Basudha Data Paper No. 5

An Examination of Contents of Phenolics and Flavonoids, and Antioxidant Potentials of Uncooked vs Cooked Grains of Selected Rice Landraces

Prepared by

Priya Mondal, Souvik Datta and Debal Deb

Basudha
Laboratory for Conservation

Kolkata

2020

An Examination of Contents of Phenolics and Flavonoids, and Antioxidant Potentials of Uncooked vs Cooked Grains of Selected Rice Landraces

Abstract

Rice grains contain various types of phenolic compounds and flavonoids, which are secondary metabolites, which may contribute to antioxidant potential. Our study of selected rice landraces show that the levels of total phenolics and flavonoids, and consequently, of the potential antioxidant activities of rice is reduced upon cooking. However, the degree of reduction of antioxidant potential in certain native landraces is small, and therefore cooked rice of these varieties may retain a high level of antioxidant activity.

Introduction

Rice (*Oryza sativa* ssp. *indica*) is a staple food for almost half of the world's population (FAO, 2009). Although the nutrient contents of rice may vary according to the environmental conditions and soil characters (Baxter et al., 2012), specific ranges of nutrient contents are variety-specific. Many varieties of rice contain considerably high amount of antioxidants which are secondary metabolites, including many phenolic compounds. Pigmented rice varieties generally contain high amount of phenolics in the aleurone layer of the rice grain (Walter & Marchesan, 2011).

Rice is generally consumed as cooked food. The cooking process causes degradation of many bioactive compounds which are known to be responsible for antioxidant activity in both pigmented and non pigmented rice (Chmiel et al., 2018). However, all published analyses of the antioxidants in rice were conducted on uncooked rice grains.

We present here the first report of the nutraceutical significance of cooked rice of 40 *indica* rice varieties. This report constitutes a part of the on-going investigation of micronutrient analysis of pigmented and non pigmented rice varieties of South Asia.

Materials

Forty rice landraces were analyzed for their micronutrient content. Samples were collected from the indigenous rice seed bank of Vrihi (www.cintdis.org/vrihi), located in Rayagada district of Odisha (19°33'06"N, 83°23'28.14" E), where each of these landraces are being cultivated for *in situ* conservation.

Methods

Determination of total phenolic and flavonoid contents

100g of rice grains of each sample were cooked at 100˚C for 30min and kept at 4˚C for total phenolic content (TPC) and total flavonoid content (TFC) estimation. Uncooked rice powder

and cooked rice were soaked in methanolic water (7:3 v/v) for overnight. On the following day, the methanolic extract was centrifuged to obtain the supernatant for further analysis. TPC of the sample was estimated by Folin-Ciocalteu method (Harborne, 1973). The absorbance was measured at 765 nm after 30 min of incubation. The results are expressed as mg of gallic acid equivalent (GAE)/100 g of dry weight.

TFC of uncooked and cooked rice samples was estimated by adding 10% aluminum chloride, 5% sodium nitrite and water in a ratio of 1:1:1:7 (Goufo & Trindade, 2014). The absorbance was measured at 510 nm after 25 min of incubation. The result is expressed as mg of quercetin equivalent (QE)/100 g dry weight of sample.

Determination of antioxidant activity

The sample extraction procedure and estimation of antioxidant activity was performed by following the modified method of Thuengtung & Ogawa (2020). All the rice varieties were cooked at 100˚C in water bath for 30 min. Before cooking, 40 mg of rice grains was soaked in water and drain out. Completely cooked rice was kept at 4˚C for further analysis. On the following day, the uncooked rice powder and cooked rice samples were extracted in 2 ml of 80% (v/v) methanolic water at room temperature for 6 hr under continuous shaking. The extracted samples were then centrifuged to get the clear supernatant for 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Ferric reducing antioxidant power (FRAP) analysis.

For DPPH assay, 60 µM DPPH (975 µl) with extracted sample (25 µl) was incubated in dark for 30 min at room temperature. The absorbance was measured at 517nm using methanol as blank. The results are expressed as µmol of ascorbic acid equivalent (AAE)/100g of dry weight of sample.

For FRAP analysis, 200 µl extracted sample was mixed with 1.3 ml of freshly prepared FRAP reagent [300mM acetate buffer (pH 3.6), 10mM TPTZ in 40mM Hcl and 20 mM $Fecl_3$ at 10:1:1 v/v/v ratio], incubated at 37˚C for 30 min. The absorbance was measured at 517 nm. The results are expressed as µmol of ferrous sulphate ($FeSo_4$)/100g of dry weight of sample.

Results

Table 1. Total Phenolic and Flavonoid Content of Uncooked Grains of 40 Rice Landraces

Landrace Accession Code	TPC (mg GAE/100g dry weight)	TFC (mg QE/100g dry weight)
AA23	33.3	55.9
AA24	73.5	293.3
AA31	82.8	190.9
B20	24	39.6
B22	88	310.8
B72A	98.3	438.8
B75	21	39.6
C19	18.5	44.2
C20	85.7	294.5
D05	35.8	48.9
DH05	48.2	29.1
G39	10.3	43.1
G44	34.5	40.7
H05	19.7	40.7
H07	44.3	43.1
J15	93.2	46.6
K08	19.3	32.6
K105	31.7	33.8
K126	48.5	48.9
K24	26.8	20
K49	69	309.6
K73	65.2	264.2
K79	15.2	40.7
L27	20.8	54.7
L31	40.7	31.4
M02	95.8	267.7
N36	30.3	38.4
S39	41.2	140.8
SH14	37.7	32.6
SH19	57.5	39.6

The levels of total phenolic compounds and total flavonoids of uncooked grains in most of the rice landraces are moderate to high (Table **1**). However, when we compared these levels in the grains of 10 randomly selected rice landraces before and after cooking, concentrations of both the phenolics and flavonoids in rice grains were found to have drastically reduced after cooking, as shown in Table **2**.

Table 2. A Comparison of Total Phenolic and Flavonoid Contents of Selected Uncooked and Cooked Grains of Selected Rice Landraces

Landrace Accession Code	Uncooked Rice		Cooked Rice	
	TPC (mg GAE/100g dry weight)	TFC (mg QE/100g dry weight)	TPC (mg GAE/100g dry weight)	TFC (mg QE/100g dry weight)
C14	105	451.6	22.2	36.3
K05	98.7	90.8	15.7	27
K10	32.2	79.1	3	20
K131	21.5	48.9	0.7	23.3
K39	31.3	40.7	0.8	19.5
K50	15.2	14.9	1.7	26.5
M17	68	327.1	37.3	43.3
P40	51.8	67.5	14.2	37.7
V01	153.3	134.6	42.8	65.2
W01	115.5	396.9	18.8	40

Table 3. Antioxidant Activity of Cooked Grains of 40 Rice Landraces

Basudha Accession Code	DPPH radical scavenging activity (µM AAE/ 100 g dry weight)	Ferric reducing antioxidant power (µM $FeSo_4$/ 100g dry weight)
AA23	651.5	2,870.1
AA24	426.5	8,920.5
AA31	1,071.5	3,529.4
B20	801.5	4,518.4
B22	1,496.5	11,170.1
B72A	1,646.5	11,034.3
B75	806.5	775.7
C19	841.5	1,784.1
C20	1,201.5	5,410.5
D05	441.5	4,033.6
DH05	746.5	1,803.5
G39	806.5	2,036.2
G44	666.5	1,357.5
H05	826.5	1,105.4
H07	401.5	4,343.9
J15	1,281.5	7,698.8
K08	776.5	2,773.1
K105	871.5	3,219.1
K126	601.5	4,072.4
K24	1,281.5	8,396.9
K49	876.5	7,504.9
K73	866.5	3,626.4
K79	876.5	3,180.4
L27	876.5	3,374.3
L31	446.5	1,570.8
M02	931.5	9,482.9
N36	836.5	2,327.1
S39	611.5	6,942.5
SH14	391.5	2,327.1
SH19	821.5	833.9

Data presented in Table **3** show high levels of antioxidant potential in most of the rice landraces examined. However, these levels are drastically reduced when the rice is cooked. Our comparison of the antioxidant potentials of 10 randomly selected rice landraces is presented in Table **4**, which indicate that the degree of reduction in the antioxidant activity is not uniform, and the cooked grains of certain landraces (namely K39, K131 and V01) may retain much of their high antioxidant potential.

Table 4. A comparison of Antioxidant Activity of Uncooked and Cooked Grains of Selected Rice Landraces

Basudha Accession Code	**Uncooked rice**		**Cooked rice**	
	DPPH radical scavenging activity (µM AAE/ 100 g dry weight)	**Ferric reducing antioxidant power (µM $FeSo_4$/ 100g dry weight)**	**DPPH radical scavenging activity (µM AAE/ 100 g dry weight)**	**Ferric reducing antioxidant power (µM $FeSo_4$/ 100g dry weight)**
C14	2,016.5	18,422.9	776.5	7,834.6
K05	871.5	9,133.9	681.5	7,214
K10	1,566.5	3,607	836.5	1,687.1
K131	961.5	2,811.9	861.5	2,191.3
K39	441.5	2,672.2	341.5	2,656.8
K50	646.5	2,327.1	526.5	1,842.3
M17	1,556.5	15,882.5	871.5	9,424.7
P40	586.5	6,787.4	566.5	5,332.9
V01	2,096.5	16,599.9	1,421.5	10,898.6
W01	2,066.5	14,835.3	1,501.5	8,183.6

References

Baxter, I., Hermans, C., Lahner, B., Yakubova, E., Tikhonova, M., Verbruggen, N., Chao, D.Y., & Salt, D.E. 2012. Biodiversity of mineral nutrient and trace element accumulation in Arabidopsis thaliana. *PLoS One* 7(4), p.e35121.

Chmiel, T., Saputro, I. E., Kusznierewicz, B., & Bartoszek, A. (2018). The impact of cooking method on the phenolic composition, total antioxidant activity and starch digestibility of rice (Oryza sativa L.). *Journal of Food Processing and Preservation* 42(1), e13383. https://doi.org/10.1111/jfpp.13383

FAO 2009. *Food and Population: FAO Looks Ahead*. Food and Agricultural Organization of the United Nations. Rome.

Goufo, P., & Trindade, H. (2014). Rice antioxidants: phenolic acids, flavonoids, anthocyanins, proanthocyanidins, tocopherols, tocotrienols, g-oryzanol, and phytic acid. *Food Sci. Nutr*. 2: 75–104.

Harborne, I.B. 1973. *Phytochemical Methods: A guide to modern techniques of plant analysis*. 2nd Edition. Chapman and Hall. New York, 88-185.

Thuengtung, S. & Ogawa, Y. Comparative study of conventional steam cooking and processing microwave cooking on cooked pigmented rice texture and their phenolic antioxidant. *Food Sci Nutr*. 2020; 00:1–8. https://doi.org/10.1002/fsn3.1377

Walter, M., & Marchesan, E. (2011). Phenolic compounds and antioxidant activity of rice. *Brazilian Archives of Biology and Technology* 54: 371–377. https://doi.org/10.1590/S1516-891320 11000 200020