94^{M} ± 0.07	41.03^{JK}±2.12	0.46^{G}±0.04	40.50^{HIJ}±2.65	0.74^{B} ± 0.01	0.97^{DE}±0.07	1.91^{P}±0.06
	MN	1.41^{Q}±0.08	42.50^{J} ± 2.35	0.34^{J} ± 0.05	42.55^{FGH}±1.54	0.64^{D}±0.02	0.31^{JKL}±0.12	0.44^{U} ± 0.03
	SW	1.37^{R} ± 0.14	37.34^{K} ± 0.98	0.28^{KL}±0.01	15.20^{M} ± 1.03	0.24^{IJK}±0.20	0.36^{JK}±0.03	0.41^{U} ± 0.02
Mean		1.85	43.78	0.37	34.50	0.57	0.59	4.28
Milled Rice	CH	3.14^{F} ± 0.13	38.78^{K} ± 0.97	0.57^{E}±0.07	58.00^{B} ± 3.58	0.63^{D}±0.03	0.67^{HI}±0.14	23.18^{F} ± 0.97
	KB	3.21^{C} ± 0.09	51.37^{H} ± 1.54	0.69^{C} ± 0.03	58.00^{B} ± 3.69	0.63^{D}±0.04	1.15^{C} ± 0.02	57.23^{A} ± 4.21
	MA	3.21^{B} ± 0.07	90.53^{A} ± 1.68	0.85^{A} ± 0.01	62.75^{A} ± 3.47	0.69^{C} ± 0.05	0.58^{I}±0.03	34.27^{B} ± 3.21
	MN	3.22^{A} ± 0.07	20.81^{N} ± 2.31	0.63^{D}±0.04	56.65^{B} ± 2.67	0.57^{E}±0.01	1.39^{B} ± 0.08	3.50^{N} ± 1.30
	SW	1.79^{N} ± 0.01	15.61^{O}±1.02	0.28^{K} ± 0.02	19.65^{L}±1.32	0.16^{L}±0.05	0.87^{EF}±0.52	0.82^{T} ± 0.04
Mean		2.91	43.42	0.60	51.01	0.54	0.93	23.8
p-Value of Product		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
p-Value of cultivars		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
p-value of Product X cultivars		<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Tukey HSD at 1%		0.0	0.0	0.0	0.0	0.0	0.0	0.0

3.2.1. Antioxidant activity

Popped and boiled rice exhibited higher and lower antioxidant activities respectively in all the assays, while the rest of the products produced a dissimilar rank order, which probably result from difference in chemical mechanism of antioxidant reaction with the possibility of natural synergism or antagonism (Vinson et al., 1998). Interestingly, in DPPH assay, boiled rice showed highest values as compared to other products. Moreover, MA-milled rice and MA-popped rice demonstrated more or less similar antioxidant activity under DPPH assay. On the other hand, popped rice from KB and MA displayed almost similar antioxidant activity under ABTS assay. In case of FRAP, milled rice showed highest value followed by popped rice. It has been observed that thermal treatment caused a decline in content of phenolic compounds with consequent decline in antioxidant activity (Massaretto et al., 2011).

3.2.2. Vitamin-E content

The vitamin-E content in different products varied from 13.60 to 62.75 μg/g with higher mean value observed in milled and popped rice. Moreover, MA milled and MA popped rice registered higher amount of vitamin-E, while SW beaten rice had the lowest. Compared to milled rice, the reduction in vitamin-E content was more pronounced with boiled rice (42%) followed by puffed rice (32%), beaten rice (24%) and popped rice (6%). Similar reduction in vitamin-E content is observed in rice bran under different cooking condition with varying rice-water ratio (Kumar et al., 2006), parboiling (Pascual et al., 2011; Khatoon and Gopalakrishna, 2004)). Thus, it seems that unlike popped rice, hydrothermal process involved in the preparation of puffed rice, beaten rice and boiled rice caused a substantial reduction in vitamin-E in these products.

3.2.3. γ-oryzanol content

The mean γ-oryzanol content in different rice products varied from 0.22 to 0.68 mg/g with the highest amount in popped rice followed by puffed rice, whereas boiled rice had the least. Among the various rice products, MA-popped rice of (0.88 mg/g) and CH- popped rice (0.84 mg/g) showed higher amount of γ-oryzanol, which is marginally higher over milled rice. In this context Pascual et al. (2011) reported that parboiling followed by storage caused a 40% reduction in γ-oryzanol. The marginal increase in γ-oryzanol content during popping is probably related to presence of bran layer with popped kernel surface and higher concentration of dry matter as compared to other products.

3.2.4. Phytic acids content

Though phytic acid (PA) is generally recognised as anti-nutritional factor, it may act as antioxidant up to a certain level. It was reported that PA has some anticancer and antioxidant functions and prevents coronary disease (Febles et al., 2002). The optimum level of PA in cereals and their products is not yet standardised. Here, the PA content in beaten rice was higher than in other products and MA and SW showed highest level (1.63 and 1.68% respectively). Lowest content of PA was found from Boiled rice of MA and CH (0.13 and 0.14% respectively). Soaking of grains in water resulted in lower PA levels. Two factors could be responsible for the retention of PA i.e. endogenous phytase activity and diffusion of PA into the soaking medium. The endogenous phytase is one of the key factor for lowering the PA level in different rice-based products (Perlas and Gibson, 2002). The lowest PA content in boiled rice was due to the diffusion of salt of phytate (with minerals) to the boiling medium (water). However, in case of beaten rice and raw rice, rice grains were exposed to minimum hydrothermal processing resulting higher PA level than other products.

3.2.5. Anthocyanin content

Table 2 also showed that mean anthocyanin content increased in the order: boiled rice < puffed rice < popped rice < beaten rice < milled rice. KB-Milled rice had higher anthocyanin content (57.23 mg/100 g). Interestingly, three of the products of beaten rice, those derived from CH, MN and SW showed higher values for anthocyanin than the corresponding milled rice. Anthocyanin is mainly present in bran layers. In milled raw rice and beaten rice 10–15% bran layers are present, which was responsible for higher anthocyanin content whereas in other products, it is lost in greater amount due to grain processing with higher temperature. It was reported that drum drying and extrusion process reduces anthocyanin content in black rice CH and cyanidin-3-glucoside is converted to Cyanidin and protocatechuic acid (Qiu et al., 2021). Thermal cooking also decreased total anthocyanin and cyanidin-3-glucoside contents but increased protocatechuic acid content in black rice (Bhawamai et al., 2016).

3.3. Principal component analysis

Principal component analysis (PCA) was performed using all the chemical variables examined in the present study (Fig. 3). From PCA, three principal components viz. PC1, PC2 and PC3 each with eigen value > 1 were chosen, which explained respectively 45.82, 13.60 and 9% of total variability. PC1 and PC2 due to their higher cumulative variance (59.42%), was considered for interpretation of results. The parameters whose curves lie close to one another and run in opposite direction on the loading plot show positive and negative relation between them respectively. Further, narrower the angle between the variables, the stronger is the correlation. Therefore, variables such as vitamin-E, γ-oryzanol, MYR, Anthocyanin, FER etc. are highly correlated whereas DPPH, CIN, LUT, CHL are poorly correlated. From the PCA biplot, it was evident that the purple circle with lower PC1 and higher PC2 score represented Milled rice (CH, KB, MA and SW) and popped rice (SW). Green circle with relatively lower both PC1 and PC2 score represented boiled rice (CH,KB, MA and MN), puffed rice (SW, CH and MN) and MN beaten. The blue circle with higher PC1 score represented MN popped, MA-puffed, KB-beaten, MA-beaten, KB-puffed and MN-beaten, which showed proximity towards the maximum number of correlated variables. From comparison of relationship between products and chemical variables in blue circle region, MN-popped followed by MA beaten and KB-beaten were identified as promising products. In addition, popped (CH and KB) with their higher both PC1 and PC2 score, fell outside the encircled region but showed proximity only towards fewer variables (LUT, CHL and CAF). Thus, blue circle region, in contrast to purple and green circle region, appears to be important to impart nutritional quality to the products.

4. Conclusion

The phenolic composition, anthocyanin, vitamin-E, γ-Oryzanol and antioxidant activity differed significantly in the investigated rice products. The level of these chemical variables was higher with black rice based products than that of white rice. Moreover, popped rice had higher amount of all these chemical parameters except anthocyanin. PCA disclosed MN popped a nutritionally superior product for its correlation with greater number of chemical variables. This finding may assist the rice consumers, growers and millers with new opportunities to promote the black rice for its enhanced levels of antioxidant components even under different method of grain processing as compared to white rice.

Author statement

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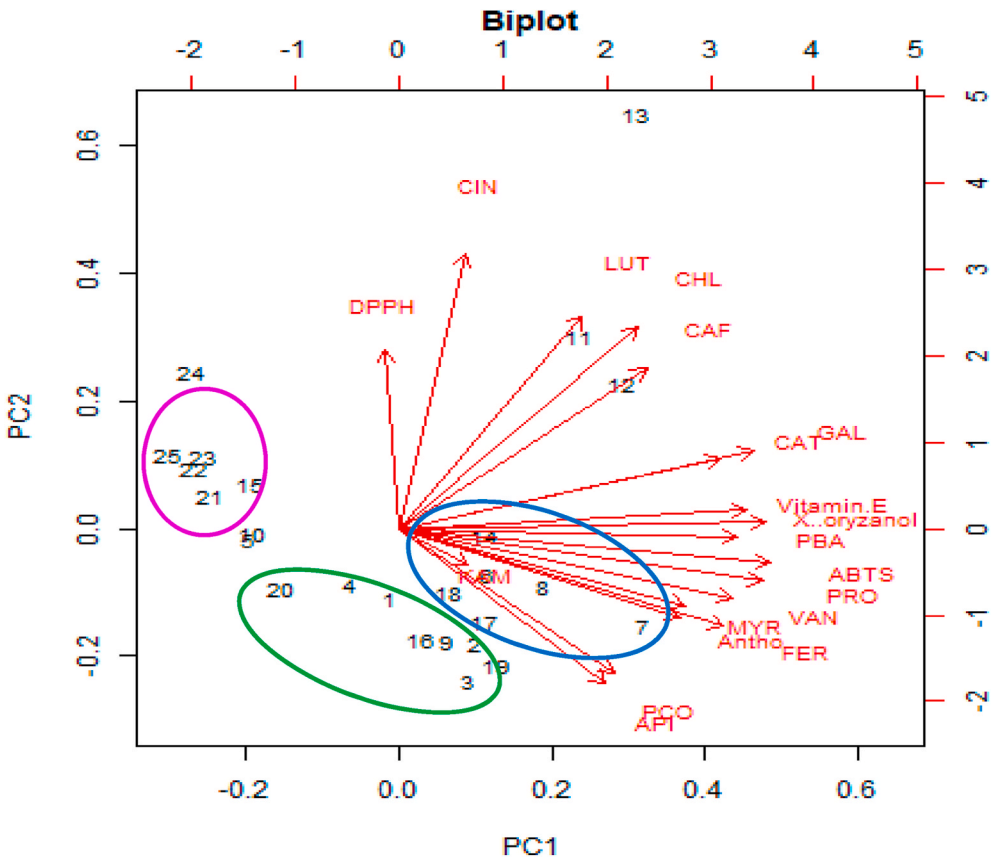

Fig. 3. PCA Biplot of antioxidant activity and phenolic compounds derived from twenty five pigmented and white rice products.
*1-CH Boiled; 2-KB Boiled; 3-MA Boiled; 4-MN Boiled; 5- SW Boiled; 6-CH Beaten; **7-KB Beaten*; 8-MA Beaten****; 9-MN Beaten; 10-SW Beaten; 11-CH Popped; 12-KB Popped; 13-MA Popped; **14-MN Popped*****; 15-SW Popped; 16-CH Puffed; 17-KB Puffed; 18-MA Puffed; 19-MN Puffed; 20-SW Puffed; 21-CH Milled; 22-KB Milled; 23-MA Milled; 24-MN Milled; 25-SW Milled.

Krishnendu Chattopadhyay: Editing and Supervision.
M. Sivashankari: Resources and Methodology.
Sankhajit Roy: Formal analysis of biochemical parameters.
Awadhesh Kumar: Data Curation and Validation.
Tufleuddin Biswas: Software analysis.
Srikumar Pal: Writing Review, Visualization, Funding acquisition and Project administration.

Declaration of competing interest

We have no conflict of Interest regarding this experiment.

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