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A PRELIMINARY INVESTIGATION ON BIOACTIVE POTENTIAL OF MILLING FRACTIONS OF *KOMAL CHAUL* OF ASSAM PROCESSED FROM PIGMENTED *KOLA CHOKUA* PADDY

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ABSTRACT- *Komal chaul, a traditional ready-to-eat rice product of Assam state in India requiring no cooking was processed from pigmented Kola chokua paddy. The paddy obtained after pressure parboiling and de husking was polished to obtain the product. Milling fractions were obtained after four different degrees of polishing from both raw and processed rice and studied for phytochemical content and antioxidant activities. Parboiling caused marked damage and dislocation of the natural bioactive compounds of the bran layers as revealed by drop in total phenolic and flavonoid contents. These compounds were found to be responsible for the ferric reducing antioxidant potential of the milling fractions. Migration of sugars and peptides were also evident. Maillard compounds were formed due to high heat processing which showed high DPPH scavenging and metal chelating properties. Non uniform migration of these and native pigments are responsible for the color of parboiled rice. Milling fractions of native parboiled kola chokua rice possess specific bioactive potential.*

Keywords- *pigmented rice; parboiling; milling fractions; antioxidant*

I. INTRODUCTION

Pigmented rice varieties have attained much scientific attention due their phytochemical contents and antioxidant properties [1]. The bioactive compounds in pigmented rice are primarily located in the surface and bran layers of the kernels that are removed during milling (polishing). LAMBERTS et al (2007) reported that the pigment compounds quantitatively drop in quantity from the surface of the brown rice to the middle of the endosperm [2]. The milling fractions of rice have attained commercial uses in food, feed and cosmetic industries due to their nutritive values and non-nutritive bioactive compositions [3,4]. The bioactive phenolic compounds in rice include ferulic acid and diferulates, anthocyanins, anthocyanidins and polymeric proanthocyanidins [5]. They act both as reducing agents and singlet oxygen quencher, thereby protect the cells against oxidative carcinogenic affect [1,6].

Thermal treatments cause depletion of natural bioactive compounds in foods [7]. Paddy is often parboiled while in the husk and cooked after milling prior to consumption and both the processes involve high temperature conditions. Two varieties of 6% polished pigmented aromatic rice on cooking at 100ºC for 10

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min resulted in variable drop in total phenolic content (TPC), total flavonoid content (TFC) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging antioxidant potentials [6]. Leaching of the pigments into the cooking water or deeper into the starchy endosperm may result in the drop of the values [2]. Pigment migration from the husk and bran layers into the kernel have also been suggested by BHATTACHARYA (1996) and LAMBERTS et al (2006) [8,9]. Parboiling involves very high processing temperature and sufficient processing time for occurrence of non-enzymatic Maillard browning which contributes to the amber coloured kernels [9]. Amongst numerous numbers of Maillard reaction products (MRPs), the melanoidins are reported to have scavenging hydroxyl radical, superoxide and hydrogen peroxide antioxidant capacities combined with metal chelation activity [9-11]. Four rice varieties when subjected to three stages of steaming showed reduction of carotenoids to trace levels [9]. The authors hence nullified the contribution of pigments to the final colour of the parboiled rice samples.

The state of Assam in India is a hub of rice germplasm [12]. Traditionally cultivated pigmented *kola chokua* paddy was found to be suitable for processing into traditional ready-to-eat *komal chaul* product by a developed laboratory-scale steam parboiling method. *kola chokua* falls under the low amylose class of rice with apparent amylose content (w.b.) of 12.6% [12]. The peculiarity of *komal chaul* is that it attains texture comparable to cooked rice on soaking in lukewarm water for a few minutes (DUTTA and MAHANTA, 2014) [13]. The developed process uses controlled single-stage steaming of the pigmented paddy followed by drying and milling. This work involved an investigation on the changes in the phytochemical content and antioxidant properties of the milling fractions of *kola chokua* rice on processing into *komal chaul* product.

II. MATERIALS AND METHODS

A. Materials

Kola chokua paddy from the recent harvest of 2014 was purchased from farmers of Jorhat district of Assam. The paddy was kept for 24 h at room temperature (RT, 27±2ºC) before storing at 4ºC till processing. Chemicals were purchased from Merck (India) and Sigma (US). Acidified methanol was prepared by mixing 1.0 mL hydrochloric acid in 9.0 mL water.

B. Parboiling and coding

Four hundred grams of paddy was brought out, kept at RT for 6h and then soaked in water at 100ºC for 1 min as described elsewhere [14]. The vessel containing the soaked paddy was then covered with a thick gunny bag and kept at RT for 18h allowing the paddy to hydrate. The excess water was then decanted and the moistened paddy was immediately steamed in an autoclave fitted with a pressure gauze (Equitron 7407ST, India) for 15 min at 103.42 kPa and 121 °C. This was followed by drying at RT for 48h. This method with further milling generated *komal chaul*. The milling fractions were coded as- R followed by the degree of milling and P followed by the degree of milling of for raw and parboiled rice fractions respectively.

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C. Milling

The rough rice was dehusked using a huller (Satake, Japan). The brown rice was then milled under manual control in an abrasive polisher (Satake, Japan). After repeated polishing trials, powdered fractions representing 3%, 6%, 9% and 12% (w.b.) of the brown rice weight were obtained. The samples were sieved through 100µm sieves and stored at 4^{o}C in sealed polypropylene pouches.

D. Colour analysis

Colour of milled parboiled rice is considered as an indicative parameter of the extent of parboiling [8]. A color measurement spectrophotometer (Hunter Color-Lab, Ultrascan Vis, US) was used to measure the values for L (lightness), a (red-green), and b (yellow-blue) values. Each sample was analysed in triplicates and the mean value was taken.

E. Sample extraction

One gram powder sample was treated with 10 mL a 10% acidified methanol solution (90:10, methanol: acidified water, v/v). The mixture was shaken in water bath at 25^{o}C for 180 min followed by centrifugation at 3000 rpm for 15 min (Hettich centrifuge, Germany). The supernatant was stored at -20^{o}C until further analysis of total phenolics, flavonoids, anthocyanins and antioxidant activities.

F. Total Phenolic Content (TPC)

TPC in the bran extracts were assessed using a method modified from the Folin–Ciocalteu assay [15]. Gallic acid was used as a standard. An aqueous gallic acid solution (500 mg L^{-1}) was diluted with deionized water to give appropriate concentrations for a standard curve. For the analysis, 20µL each of extract, gallic acid standard or blank were taken in separate test tubes and to each 1.58 mL of distilled water was added, followed by 100µL of Folin–Ciocalteu reagent and mixed thoroughly. 300 µL of sodium carbonate was added after 5 min and vortexed immediately before incubating the tubes in the dark for 30 min at 40˚C. The absorbance was measured at 765 nm in a UV-Vis spectrophotometer (Cecil, Aquarius 7400, UK). The results were expressed in milligram of galic acid equivalent (mgGAE) per 100g of sample.

G. Total Flavonoid Content (TFC)

A method used elsewhere was used [6]. Briefly, 0.5 mL of the extract was mixed with 1.5 mL of 95% ethanol, 0.1mL of 10% aluminum trichloride, 0.1 mL of 1M potassium acetate, and 2.8 mL of deionised water. After incubation at RT for 40 min, the absorbance of reaction mixture was measured at 415 nm against deionised water taken as blank in the UV-Vis spectrophotometer. TFC was expressed as quercetin equivalent (mgQE) per 100g of sample.

H. Ferric Reducing Antioxidant Potential (FRAP)

FRAP of the acidified methanolic extract was measured by the method of BENZIE and STRAIN (1996) [16]. FRAP solution was freshly prepared by mixing 2.5 mL of a 10 mM 2,4,6-TPTZ [2,4,6-tri(2-pyridyl)-1,3,5-triazine] solution in 40mM hydrochloric acid with 2.5 mL of 20mM ferric chloride and 25 mL of 0.3M acetate buffer (pH 3.6). The solution was pre warmed at 37˚C. 40 µL of sample extract was mixed with 3.0 mL of FRAP

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solution and incubated at 37˚C for 4 min and the absorbance was determined at 593 nm in the UV-Vis spectrophotometer against a blank that was prepared using distilled water. A calibration curve was prepared, using an aqueous solution of ferrous sulfate (1-10 mM). FRAP value was expressed as μM of ferrous equivalent Fe (II) per 100g of sample.

I. DPPH Radical Scavenging Activity

2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of the extract was measured by determining the inhibition rate of the [17,18]. Briefly, 100 μL of extract was added to 1.4 mL of 10^{-4} M DPPH radical prepared in methanolic solution. After 30 min, the absorbance at 517 nm was measured against a blank containing a mixture of 100 μL methanol and 1.4 mL of DPPH radical solution. The results were expressed in terms of radical scavenging activity using the following equation

Radical scavenging activity % = [(Ao-As)/ Ao] × 100.

Where, Ao is absorbance of blank and As is the absorbance of sample extract.

J. Metal chelating Capacity (MCC)

Metal chelating capacity was determined by the method of DINIS et al (1994) [19]. For this, 1.0 mL of 0.125 mM ferrous sulphate and 1.0 mL of 0.3125 mM Ferrozine were mixed with 0.2 mL of extract. The mixture was allowed to equilibrate for 10 min at room temperature and the absorbance was read at 562 nm. The control contained all the reagents other than the extract. Decreased absorbance of the reaction mixture indicated increased activity. MCC was determined by the following formula

MCC % = [(Ao-As)/ Ao] × 100

Where, Ao is absorbance of control blank, and As is absorbance of sample extract

K. Statistical analysis

All the experiments were carried out in triplicates. Tests of significant differences between means of colour values were determined by Duncan's multiple range tests at a significance level of 0.05 using statistical package for the social sciences SPSS 11.5 (SPSS Inc., Chicago,
IL, USA). Pearson correlation coefficient among TPC, TFC, FRAP, DPPH scavenging activity, MCC and L, a, b colour values of the milling fractions were also analyzed.

III. RESULTS AND DISCUSSION

While the bran layers of raw rice was darker than the parboiled sample (Table 1) indicating destruction of the pigments on parboiling or possible migration to the inner layers. This was suggested by marginal increase in the 'L' values of the milling fractions of parboiled rice as compared to raw rice. The changing pattern of 'a' values however did not correlate with change in 'L' for both raw and parboiled samples (Table 2). The increased yellowness (b) with milling of raw rice indicated higher concentration of carotenoid pigments in the inner layers of the raw rice kernels showing correlation with changes in both L and a values. The b values dropped extensively after parboiling and showed irregular pattern indicating destruction or non-uniform migration of the pigments in the gelatinized kernel during parboiling. While the notable change in colour was reflected in the

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change of the H values, decreased values of C indicated that the colour compounds were irregularly distributed in the milling fractions of the *komal chaul* (x-rite).

N3% showed the highest TPC, TFC and FRAP values of 1942.0 mg GAE/100g, 201.0 mgQE/100 g and 7239.6 mM/ 100g respectively (Fig 1a,b,c). The second milling fraction, N6% also showed to possess appreciable amount of phenolic compounds. The results hence indicated that the FRAP activity of the raw rice fractions were basically attributed to the phenolic contents in those. The later milling fractions, namely N6%, N9% and N12% were supposed to be with higher starch content [2], thereby giving lower values for these parameters. Parboiling resulted in partial destruction of the phenolic compounds as exhibited by significant drop in their values. Further reduction in these values with degree of milling was also observed for the milling fractions of the parboiled rice.

DPPH radical scavenging activity and metal chelating properties however did not show significant fall after parboiling as in raw samples (Fig 1d). Values higher that the raw samplesand negative correlation with TPC, TFC and FRAP indicated that the phenolics did not have any significant influence on the antioxidant activity of the milled layers and newer active compounds were formed on processing. High temperature parboiling reportedly produces Maillard compounds and destruction of the natural carotenoids [9]. Probable melanoidin formation in the rice kernel might have resulted in the notable increase in these two parameters. DPPH scavenging activity was highest (95.85%) for the 3% milled fraction. SMANIOTTO et al (2009) reported that melanoidin compounds form due to reaction of intact protein with the maltodextrin produced by breakdown of starch due to the high heat processing [20]. These simpler polysaccharides may have moved out from the endosperm as occurs during cooking but could not release out from the kernel due to the husk and firmer bran layers and hence got accumulated in the surface layers. They simultaneously took part in the Maillard's reaction. Notably higher concentration of these compounds was also seen in the 6% and 12% milled fractions, which indicates inward migration of peptides into the endosperm and formation of the compounds [21]. Melanoidin compounds also have antimicrobial activity which is related to their metal chelating activity on cations like ferrous, zinc and cuprous etc [20]. MCC of the milling fractions of the parboiled sample hence showed significant correlation with the DPPH scavenging activity (Table 2, Fig. 1e). As DPPH values showed negative correlation with the colour value, it can be opined that the colour of parboiled rice is not principally attributed to melanoidin but to some other browning compound without having the radical scavenging properties.

IV. CONCLUSIONS

Migration of pigments and bioactive compounds occur during parboiling, thereby altering the native compositional structures of the milling fractions of rice. Pressure parboiling caused destruction of natural bioactive compounds with formation of Maillard browning compounds which showed developed DPPH scavenging and metal chelating properties and colour

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development in milling fractions of *komal chaul*. Further studies on chromatographic isolation and identification of these compounds with *in vivo* experiments may provide more insight into the bioactive composition of *komal chaul* and other parboiled rice and rice products.

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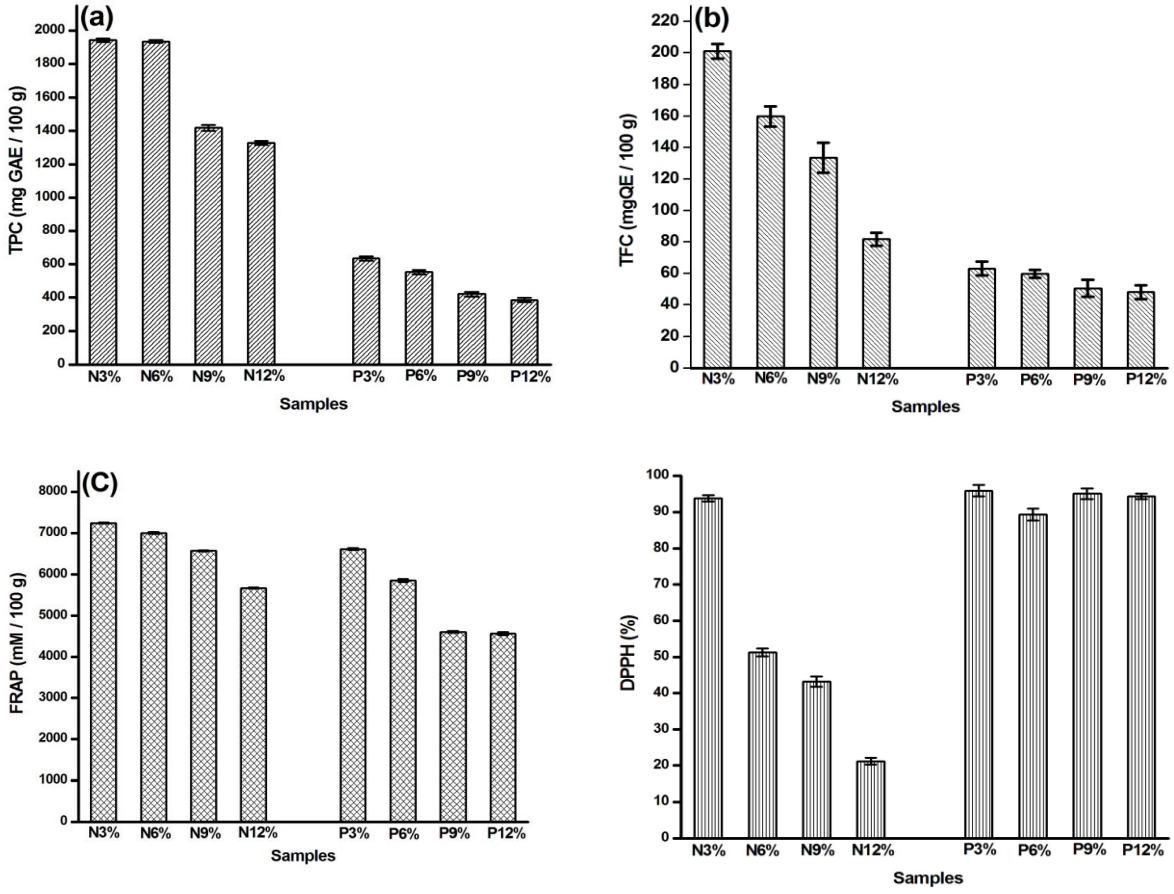
TABLES AND FIGURES



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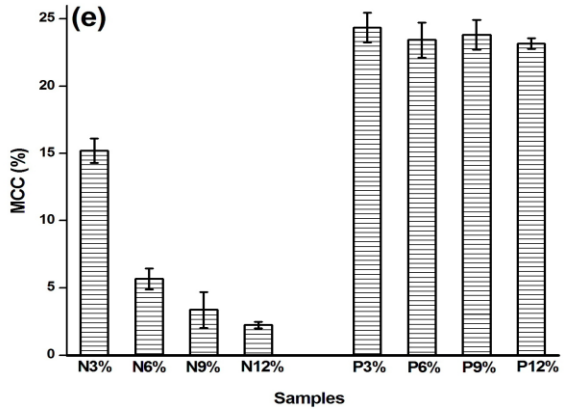

Fig. 1. a) Total phenolic content (mgGAE/100 g) (b) Total flavonoid content (mgQE/100 g) (c) Ferric reducing antioxidant potential (μM/100 g) (d) DPPH radical scavenging activity (%) and (e) Metal chelating capacity (%) of the milling fractions.

*Table 1.*Colour values of milling fractions of raw and parboiled *kola chokua* rice

Sample	L	a	b	H	C
R3%	37.0±0.1^{d}	7.6±0.2^{f}	7.9±0.3^{d}	55.64±0.2^{d}	11.0±0.3^{d}
R6%	37.6±0.2^{e}	7.5±0.3^{e}	8.1±0.1^{e}	53.00±0.2^{c}	11.1±0.4^{d}
R%	38.6±0.1^{f}	7.4±0.2^{d}	8.2±0.4^{e}	52.06±0.3^{ab}	11.1±0.2^{d}
R%	43.6±0.4^{g}	7.4±0.1^{d}	8.4±0.2^{f}	50.13±0.2^{a}	11.2±0.6^{d}
P3%	35.0±0.3^{ab}	4.7±0.3^{c}	5.4±0.4^{c}	50.22±0.3^{a}	7.2±0.3^{c}
P6%	34.8±0.2^{a}	4.7±0.2^{c}	4.9±0.1^{b}	55.55±0.2^{d}	6.8±0.4^{b}
P9%	35.8±0.3^{b}	4.6±0.2^{bc}	5.0±0.5^{b}	52.43±0.4^{b}	6.8±0.1^{b}
P12%	36.6±0.1^{c}	4.2±0.3^{a}	4.8±0.2^{a}	49.97±0.1^{a}	6.4±0.4^{a}

Table 2. Pearson correlation coefficient values for relation between changes in different quality parameters of the milling fractions

	TPC	TFC	FRAP	DPPH	MCC	L	a	b
TPC		0.943*	0.792*	-0.522	-0.765*	0.447	0.954**	0.917**
TFC			-0.881*	-0.245	-0.559	0.186	0.843**	0.774*
FRAP				-0.214	-0.436	0.032	0.686	0.641
DPPH					0.936**	-0.882*	-0.713*	-0.784*
MCC						-0.816*	-0.898**	-0.938**
L							0.650	0.726*
a								0.989**
b								