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EFFECT OF PROCESSING METHODS ON THE NUTRACEUTICAL AND ANTIOXIDANT PROPERTIES OF RED RICE (*ORYZA NIVARA*)

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ABSTRACT

The effect of roasting, microwave cooking and pressure cooking/autoclaving on the nutraceutical and antioxidant properties of red rice (*Oryza nivara*) was investigated. The nutraceutical properties were determined by evaluating the total phenolic, flavonoid and phytic acid contents while the antioxidant properties were studied by the DPPH free radical scavenging activity and the iron reducing power assay. The results showed that the total phenolic and flavonoid of processed red rice increased, while phytic acid content was decreased when compared to native sample. The DPPH radical scavenging activity and the iron reducing power of roasted red rice extract were the highest compared to the other processed red rice. A significant increase in TPC, FRAP, reducing power and DPPH content was found in all the selected cereals while decrease in TFC was observed after microwave & roasting. The roasted flour exhibits higher AOA then microwave treated flour. The results indicate that processing has significant effects on the nutraceutical and antioxidant properties of selected cereals.

Keywords: Red rice, pressure cooking, Microwave treatment, Steaming, Roasting, Nutraceutical, Antioxidant

INTRODUCTION

Rice (*Oryza sativa L.*) is considered a basic source of energy for more than half of the population worldwide (Monks *et al*., 2013). Consumer preference of rice depends on the cultural tradition of each region of the world and it is associated with the amylose content of the grains. Regardless of the amylose content, the most consumed rice is milled rice, which is industrially prepared by removing about 7–12% of the bran fraction by mechanical milling. The milling process removes most of the fibre and fat from the grain, improving its sensory properties and storage stability, respectively. Recently, pigmented rice, such as red rice, black rice and wild rice, have received the attention of consumers looking for healthier foods. The health benefits of pigmented rice are attributed to the presence of phenolic compounds that possess antioxidant, anticarcinogenic, antiallergic, anti-inflammatory, antiatherosclerosis and hypoglycaemic activities (Deng *et al*.,2013).

A variety of heat-processing methods are applied to grains in preparation for consumption. Heat processing affects the level of phytochemicals (Xu *et.al.* 2007), antioxidant activity (Xu *et al.* 2007; Queiroz *et al.* 2009), functional properties (Muyonga *et al.* 2001; Naveena and Bhaskarachary, 2014), and nutritional value (Rehman and Shah 2005) of foods. Rheological properties of grain have also been shown to vary among species (Kong *et.al.* 2009). Attention is currently being given to the antioxidative and radical- scavenging properties of coloured rice cultivars because of their potential to provide and promote human health by reducing the concentration of reactive oxygen species and free radicals (Nam *et al.*, 2006; Oki *et al.*, 2005). However, information on the effect of processing on the phenolic compounds and the antioxidant properties of red rice is rather scarce. Hence, this study was carried out to investigate the effect of different processing (pressure cooking, microwave cooking and roasting) on the nutraceutical (total phenolics, flavonoids and phytic acid) and antioxidant properties (DPPH free radical scavenging activity and reducing power assay) of red rice.

MATERIAL AND METHODS

MATERIAL

GRAIN MATERIALS

The cereal crop red rice (*Oryza nivara*) was selected for the study as they are adapted to the growing conditions in the northern India. The identified cereal red rice was purchased from shops of Allahabad district. A composite sample of about 5 kg of grain representing different shops/seed centres was used in the present study. The red rice was ground using a laboratory mixer and the whole grain meals were kept in a refrigerator until analyzed.

METHODS

ROASTING

In dry heat treatments, the dry, whole grains were heated in a hot air oven at 110^{o} C for an hour (Nathya *et al* 2007).

MICROWAVE COOKING

Red rice (50 g) was taken in a glass beaker (250 ml) and placed at the center of the microwave oven (LG, Intellocook, 2450 MHz, 900W) and roasted for 120 s at 900W. Both the sand roasting andmicrowave cooking treatments were carefully optimized in such a way that it resulted in grain with maximum expansion and no burning.

PRESSURE COOKING/AUTOCLAVING

The rinsed seeds were autoclaved using vertical autoclave at 15 lb pressure (121°C) in tap water (1:10, w/v) for 15 minutes until seeds were soft when felt between the fingers. Autoclaved seeds were dried in hot air oven at 55°C for 24 h and made to flour using electric grinder until to pass 0.425 mm sieve.

SAMPLE PREPARATION

The above processed samples were dried in a hot air oven at 50 ± 2^{o}C for 6 h. Native (Unprocessed) and all processed red rice samples were pulverized to pass through 60 BSS (250 µm) sieve to obtain red rice flour. Any fraction retained on the sieve was again ground in the electric grinder so that all of it passed through the 60 BSS sieve, the process was repeated till all the flour passed through the sieve. The flour was stored in airtight container at −20 °C for further analysis.

CHEMICAL COMPOSITION

The red rice processed and native flour samples were analyzed for moisture, ash and fat. The moisture content of the multigrain composite mixes was determined by drying at 105 °C until a constant weight was attained as per AOAC 2005. The micro Kjeldhal method was employed to determine the total nitrogen and the crude protein content (Nx6.25) (AOAC 2005). Crude lipid was estimated by extraction with petroleum ether (60–80 °C), using Soxhlet apparatus and ash contents was determined as per AOAC 2005. The mineral content calcium and iron was determined using spectrophotometer as per the procedure given by Rangana,(2000). Total carbohydrate was obtained by difference method, after estimating all other components by proximate analysis.

DETERMINATION OF TOTAL PHENOL CONTENT

Total polyphenols were estimated as per procedure described by Singleton *et al.*, (1999) using Folin -Ciocalteu method, where 250 mg sample was taken in 10 ml of methanol and water (80:20 v/v) solution in a graduated test tube and heated on water bath at 70^{o}C for 10 min. The sample was brought to room temperature, centrifuged at 3000 rpm for 10 min. The supernatant (0.2 ml) was made up to 10 ml with distilled water. This solution was diluted 10 fold and sample solution (5 ml) was mixed with saturated sodium carbonate (0.5 ml) and Folin-Ciocalteaue reagent (0.2 ml) and made up to 10 ml with distilled water. The absorbance was read at 765 nm after 60 min by UV visible double beam spectrophotometer (Model Evolution 600, Thermo Electron, US).

REDUCING CAPACITY (RC)

The reducing power was measured as described by Sharma and Gujral with slight modification. Cereal flour (1.0 g) was extracted with 80 % methanol (3.0 ml) for 2 h. The extract was mixed with phosphate buffer (2.5 ml, 0.2 mol/L, pH 6.6) and 2.5 ml potassium ferricyanide (1.0 %) was added followed by incubation at 50° C. Then 2.5 ml trichloroacetic acid solution (10%) was added to mixture, which was then centrifuged at 10,000 g for 10 min. The upper layer of solution (2.5 ml) was mixed with 2.5 ml deionized water and 1.0 ml ferric chloride (0.1%). The absorbance of the mixture was measured at 700 nm. The results were reported as µmol ascorbic acid equivalent (AAE)/g of flour using standard curve of ascorbic acid.

RADICAL SCAVENGING ACTIVITY

The antioxidant activities of native and processed raw materials were also measured by the stable 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) DPPH radical scavenging method (De Ancos, *et al*; 2002). An aliquot (0.10ml) of sample extract in 80 % methanol was mixed with 2 ml of methanolic 0.1 mM DPPH solution and the volume was made up to 5 ml with distilled water. The mixture was thoroughly vortex-mixed and kept in dark for 30 min. The absorbance was measured at 515 nm. The result was expressed as percentage of inhibition of the DPPH radical. The percentage of inhibition of the DPPH radical was calculated according to the following equation:

$$\text{Per cent (\%) antiradical activity} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100$$

STATISTICAL ANALYSIS

All experiment was conducted in triplicates, and the data were expressed as mean± standard deviation (S.D). One-way analysis of variance (ANOVA) and Duncan's multiple range test were carried out to determine significant difference ($p<0.05$) between the means by

Statistical Packages for Social Sciences (SPSS version 12.0).

RESULT AND DISCUSSION

NUTRACEUTICAL PROPERTIES

Total phenol content by FCR and in vitro antioxidant capacity assays, such as the DPPH and FRAP assays (which were used in this study), represent convenient methods for the identification of potential sources of antioxidant compounds (Stratil *et al*., 2006). As already mentioned, antioxidants, such as polyphenols, have significant potential health benefits; they may protect cell constituents against oxidative damage and therefore limit the risk of various degenerative diseases associated to oxidative stress such as cancer, cardiovascular disease and osteoporosis. However, the value of in vitro antioxidant capacity assays for assessing the health-related implications of a food extract has been limited for a number of reasons, mainly due to the lack of standardization amongst these methods, the changes in the antioxidant activity of polyphenols following extensive metabolization in the body and the large variation in bioavailability existent among the different types of polyphenols (Scalbert *et al*., 2005). Nevertheless, this does not exclude antioxidant properties (redox properties) of polyphenols from being one of the key parameters in determining their biological effects (Scalbert *et al*., 2005).

Table 1 shows the total phenolic contents (TPC) of native and processed red rice. The TPC of native red rice was 163 mg GAE/100 g and processing increased it significantly from 246.3 to 277.86 mg GAE/100 g. The order of increase in TPC was roasted > pressure cooking) > microwave > native. The increase in TPC during roasting may be due to the increase in the extractability of bound phenolics by the thermal degradation of cellular constituents. The increase in TPC during processing has also been reported by other investigators (Boateng, *et al*, 2008; Duenas *et al*, 2009; Fernandez *et al*., 2006; Gallegos *et al*, 2010). The increase in phenolic compounds in germinated brown rice could be explained as an increase in the free forms due to the breakdown of the cell wall during germination. Insoluble phenolic compounds have been found to be cell wall components, (Shibuya; 1984) which are bound to polysaccharides in the cell wall of rice grains. During germination induced saccharolytic enzymes breakdown endosperm which release bound phenolic compounds. Tian; 2004, & Yin and Mei; 2006; reported that after long-term germination of legumes the total phenolic and flavonoid content increased and contributed to higher antioxidant activity. Sharma and Gujral; 2010, reported that germination led to increase in TPC in different barley cultivars. Gumul *et al*. 2007. Total Phenolic Content Plant phenolic compounds are nowadays getting increased attention in the diet due to their natural antioxidant potential. Increased consumption of phenolic compounds has been associated with the reduced risk of cardiovascular diseases and certain cancers (Liu 2004, 2007; Dykes and Rooney 2007).

The Flavonoid content of processed and native red rice was presented in table 1. There was a decrease in flavonoid content during roasting and microwave cooking while increase in flavonoid content was found during pressure cooking when compared to the native sample. The increase in flavonoid content during pressure cooking may be attributed to the biochemical changes of red rice during processing, which leads to the production of these plant secondary metabolites. The flavonoid contents of red rice increased by 13.5 % during pressure cooking. The results were in agreement with Pradeep and Guha, 2011 for the little millet extract. Similar increase in the flavonoid contents was also observed during the thermal treatment of oak acorns and Brazilian bean, respectively (Rakic *et al*., 2007; Ranilla *et al*, 2009).

Apart from its antinutrient activity, phytic acid also exhibit antioxidant activity, because it chelates metal ions such as iron and copper and in this way it prevents various oxidative processes Singh *et al*; (2013). The phytate molecule is negatively charged at the physiological pH and is reported to bind essential, nutritionally important divalent cations, such as iron, zinc, magnesium and calcium. Table 1 shows that highest reduction in phytic acid content was found in case of microwave cooking followed by roasting and pressure cooking. Similarly, a signifcant reduction of phytic acid contents by thermal processing (roasting, autoclaving and microwave) has been observed in other plant foodstuff (Habiba, 2002; Fagbemi *et al*., 2005; Frontela *et al*., 2008; Wang *et al*., 2008, Nazni *et.al*., 2014). The apparent decrease in phytate content during thermal processing may be partly due either to the formation of insoluble complexes between phytate and other components, such as phytate - protein and phytate-protein-mineral complexes or to the inositol hexaphosphate hydrolyzed to penta- and tetraphosphate (Siddhuraju and Becker, 2001). On the other hand, some authors reported that phytic acid contents were unaffected or increased after heat treatments (Yagoup and Abdalla, 2007; Martín-Cabrejas *et al*., 2009; Embaby, 2010).

Table 1: Nutraceutical properties of native and processed Red rice flour

Samples	TPC (mg/100 g gallic acid Eq.)	Total Flavonoid content (mgQE/g)	Phytic acid (mg/100 g)
Native	163 ±0.56 [a]	0.74 ±0.1 [c]	168.1 ±1.15 [d]
Roasted	277.86 ±0.55 [d]	0.53 ±0.20 [a]	91.6 ±1.20 [b]
Pressure cooked/autoclaved	263.77 ±0.48 [c]	0.84 ±0.16 [d]	98.38 ±1.30 [c]
Microwave treated	246.3 ±0.60 [b]	0.56 ±0.18 [b]	83.36 ±1.40 [a]

Results are mean of three determinations ± SD. Values with same letters (a, b, c, d within column) are not significantly different at $p < 0.05$.

AOA

From Table 2, it is observed that the radical scavenging activity of processed red rice increased compared to the native sample. The roasted cereal showed the highest radical scavenging activity (84.61%), compared to microwave (83.24%) and pressure cooked (80.6%). This may be due to the presence of highest TPC in roasted red rice, since the %DPPH inhibition is directly correlated with TPC (Alothman *et al*, 2009). The higher antioxidant properties of roasted red rice may be due to formation of higher Maillard products during the high temperature short time processing. Similar findings have been reported by Nicoli *et al*; (1997) during roasting of coffee brews and also by Rocha *et al*; (2007) during pressure cooking of common bean.

The iron reducing power of native and processed red rice is shown in Table 2. In FRAP method, ferric 2,4,6-tripyridyl-s-triazine complex gets reduced to its ferrous form (Prajapati *et al*, 2013). It is observed that the results followed a similar trend to the DPPH free radical scavenging activity. The roasted rice sample showed increase in the reducing power (35.06 mg AAE/g of flour) as compared to the native sample. Similar results were reported for roasted apricot kernels (Durmaz and Alpaslan, 2007). Microwave and pressure cooked samples reducing power ranged from 33.41 to 34.1 mg AAE/g of flour respectively. The processed red rice exhibited a higher iron reducing power indicating greater antioxidant property, which is desirable for use as a base material in health food formulations.

Table 2: Antioxidant properties of native and processed red rice flour

Samples	DPPH % radical scaving activity	Reducing power (mg AAE/g of flour)
Native	61^{a} ±0.59	26.42^{a} ±1.15
Roasted	84.61^{d} ±1.20	35.06^{d} ±1.66
Pressure cooked/autoclaved	80.6^{b} ±1.22	34.1^{c} ±1.50
Microwave treated	83.24^{c} ±1.40	33.41^{b} ±1.80

Results are mean of three determinations ± SD. Values with same letters (a, b, c, d within column) are not significantly different at $p < 0.05$.

CONCLUSIONS

Polyphenols are well known to be important nutraceuticals having antioxidant properties. The antioxidant activity of native and processed red rice polyphenols was determined in terms of the DPPH reduction capacity as well as the reducing power of ferrous to ferric iron state. The present study showed that roasting significantly improved the nutraceutical properties of red rice by increasing its content in phenolic compounds and also its antioxidant activities. Therefore, roasted red rice holds a good potential as a source of nutraceuticals in food formulations.

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