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ANTIMUTAGENIC PROPERTIES OF SEVERAL KINDS OF RICE AS TESTED UPON YEAST STRAIN *SACCHAROMYCES CEREVISIAE* D7

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The aim of this work was to study antimutagenic and antioxidant properties of water extracts of various types of rice. Antioxidant activity, total phenolic and flavonoid contents were measured spectrophotometrically. Individual phenolics – catechins, catechin gallates, ferulic acid – were analysed by HPLC/PDA. Antimutagenic activity of rice extracts was tested using unicellular eukaryotic yeast strain *Saccharomyces cerevisiae* D7 the first time.

Non-processed rice types contained substantially higher amount of soluble total and individual phenolics and had higher antioxidant activity than peeled rice. Parboiled rice, in which technological processing helps to maintain active substances inside the grains, exhibited also relatively high phenolic levels. The highest antimutagenic effect was proved in Indian Rice, Arborio, Jasmine Rice and Three Colours Rice independently of rice extract concentration. Because of relatively high values of phenolics and high antioxidant activity, the antimutagenic activity of these samples could be attributed to biologically active phenolics present mainly in the bran layer of whole rice grain.

Keywords: rice, antioxidant activity, phenolics, flavonoids, antimutagenicity

Cereals provide the main part of human nutrition, they are used as feed for farm animals and a small amount is used for technical applications. Rice (*Oryzae sativa*) is cultivated in over one hundred countries as staple food for more than half the population of the world. Pearled rice, the husk and bran being removed during the rice-milling process, is the most common type of rice consumed because of its high content of starch. Recently, studies evidenced that many bioactive compounds do not uniformly exist in a cereal grain but are concentrated in the bran layer (Butsat & Siriamornpun, 2010). Thus, the consumption of whole grain in regular meals is strongly recommended, to provide desirable health benefits beyond basic nutrition and to reduce the risks of many chronic diseases (Liu, 2007).

The beneficial components of rice bran comprise sterols, γ-oryzanols, tocopherols, tocotrienols and phenolic compounds (Aguilar-Garcia et al., 2007). Some special phenolic acids, such as ferulic acid and *p*-coumaric acid, found in rice bran are not present in significant quantities in fruit and vegetables (Adom & Liu, 2002). Recently, ferulic acid was related to the prevention of diabetic complications (Yawadio et al., 2007). γ-Oryzanol, a mixture of lipophilic phytosterols with ferulic acid esters, exhibits antioxidant activity and cholesterol-lowering effect (Miller & Engel, 2006). Tocopherols, tocotrienols and several phenolic compounds in rice bran have potentially beneficial effects, such as antioxidant activity, antimutagenic and anticancer effects (Nam et al., 2005), that play important roles in maintaining health.

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Ferulic acid and *p*-coumaric acid are the major phenolic compounds in rice, which exist in free, soluble-ester, and insoluble-bound form (Sosulski et al., 1982). Several studies reported that only free and soluble-ester ferulic acids could be absorbed from the stomach and intestine (Kern et al., 2003). Small amounts of bound ferulic acids, which are mainly conjugated with insoluble polysaccharides arabinoxylan and lignin, would be released by intestinal esterase and colonic microflora (Zhao et al., 2003). Considerable metabolic turnovers of hydroxycinnamic, *p*-coumaric and sinapinic acids during brain development were reported. The levels of these phenolics decreased in the soluble-ester fraction, but steadily increased in the insoluble-bound fraction as the grain developed (Lin & Lai, 2011).

Rice is the most common cereal for direct consumption. It is formed from complex saccharides, it contains high amounts of fibre, low amounts of fat and no cholesterol, sodium or gluten. Rice also contains many types of phenolic compounds, which have significant antioxidant properties. Cereal grains are milled into flour used for various bakery products. New types of rice have appeared recently, e.g. Arborio, Basmati, Jasmine Rice, Three Colours Rice, etc. Some of them offer new flavours and culinary processing. There is also the question what the difference is between the various rice species in content of flavonoids, catechins, saccharides, etc.

The aim of this work was to analyse antimutagenic and antioxidant properties in water extracts of selected kinds of rice in relation to content and composition of phenolic compounds. For genotoxicity/antimutagenicity assay eukaryotic test system *S. cereviviae* D7 was used. This mutant yeast strain was constructed to simultaneous testing of toxicity, reverse mutations in ilv1 locus, mitotic crossing-over and total aberrations at the ade2 locus and mitotic gene conversion at the trp5 locus (Zimmermann et al., 1984; Vlckova et al., 2006; Miadokova et al., 2010).

1. Materials and methods

1.1. Chemicals reagents

All reagents were of analytical grade. Acetonitrile (HPLC gradient grade), methanol (HPLC, gradient grade) and ethanol for HPLC were purchased from Sigma-Aldrich (St. Louis, MO, USA). Deionised water was supplied by a Milli-Q water purified system from Millipore (Bedford, MA, USA). Phenolic standards were obtained from Sigma-Aldrich (St.Louis, MO, USA). The standards used were: (–)-catechin, epicatechin, (–)-catechin gallate, epicatechin gallate, ferulic acid, morin, rutin and naringenin, all of them with purity higher than 95%. The mutagen 4-nitroquinoline-N-oxide was purchased from Sigma-Aldrich. YPD medium was obtained from HiMedia, tryptophan, isoleucine and specific components of *S. cerevisiae* D7 medium were purchased from Sigma-Aldrich. Rest of the chemicals used in the study was of analytical grade purity and obtained from local distributors.

1.2. Raw materials

For our study 17 samples of rice were used. All samples were bought in the local market. Analysed samples of rice are summarized and described in Table 1. Extracts for analysis of contents of total phenolics and flavonoids and antioxidant activity were prepared from 10 g of ground rice grains extracted to 25 ml of water.

1.3. Analysis of total phenolics and flavonoids

Total phenolics were analysed colorimetrically with Folin-Ciocalteu reagent (750 nm). Total flavonoid content was analysed colorimetrically with $NaNO_2$+$AlCl_3$ (510 nm). Total antioxidant capacity was measured by ABTS Randox kit (Marova et al., 2011). In this test ABTS® (2,2'-Azino-di-[3-ethylbenzthiazoline sulphonate]) is incubated with a peroxidase (metmyoglobin) and H_2O_2 to produce the radical cation ABTS®*+. This has a relatively stable blue-green colour, which is measured at 600 nm. Antioxidants in the added sample cause suppression of this colour production to a degree which is proportional to their concentration.

1.4. Chromatographic analysis of individual phenolics

Samples of rice (10 g) were homogenized using laboratory mill. The samples were acidified by 2% HCl and extracted by ethyl acetate. After the vacuum evaporation of ethyl acetate, extracted phenolic compounds were dissolved in acetonitrile:water mixture (90:10, v/v) and in ethanol for HPLC, respectively, transferred into Eppendorf tubes, centrifuged (10 000 r.p.m., 10 min) and filtered through PTFE filter (0.45 mm). These purified extracts were used for chromatographic analysis (Marova et al., 2011).

Separation and identification of individual phenolics were performed by on-line LC/PDA/ESI-MS analysis (MS Pump Plus, Surveyor Finnigan, USA; PDA Plus Detector Surveyor Finnigan, USA; mass spectrometer LCQ Advantage Max, Thermo Fisher Scientific, USA). Solvents were filtered through a 0.45 mm Millipore filter. Standards were dissolved in acetonitrile:water mixture (90:10, v/v).

For final analysis, rice extracts (10 µl) in ethanol were injected into the chromatography column Restek Ultra Aqueous, C18, 5 mm, 250×4.6 mm (Restek Corporation, Bellefonte, PA, USA) heated to 30 °C. Gradient elution was performed using optimized linear gradient aqueous acetonitrile acidified by 1% acetic acid in range of 60–57%: 40–43% (3 min), 57–55%: 43–45% (20 min), 55–45%: 45–55% (10 min) and 45%: 55% (30 min) at flow rate 0.4 ml min^{-1}. PDA detection and quantification of individual phenolic: (–)-catechin, epicatechin and their gallates, ferulic acid, morin, rutin, and naringenin was done using external standards.

1.5. Antimutagenicity assay – the Saccharomyces cerevisiae *D7 test*

Antimutagenicity test was performed using unicellular test strain *S. cerevisiae* D7 (Zimmermann et al., 1984; Marova et al., 2010). The test strain was obtained from the Department of Genetics, Comenius University, Bratislava. The test was performed according to the modified procedure of Zimmermann and co-workers (1984). This "Mitotic Gene Conversion Assay in *S. cerevisiae*" is one of the standard protocols recommended to test for mutagenic/antimutagenic potential of a compound. The assay uses the D7 strain of *S. cerevisiae*, initially constructed and described by Zimmermann and co-workers (1984). When compounds mutate the DNA of this diploid yeast strain, specific phenotypes are produced at three separate genomic sites. Additionally, each one of these phenotypic changes specifies a separate type of mutation and repair mechanism. At the heteroallelic *ade2* site, the two mutant alleles demonstrate intergenic complementation, producing white colonies of yeast. Mitotic crossing-over between the centromere and the ade2 locus induced by mutagen is manifested as twin spotted (pink/red) colonies among white colonies (Vlckova et al., 2006). The

heteroallelic *trp5* locus results in tryptophan auxotrophy, and thus D7 cells are not able to grow on synthetic media lacking tryptophan. Mitotic gene conversion at the *trp5* locus, caused by repair mechanisms induced after particular types of mutagenic treatments, produces colonies that are able to grow on synthetic media lacking tryptophan. Furthermore, reverse mutations can be identified by assaying for isoleucine prototrophy. The D7 strain is auxotrophic for isoleucine because of its homoallelic loss-of-function mutations in the *ilv1* locus. This isoleucine auxotrophy can be overcome by a reverse point mutation at the *ilv1* locus (Vlckova et al., 2006; Miadokova et al., 2010).

Water extracts of selected rice samples (0.5 g and 2 g of milled grains per 10 ml) were used for antimutagenicity testing. After 16–18 hours of cultivation of the yeast *S. cerevisiae* D7 in the liquid YPD medium, the cell suspension in the logarithmic phase of growth was divided into the centrifuge tubes (10 ml) and centrifuged for 5 min at 5000×*g* and 20 °C. The cell sediment was suspended twice in phosphate buffer pH 6.98. The 10 ml of cell suspension in phosphate buffer pH 6.98 was treated with 0.1 ml of a 0.06 mg ml^{-1} 4-nitroqunoiline-N-oxide (4-NQO), which was used as a mutagen (Bunkova et al., 2005), and inoculated simultaneously with 0.5 ml of the rice sample dissolved in DMSO.

Onto the selective medium for the tryptophan conversions 0.1 ml of the suspension of 10^6 cells/ml was spread. Onto the selective medium for isoleucine reverse mutations 0.1 ml of the 10^7 cells ml^{-1} was spread. The number of the yeast colonies in the Petri dishes was counted after 5–10 days of incubation at 28 °C.

All measurements were done in triplicates. The percentage of the inhibition of 4-NQO effect was calculated according to the equation:

$$\text{Per cent of the inhibition (\%)} = 100–[(X_1/X_2\times100],$$

where X_1 is the number of the yeast colonies in the presence of 4-NQO and selected rice water extract, X_2 is the number of the yeast colonies in the presence of the 4-NQO without rice solution. The results are expressed as means±SD from three (five, respectively) measurements. Results were analysed by the Student *t*-test using Statistica for Windows 5.0 (Statsoft, USA). The differences of $P<0.05$ were regarded as statistically significant (Marova et al., 2010).

1.6. Statistical evaluation

The limits of detection and quantitation have been calculated as 3 and 10 times signal-to-noise ratios, respectively. Regression equations, correlation coefficients and relative standard deviations were calculated using Excel 2007 software (Microsoft, Redmond, WA, USA).

2. Results and discussion

2.1. Sample description

White rice samples and types of parboiled rice were selected according to producer, price and expected frequency of use by the population. Some specific types of white rice (Jasmine Rice, Arborio and Basmati) as well as coloured or whole grain rice (Red Rice Natural, Indian Wild Rice) were analysed, too.

2.2. Total phenolics and flavonoids

From an analytical view, phenolics can be classified into soluble (free and conjugated) and insoluble bound forms (Naczk & Shahidi, 2004). In non-pigmented rice, soluble phenolics contribute 60% of total phenolics, whereas in pigmented rice, due to the presence of high amounts of flavonoids, their contribution reaches 80% (Massaretto et al., 2011). In black rice, the major flavonoids are anthocyanins and in some cultivars proanthocyanidins can also be found. In red rice the most abundant flavonoids are proanthocyanidins with different degrees of polymerization, followed by variable amounts of anthocyanins. Insoluble phenolic compounds occur mostly bound to polysaccharides of the cell wall and may be partially released and transformed in the colon by microflora enzymes before absorption. This indicates that polyphenols may exert both systemic and local antioxidant effects, modulated by the microbial metabolism (Abdel-Aal et al., 2006).

In this study most parameters for soluble phenolics (=water extracts) were determined only to compare them with the results of the antimutagenicity test. The content of total soluble phenolics ranged from 3.40 to 22.49 mg/100 g of rice, the highest values were measured in Red Rice Natural, Arborio Rice and Indian Wild Rice (Table 1). The content of total soluble flavonoids ranged from 1.16 to 13.33 mg/100 g. The highest content was found in the same kinds of rice as the highest values of total phenolics. The content of total flavonoids in Red Rice Natural was twice higher than in Arborio. The lowest contents of these active compounds were measured in Long Grain White Rice and Jasmine Rice. Total flavonoids in most rice samples exhibited approximately 35% of total polyphenols on the average except in Red Rice Natural (Table 1). The levels of total phenolics and total flavonoids exhibited strong direct correlation (R^2=0.896; $P<0.05$).

In the study of cooked rice, similar differences were found between the phenolic contents of white and pigmented rice as described above (Massaretto et al., 2011). The average content of phenolics in the pigmented group (409.7±62.9 mg FA eq./100 g) was about 4 times as high as that in the non-pigmented rice group (99.4±19.1 mg FA eq./100 g). Phenolic content differed widely among pigmented rice cultivars (343.7–504.9 mg FA eq./100 g) and may reflect the differently coloured bran of the red rice genotypes analysed, since non-pigmented rice varieties showed lower variation (75.7–134.0 mg FA eq./100 g).

In general, rice lines with darker bran exhibit higher content and broader variation in soluble phenolics (Massaretto et al., 2011). In general, results of our study as well as other works indicate that non-processed rice types (e.g. Natural Rice, Indian Wild Rice, etc.) contain substantially higher amounts of soluble phenolic compounds than peeled rice. Technological preparations of parboiled rice help to maintain active substances inside the grains.

2.3. Antioxidant activity

The antioxidant activity of rice water extracts ranged from 6.24 to 75.72 mg/100 g. The highest value was found in Natural Red Rice (75.72 mg/100 g), followed by Indian Wild Rice and Three Colours Rice (Fig. 1).

The antioxidant activity was associated with the content of total phenolics and total flavonoids. The Red rice and Indian rice, where the highest values of total phenolic and flavonoid compounds were found, exhibited the highest antioxidant activity as well. Nevertheless, no direct correlation of total phenolics/flavonoids and total antioxidant activity was found.

Table 1. Total phenolics and total flavonoids content in rice grain

Rice sample	Total phenolics (mg/100 g)	Total flavonoids (mg/100 g)	% of total flavonoids in total polyphenols
Special and white rice samples:			
Long Grain White Rice	7.07±0.21	2.20±0.05	31.12
Long Grain White Rice, BIB*	15.23±0.20	5.14±0.05	33.75
Bask, 1 kg	10.88±0.14	3.53±0.04	32.44
SOS Classic, 1 kg	13.13±0.38	4.24±0.03	32.29
SOS Long, 1 kg	10.23±0.18	3.20±0.03	31.28
Long Grain Husked Rice	8.33±0.11	3.14±0.03	37.70
White rice "Menu Zlaté"	14.05±0.39	4.95±0.09	35.23
White rice "Albert Bio"	3.40±0.08	1.16±0.04	34.11
White rice "Lagris", Long Grain	12.68±0.14	5.51±0.22	43.45
"Lagris", Arborio rice	18.33±0.27	6.76±0.08	36.88
Basmati Rice, "Lagris"	13.54±0.14	4.86±0.14	35.89
Jasmine Rice, "Lagris"	8.56±0.13	2.56±0.13	29.90
Parboiled rice samples:			
Parboiled "Menu Zlaté"	13.41±0.51	4.75±0.05	35.42
"Lagris", Parboiled rice	14.15±0.06	4.64±0.03	32.79
Coloured and raw rice samples:			
"Lagris", Three Colours rice	12.53±0.20	3.93±0.06	31.36
"Lagris", Indian Wild Rice	17.79±0.14	6.57±0.12	36.93
Red Rice Natural	22.49±0.31	13.33±0.05	62.26

*BIB: Boil-In-Bag

Several epidemiological and experimental studies have clearly indicated that the increased consumption of whole grain products have been associated with the reduced risk of cardiovascular diseases, cancer and other chronic diseases (Veenashri & Muralikrishna, 2011). The beneficial health effects derived from the intake of diets rich in cereals and millets have mainly been ascribed to dietary fibre or to some of the components associated with fibre including phenolic acids such as ferulic, vanillic, *p*-coumaric, caffeic, chlorogenic and syringic acids (Andreasen et al., 2001), which are found to be present in free and bound forms. They are concentrated in the bran portion of cereal kernels and millets. Soluble dietary fibre consists of b-1,3/1,4 glucans, water soluble arabinoxylans, arabinans, arabinogalactan-proteins with small amount of total bound phenolic acids (about 10%), whereas insoluble dietary fibres have water insoluble arabinoxylans, cellulose–lignin complexes as main constituents with high amount of bound phenolic acids (Veenashri & Muralikrishna, 2011).

Hydroxycinnamic acids exhibited in vitro chemoprotective and antioxidant properties that may also contribute to the beneficial effects of a diet rich in cereal brans (Andreasen et al., 2001). Ferulic acid and other hydroxycinnamic acid derivatives exhibit very strong antioxidant activity since they form resonance-stabilized phenoxy radicals, terminate free radical chain reactions and also known to inhibit lipid peroxidation in rat liver microsomal

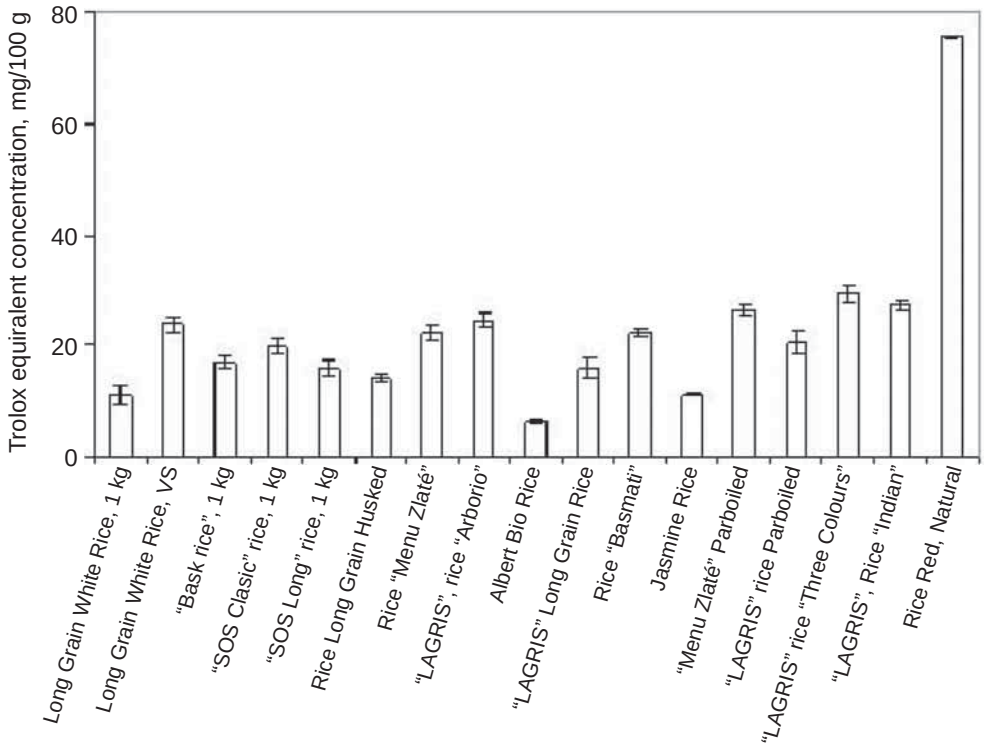

Fig. 1. Antioxidant activity of rice samples

membranes. Ferulic acid in the bound form is known to decrease total cholesterol and increases vitamin E bioavailability; increases vitality of sperms and also it is a good protective agent against UV radiation-induced skin damage. In the study of Rao and Muralikrishna (2007) it was demonstrated for the first time regarding the antioxidant activity of water-soluble arabinoxylans (feraxans) isolated from rice and ragi (finger millet) that they can exhibit very strong antioxidant activity, which could be 5000 times higher than the activity exerted by sulphated polysaccharides (Adom & Liu, 2002).

In our study the antioxidant activity of the soluble portion of rice extracts was evaluated. It was clearly demonstrated that antioxidant activity of Red Rice Natural is substantially higher than of other analysed samples, probably due to a different phenolic composition and the presence of proanthocyanins and anthocyanins (Massaretto et al., 2011). In whole grain and parboiled rice samples higher antioxidant activity was demonstrated than in peeled grain samples.

2.4. Analysis of individual phenolic compounds

Similarly to other parameters, individual flavonoids were determined in the soluble portion of rice homogenate as described in Materials and methods. Catechins, catechin gallates, ferulic acid and chlorogenic acid were found in higher quantities. Rice Parboiled, Three Colours and Indian Wild Rice had high contents of catechin and catechin gallate (Table 2).

The highest values of minor individual flavonoids (morin, rutin, naringenin) were measured in Indian Wild Rice and Parboiled Rice (Table 3).

Table 2. Content of individual catechins and phenolic acids in rice samples

Rice species	Catechin (mg/100 g)	Epicatechin (mg/100 g)	Catechin gallate (mg/100 g)	Epicatechin gallate (mg/100 g)	Ferulic acid (mg/100 g rice)
Special and white rice samples:					
Long Grain White Rice	1.25±0.03	1.00±0.02	2.12±0.01	0.55±0.02	61.12±0.91
Long Grain White Rice, BIB*	5.28±0.05	1.16±0.10	3.19±0.02	0.56±0.01	46.73±0.23
Bask, 1 kg	1.65±0.01	1.17±0.02	2.77±0.04	0.74±0.01	114.89±1.33
SOS Classic, 1 kg	1.59±0.05	0.91±0.09	3.18±0.01	0.65±0.02	202.20±0.44
SOS Long, 1 kg	2.18±0.03	0.78±0.02	3.03±0.01	0.74±0.01	109.7±3.70
Long Grain Husked Rice	1.04±0.02	0.89±0.06	3.23±0.02	0.48±0.08	84.56±2.94
White rice "Menu Zlaté"	2.37±0.01	1.39±0.04	4.06±0.05	0.93±0.01	213.58±6.91
White rice "Albert Bio"	0.80±0.04	0.40±0.01	1.95±0.01	0.17±0.01	44.83±0.11
White rice "Lagris", Long Grain	1.75±0.06	2.45±0.05	2.36±0.01	0.75±0.08	111.40±1.15
"Lagris", Arborio rice	2.45±0.01	0.85±0.03	5.13±0.10	0.82±0.02	175.80±0.66
Basmati Rice, "Lagris"	2.83±0.02	1.11±0.05	3.47±0.08	0.82±0.06	98.34±0.35
Jasmine Rice, "Lagris"	2.69±0.07	1.07±0.02	4.37±0.06	0.85±0.03	54.27±1.60
Parboiled rice samples:					
Parboiled "Menu Zlaté"	22.44±0.05	4.71±0.03	43.96±0.48	1.00±0.04	153.00±9.84
"Lagris", Parboiled rice	16.79±0.13	2.44±0.01	23.92±0.60	0.43±0.02	104.79±0.51
Coloured and raw rice samples:					
"Lagris", Three Colours rice	27.60±0.29	3.25±0.04	41.10±1.41	0.63±0.02	191.89±0.33
"Lagris", Indian Wild Rice	19.20±0.17	4.62±0.03	26.15±0.04	5.92±0.07	556.09±4.74
Red Rice Natural	5.64±0.16	1.36±0.01	1.52±0.04	0.27±0.01	82.81±1.28

*BIB: Boil-In-Bag

The above-mentioned recent studies documented positive health effects of whole-grain rice consumption. Especially the red and black pigmented genotypes have aroused considerable interest because of their wide range of phenolic acids and derivatives (Aguilar-Garcia et al., 2007; Lin & Lai, 2011; Massaretto et al., 2011).

In general, the biological activities of phenolic compounds depend on their absorption and metabolism, which are closely related to their chemical structure ranging from simple phenolic structures to highly polymerized compounds. Food anthocyanins are poorly absorbed from both stomach and small intestine and plasma concentrations range from nanomol to micromole dm^3. Methylated, glucuronidated and sulfo-conjugated anthocyanin metabolites can be found in the plasma at levels more than twice as high as in the intact compounds. Indeed, these compounds may have direct protective effects on the intestinal mucosa against oxidative stress or the action of carcinogens.

Table 3. Content of individual flavonoids in rice samples

Rice species	Rutin (mg/100 g rice)	Morin (mg/100 g rice)	Naringenin (mg/100 g rice)
Special and white rice samples:			
Long Grain White Rice	5.22±0.01	0.21±0.02	1.49±0.01
Long Grain White Rice, BIB*	21.27±0.03	1.96±0.08	1.36±0.06
Bask, 1 kg	10.38±0.05	0.35±0.01	2.17±0.17
SOS Classic, 1 kg	14.56±0.14	2.11±0.07	1.66±0.01
SOS Long, 1 kg	12.90±0.04	1.72±0.02	6.21±0.13
Long Grain Husked Rice	4.70±0.07	0.91±0.04	2.14±0.01
White rice "Menu Zlaté"	14.86±0.01	1.49±0.05	1.93±0.02
White rice "Albert Bio"	8.12±0.04	5.85±0.01	7.77±0.03
White rice "Lagris", Long Grain	4.90±0.04	0.44±0.02	2.07±0.03
"Lagris", Arborio rice	10.05±0.04	1.25±0.04	2.19±0.05
Basmati Rice, "Lagris"	11.02±0.06	2.25±0.02	1.34±0.04
Jasmine Rice, "Lagris"	8.96±0.02	1.19±0.01	1.82±0.01
Parboiled rice samples:			
Parboiled "Menu Zlaté"	21.15±0.05	37.69±0.63	9.55±0.27
"Lagris", Parboiled rice	31.70±0.09	31.58±0.29	1.41±0.01
Coloured and raw rice samples:			
"Lagris", Three Colours rice	24.93±0.35	21.42±0.26	6.84±0.30
"Lagris", Indian Wild Rice	178.28±1.91	42.17±0.57	325.8±7.06
Red Rice Natural	25.04±0.32	3.42±0.04	8.30±0.11

*BIB: Boil-In-Bag

In our study remarkable levels of antioxidant activity and of total as well as some individual phenolics were found in whole grain, parboiled and coloured rice samples. Because of expected association between phenolics content and antioxidant activity, the antimutagenic effect of these samples was studied in detail.

2.5. Antimutagenicity assay

Antimutagenic activity was studied in selected samples of rice using simple eukaryotic test with *S. cerevisiae* D7 yeast strain (Bunkova et al., 2005; Marova et al., 2010). When this test strain is treated with a compound that is a potential mutagen, phenotypic changes can be followed to indicate mutagenesis. Depending on the type of this phenotypic change, the mutagen can be characterized (Zimmermann et al., 1984; Bunkova et al., 2005; Vlckova et al., 2006; Miadokova et al., 2010).

Table 4. Antimutagenic activity of selected kind of rice

Rice species	% inhibition; 0.5 g/10 ml		% inhibition; 2 g/10 ml	
	Trp conversion	Ile reversion	Trp conversion	Ile reversion
Special and white rice samples:				
Long Grain White Rice	11.51±0.14	30.8±0.65	41.69±2.58	59.75±1.84
Long Grain White Rice BIB*	43.51±3.72	47.32±4.12	53.12±2.89	54.78±2.64
"Lagris", Arborio rice	70.62±5.34	71.12±4.87	73.92±6.12	81.57±5.59
Jasmine Rice	56.08±4.45	73.17±5.84	71.08±3.88	90.36±7.22
Parboiled rice samples:				
"Menu gold", Parboiled	18.32±0.87	36.27±3.14	29.52±1.91	52.27±2.45
Raw and coloured rice samples:				
"Lagris", Three Colours rice	63.69±3.88	82.03±3.70	83.69±5.68	94.98±6.44
"Lagris", Indian Wild Rice	56.05±4.76	80.65±6.44	70.64±6.98	86.67±8.04
Red Rice Natural	29.11±1.98	52.48±4.25	58.99±6.55	71.66±6.52

*BIB: Boil-In-Bag

In this work antimutagenic effect was expressed as per cent of inhibition of *S. cerevisiae* D7 Trp/convertants and Ilv/revertants formation. As a positive control (100%) cultivation of test yeast strain in presence of standard mutagen 4-NQO was used. The optimal yeast colony number in positive control varied in the range of 80–120. Number of spontaneous mutations in media without Trp and/or Ile did not exceed 2–4 colonies.

Two rice extract concentrations (0.5 and 2 g rice grain/10 ml of water) were tested in each antimutagenicity test. No toxic effect of rice extracts alone was observed at either tested concentrations. As expected, no significant dilution effect was observed using the soluble part of rice phenolics. Four times concentrated extracts exhibited only a small increase of antimutagenicity. This effect can be related to the complex character of this complicated biological material, which can contain derivatives with antimutagenic as well as genotoxic activity. The ratio of these compounds can be substantially influenced by dilution. The resulting effect is expressed as a sum of these diverse activities of different components present in rice extracts. The highest antimutagenic effects were determined in Indian Rice extract, Arborio, Jasmine Rice and Three Colours Rice (Table 4). Because of the relatively high values of phenolic compounds in these samples, antimutagenicity could be related mainly to some of them with high biological activity. The result for Jasmine Rice was suprising, as it had low antioxidant activity and phenolics content. Thus, other types of natural compounds present in this flavoured rice could be responsible for its high antimutagenic activity.

To this time only limited number of studies about rice antimutagenicity was published. Study of ethanol–water (70:30 v/v) extracts from rice bran removed from seeds of two blackish-purple pigmented and one non pigmented brown rice cultivars were evaluated for antioxidant, anti-tumour-promoting, and anticarcinogenic activities in chemical assays and in mammalian cells using inhibition of 4-nitroquinoline N-oxide-induced mutagenesis and inhibition of phorbol ester-induced tumour promotion (Nam et al., 2005). The extracts from the pigmented rice seeds had generally higher activities in all tests than the extract from the non-pigmented cultivar. The results suggest that bran from pigmented rice cultivars may

provide a source of new natural antioxidants and anticarcinogens, and that such rice cultivars with high antioxidant potential also provide a genetic resource for the development of new, improved rice cultivars that may make it possible to enhance both the nutritional and medical value of rice-based diets (Zhao et al., 2003).

In another study a 3% suspension of heat-stabilized defatted rice bran was treated with papain, followed by inactivating the enzyme by heat, and centrifugation. The supernatant was subjected to ultrafiltration, and fractions with various molecular sizes were evaluated for antimutagenicity by Ames test using *Salmonella typhimurium* TA 100 against phenazine methosulfate. Among the partially purified fractions three peptide components from F3 (5–10 kDa) showed significant antimutagenicity, which were 80.2, 53.4, and 58.6% at concentration of 100 mU g/plate, respectively. These peptides are to find application in nutraceutical and pharmaceutical products (Massaretto et al., 2011).

In our study some kinds of natural and colour rice as well as some flavoured samples exhibited strong antimutagenicity, high antioxidant activity as well as high levels of phenolic compounds. It can be summarized that in rice samples with high content of biologically active phenolic substances also high antimutagenic effect was found. Additional effect of other natural compounds should be taken into account, too.

3. Conclusions

The presented study had demonstrated the antimutagenic effect of selected types of rice in connection with antioxidant properties and levels of phenolic compounds. Non-processed rice types (e.g. Natural Red Rice, Indian Wild Rice, etc.) contained substantially higher amounts of biologically active phenolics than peeled rice. The antioxidant activity was associated with total phenolic and flavonoid contents. The Red Rice and Indian Rice, where the highest contents of total phenolic and flavonoid compounds were found, also exhibited the highest antioxidant activity. The antimutagenic effect was the highest in water extracts of natural and whole grain rice with high phenolic content, though high antimutagenicity was found also in Jasmine Rice. Technological preparations of parboiled rice help to maintain active substances inside the grains.

Because of the relatively high content of biologically active compounds, antimutagenic and antioxidant effects and worldwide consumption, it can be concluded that rice (predominantly natural) belongs to important foods recommended for modern human nutrition.

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