Composition of amino Acids, fatty acids, minerals and dietary fiber in some of the local and import rice varieties of Malaysia

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Abstract

In this study, three popular, regionally grown rice varieties (Bario, brown and white) were compared with three of the most popular and highly marketed imported rice varieties (black, glutinous and basmati rice) in Penang region of Malaysia. Rice samples were evaluated for amino acids, fatty acids, minerals, heavy metals and dietary fiber composition. Overall, amino acids content among all the rice samples were comparable to each other. Results with regard to minerals showed potassium to be high in brown rice (197.41 mg/100g), while magnesium was recorded to be high in black rice (107.21 mg/100g). Heavy metals such as cadmium, nickel, mercury and lead, though present, they were in negligible amounts. Among all the rice varieties investigated, the total saturated fatty acid and unsaturated fatty acid content was highest in black rice (5.89%). The soluble dietary fiber was higher in white rice (16.39%), whereas insoluble dietary fiber was high in brown (16.51%) and black rice (14.49%), respectively. Results generated from this study is anticipated to benefit both the health wary consumers (based on their potential nutritional attributes) as well as the local food industries to choose the best rice variety while developing novel rice based food products.

Introduction

Of late, with the augmentation in worlds population, the need for potential nutritional sources from plant origin has tremendously increased (Friedman and Brandon, 2001; Bhat and Karim, 2009; Abbas et al., 2011). Among the various nutritional attributes, protein quality, minerals and fatty acids content assumes higher significance owing to their potential health benefits. It is well-accepted fact that rice is a popular staple food, consumed by a majority of world’s population (Singh et al., 2005; Cai et al., 2011; Thomas et al., 2014 a, b). Rice is a wholesome grain that can fulfill the daily nutritional/dietary requirements of the local populations. Rice grain encompasses rich amounts of carbohydrate, fiber, protein, fat and minerals. Carbohydrate (nearly 75-85%) in rice grain provides most of the calories associated, whereas protein encompassing eight of the essential amino acids can fulfill other nutritional requirements. Whereas, minerals like iron, copper, zinc, calcium, magnesium, phosphorus and manganese are also found in adequate amounts in rice (Yousaf et al., 1992; Parengam et al., 2010). In addition, adequate amounts of dietary fiber is also reported to be present in rice grains (Aune et al., 2011), thus rendering it a wholesome food.

Owing to the increased demand by consumers for a healthy, natural and a staple food, providing in-depth details on the nutritional qualities of locally grown, indigenous rice varieties assumes high importance. Hence, the present study was planned in order to provide vital foundation informations on the amino acids, minerals, and fatty acids composition of six popular rice varieties (popular as well as highly marketed; 3 locally grown and 3 imported rice varieties) of Penang region in Malaysia. This study is anticipated to benefit both the health wary consumers as well as the local food industries to choose the best rice variety while developing novel rice based food products.

Materials and Methods

Materials

In total, six commonly consumed and popular rice varieties were purchased from a local Supermarket in Penang, Malaysia. The local rice varieties selected included the Brown rice and white rice (medium grain type) (both origin of Kedah region, West Malaysia), and Bario rice varieties (grown in the Bario region of Sabah, East Malaysia). Imported rice varieties, which were used for comparison included: Black rice and glutinous rice (origin of Thailand) and Basmati...
rice (origin of Pakistan). Only whole rice grains that maintained 75% of its grain length and without any physical damage were selected for analysis. Rice samples were individually ground to a fine powder (30 mesh size) using a Waring blender (Panasonic MX-898 M, Malaysia), packed in air tight plastic bags and were stored at 4°C for future analysis.

**Amino acids analysis**

To determine the amino acids content, the method depicted by Zarkadas et al. (2007) was adopted. Briefly, individual rice samples (100 mg) was mixed with 6 N HCL (5 ml) and heated (at 110°C for 24 h in an oven). The hydrosylates obtained were mixed with 50 µmol/ml of L-α-amino-n-butyric acid as internal standard (400 µl; AABA). Further, this mixture was made-up with distilled deionized water (100 ml), followed by filtering by using Whatman No 1 filter paper.

Amino acid derivation was undertaken by using 10 µL of sample aliquot and 20 µL AccQ reagent (6-aminoquinolyl-N-hydroxysuccinimidyl carbamate) before being injected into the HPLC column. Analysis was performed using Waters-HPLC System (Milford, MA, U.S.A.) consisting of Waters 1525 binary HPLC pump, 717 plus auto sampler, 2475 multi λ fluorescence detector; wavelength excitation 250 nm, emission 395 nm and a AccQ TagTM amino acid analysis column (3.9 mm x 150 mm; packing material: silica based bonded with C18), which were all based according to the AccQ Tag TM method. The column was maintained at 37 °C and the eluent (flowed at a rate of 1 ml/min.). Chromatographic peaks were plotted and evaluated (using Breeze TM Workstation version 3.20), with two different eluents used (AccQ Tag TM concentrate and Acetonitrile/Water, 60/ 40%). Amino acids standard mixture was prepared using commercial mixture of standard H (Pierce Chemical, U.S.) which was used as a reference to calibrate the HPLC-system. Besides, methionine and cysteine were determined independently based on the performic acid method.

**Minerals content**

Mineral analysis was carried out based on the method described by Hooda et al. (2006) with slight modifications. Initially, 6 ml of concentrated HNO₃ and 1 ml of 30% (v/v) H₂O₂, which were added to 1 g, sample in a digestion flask. The sample and reagent were left to digest for 15 min using a microwave digester (Milestone Ethos 900 microwave lab station, Sorisole, Italy). The digested samples were then transferred to a 500 ml volumetric flask duplicate. The mineral analysis for potassium, calcium, magnesium, sodium, chromium, iron, manganese and zinc was conducted using atomic absorption spectrophotometer (Perkin Elmer, Waltham, Massachusetts, USA).

**Heavy metals**

Heavy metals in the rice samples were evaluated based on the standard method of AOAC (2000). In brief, one gram of rice sample was weighed individually in a conical flask followed by addition of nitric acid (10 ml; HNO₃) and the mixture was subjected to digestion. This mixture was heated on a hot plate (temperature of 70°C for 20 min.), followed by addition of 5 ml of perchloric acid (HClO₄). The mixture was allowed to boil until evaporation and once a clear solution of about 3 ml was obtained, this was removed and cooled. For determining the arsenic content, 5 ml of concentrated sulphuric acid (H₂SO₄) was added along with HNO₃ and HClO₄. The digested samples were transferred into a volumetric flask (100 ml) and were made up to the mark using distilled deionized water. After this, the contents were filtered, cooled and used for analysis. Heavy metals such as Selenium, cadmium, copper, nickel, mercury, lead and arsenic were determined using flame atomic absorption spectrophotometer.

**Fatty acids profile**

Fatty acids profile or the fatty acid methyl esters (FAMES) were determined according to the method described by Firestone (1995) with slight modifications. Approximately, 1 g of rice powdered sample was weighed in a centrifuge tube (50 ml). Before being homogenized at 8000 rpm for 30 Sec using a homogenizer (IKAR® T25D, Humboldtstraf, Königswinter, Germany), 7 ml methanol was added to the extract mixture. This was followed by addition of 14 ml of chloroform and the mixture was again homogenized for another 30 seconds. Samples were analysed using Shimadzu Gas-Chromatography (GC-17A) equipped with an integrator system (GC class VP 4.3) and fitted with a DB 20 column. The conditions inside the GC-FID were formulated according to the method described by Celik and Ercisli (2009). The peaks were identified and compared by relating them to recognized standards.

**Total dietary fiber**

Total dietary fiber analysis was performed based on the standard gravimetric dietary fiber method (AOAC Official Method 985.29, 2000). This method involves enzymatic treatment for removal of starch and protein. The analysis is separated into 2 parts: Soluble dietary fiber (SDF) and insoluble dietary fiber.
Ethanol was used to precipitate insoluble dietary fiber before being placed in the muffle furnace. A correction for protein and ash in the residue was done. The total dietary fiber, soluble dietary fiber and insoluble dietary fiber were calculated using the following formula:

\[
\text{Protein} = \frac{\text{Titration value} \times 0.28 \times 6.25}{\text{Weight of sample (g) } \times 100}
\]

\[
\% \text{ IDF} = \frac{\text{Average weight of residue (mg) } - \text{ Protein } - \text{ Ash, Blank} \times 100}{\text{Weight of sample (mg)}}
\]

\[
\% \text{ SDF} = \frac{\text{Average weight of residue (mg) } - \text{ Protein } - \text{ Ash, Blank} \times 100}{\text{Weight of sample (mg)}}
\]

Statistical analysis

Statistical analysis of the results obtained in this study were subjected to One-way analysis of variance (ANOVA) as well as Tukey’s HSD Post-hoc test by using SPSS software (SPSS version 17.0 SPSS Inc., Wacker Drive, Chicago, IL, USA). The level of significance was determined at \( P < 0.05 \).

Results and Discussion

Amino acids content

The estimated protein quality of a rice grain can be determined by measuring their respective amino acids content (Chen et al., 1986). In the present study, sixteen free amino acids were detected in rice samples, based on the retention time of standard mixture (Table 1). Among these, eight of the major essential amino acids were present in all the rice samples analyzed (except for tryptophan). These included: histidine, threonine, valine, methionine, lysine, isoleucine, leucine and phenylalanine. Essential amino acids are required in adequate amounts in the normal human diet, as it cannot be produced inside body. The highest amount of essential amino acid found in all rice samples was phenylalanine, which ranged from 33.83-33.86 g/16 g N. From the results obtained, there was not much difference in the range observed among the six rice samples analyzed. To support our observations, minor variations in the amino acid content especially that of milled rice have been previously reported (Houston et al., 1969; Hamid et al., 2007).

Besides, the eight essential amino acids, a range of various amino acids were present in the rice samples studied. These included the aspartate (11.83-11.91 g/16 g N), histidine (14.63-14.68 g/16 g N), threonine (18.33-18.47 g/16 g N), valine (28.76-28.77 g/16 g N), methionine (29.28-29.29 g/16 g N). Brown rice had the lowest, whereas, white rice had the highest level of aspartate and serine. Apart from this, aspartic and glutamic acids have been reported to influence the taste of rice, particularly the sweetness and the unique ‘umami taste (Kasai et al., 2001). Compared to other cereal varieties, rice normally has high levels of amino acids and protein content (FAO, 1992).

Earlier, Kamara et al. (2010) have reported that concentration of amino acids found in rice grains to appreciably influence the sensory qualities in cooked rice, thus affecting the overall acceptability of a rice variety. Overall, among the local rice varieties, Bario rice was found to be better, whereas among the imported rice varieties basmati rice variety was found to be paramount when amino acids was taken into consideration. According to Kerr (2010) basmati rice contains high amount of amino acids, thus rendering them to be one of the most nutritious rice varieties available in the present market.

Fatty acids profile

To facilitate differentiation between fats and oils as well as to analyze the total fat content in a food substrate, fatty acid methyl ester (FAMEs) analysis are performed. The lipid content plays a pivotal role during processing and cooking, and can have high influence on the eating quality of rice (Zhou et al., 2002). Auto-oxidation of lipid can occur when rice is stored for long time duration, thus affecting the overall quality and odour of the cooked rice (Yasumatsu and Moritaka, 1964).

Results obtained for the fatty acids composition of rice in the present study is provided in Table 3. Our results showed palmitic, oleic and linoleic acid to be present in all the rice samples with the highest being in black rice. These results are comparable with the findings of Hwang et al. (2002) and Cho et al. (2006) who have reported major fatty acids in rice to be: Palmitic, oleic and linoleic acids. Palmitic acid is a saturated fatty acid, whereas oleic acid and linoleic acid are unsaturated fatty acids. Palmitic acid is a saturated fatty acid, whereas oleic acid and linoleic acid are unsaturated fatty acids. In addition to these results, Bario rice showed high amounts of caproic acid at 0.47%.

In this study, the total saturated fatty acid was recorded to be highest in black rice (5.89%) followed by glutinous rice (0.85%). However, for the total unsaturated fatty acids content, black rice had the highest amount (2.03%) followed by brown rice (0.59%). The ratio of unsaturated fatty acid versus saturated fatty acid was recorded to be high in white rice (1.23%) and low in basmati rice (0.20%). Caprylic acid was found only in brown and black
rice varieties. Cis-11-Eicosenoic acid and Erucic acid was present in white and brown rice varieties, respectively. The fatty acid profile of rice grains is noticeably superior when compared to oats, maize, wheat, barley and other cereals. Apart from this, rice grains are reported to have lower levels of saturated fatty acids compared to other staple food or cereals, which renders them to be a healthy and better choice (Cicero and Derosa, 2005; Eshak et al., 2011). In addition, as not much research works have been conducted on the levels of dietary fats, the possibility of its use is opined to be destabilized (Oko et al., 2012).

Minerals content

Minerals are essential component required in the normal diet as it can promote health to an optimum level. Minerals are naturally found in various foodstuffs, and are at higher levels in those, which are grown directly from the soil (e.g. fruits, vegetables, cereals, grains, etc). Results on the mineral content of various rice samples evaluated in the present study are presented in Table 2. Minerals such as calcium (Ca) and iron (Fe) were high in black rice (21.38 mg/100g) and brown rice (0.81 mg/100g). Macronutrients such as sodium (Na), were low in all samples except for the black rice (10.19 mg/100g). The lower concentration level of sodium in rice can be advantageous for those people who require to restrict the intake owing to high hypertension associated problems (Umadevi et al., 2012). Among all the six rice samples analyzed, the dominant macro-mineral detected was potassium (K), which was in the range of 76.12-197.41 mg/100g and magnesium (Mg), which was in the range of 13.49-107.21 mg/100g, respectively. Magnesium and potassium content was found to be high in black (K, 186.54 mg/100g and Mg, 107.21 mg/100g) and brown rice varieties (K, 197.41 mg/100g and Mg, 95.09 mg/100g). Our results are comparable to the observations of Parengam et al. (2010). In addition to these, the essential micro-nutrients such as manganese and zinc were found in appreciable amounts in brown rice (1.93 mg/100g) and black rice (0.29) varieties, respectively. Generally, low level of zinc in polished rice is reported compared to unpolished rice (Brinch-Pedersen et al., 2007). In this study, zinc level was
higher in black rice, and lower in Bario rice. Zinc, being an essential micronutrient is required to be a part of the normal human diet, and its deficiency can lead to a flawed immune system. Above all, it is worthy to mention that nearly one third of the world’s population is affected by zinc deficiency (Hotz and Brown, 2004).

With regard to heavy metals, human intervention (such as use of fertilizers in agriculture, mining, etc) can be the route cause for their accumulation (Bhat et al., 2010; Sukreeyapongse et al., 2010). Since rice is a staple food, growing them in polluted soils can pose serious health risks to humans (Ashifa et al., 2001).

In the present study, selenium a proved antioxidant, were present in acceptable threshold levels (< 0.25 mg/100g for all samples). The cadmium content in all the rice samples were below 0.05 mg/100g and did not exceed the limit set by Codex (1995) which is set at 4 mg/100g. The other heavy metals that were detected present in rice samples analyzed as that of mercury and arsenic were below 0.05 mg/100g. The levels of arsenic content did not exceed the permissible limit based on WHO recommendation, which is set at 10 mg/100g (Rahman et al., 2007). In recent years, mercury is a common element detected in rice grains (Horvat et al., 2003). Presence of low level of mercury can be of help to local populations, as mercury toxicity is known to lead to kidney and gastrointestinal damages (permissible limit set by WHO is 10 mg/100g) (Brandt, 2005). Overall, the rice samples evaluated in this study contained low amount of heavy metals and can still be considered safe for human consumption.

**Dietary fiber (Soluble/Insoluble)**

Dietary fiber encompasses those fractions of food which are incapable of enzymatically degraded in the digestive tract of human. Rice bran has been reported to encompass high amount of dietary fiber and are routinely being added during preparation of healthy snacks, breakfast cereals, bakery products, etc (Garcia et al., 2012, Dakhara et al., 2012). Among the dietary fibers, insoluble fiber is reported to be beneficial to overcome constipation problems, whereas soluble fibers can be of help to minimize low-density lipoproteins or cholesterol in the blood. The American Dietetic Association recommends incorporating fresh produce (vegetables and fruits) as well as grains in the daily human diet for health reasons (National Research Council, 1989).

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Table 2. Minerals and heavy metal content in different rice varieties evaluated in the study (mg/100 g; n =3 ± standard deviation)

<table>
<thead>
<tr>
<th>Minerals</th>
<th>Local Rice</th>
<th>Imported Rice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>White</td>
<td>Brown</td>
</tr>
<tr>
<td>K</td>
<td>84.02</td>
<td>197.41</td>
</tr>
<tr>
<td>Mg</td>
<td>13.49</td>
<td>95.09</td>
</tr>
<tr>
<td>Na</td>
<td>6.11</td>
<td>6.61</td>
</tr>
<tr>
<td>Cr</td>
<td>&lt;0.25</td>
<td>&lt;0.25</td>
</tr>
<tr>
<td>Fe</td>
<td>0.54</td>
<td>0.81</td>
</tr>
<tr>
<td>Mn</td>
<td>0.81</td>
<td>1.93</td>
</tr>
<tr>
<td>Zn</td>
<td>0.16</td>
<td>0.19</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Heavy Metals</th>
<th>Local Rice</th>
<th>Imported Rice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>White</td>
<td>Brown</td>
</tr>
<tr>
<td>Se</td>
<td>&lt;0.25</td>
<td>&lt;0.25</td>
</tr>
<tr>
<td>Cd</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Cu</td>
<td>0.54</td>
<td>0.58</td>
</tr>
<tr>
<td>Ni</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Hg</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Pb</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>As</td>
<td>&lt; 0.05</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>
Results on soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) in various rice varieties investigated in this study is shown in Table 4. From the results, IDF was recorded to be higher than SDF in brown, black and basmati rice varieties. However, the white, Bario and glutinous rice had higher amounts of SDF. The highest amount of SDF and IDF was observed in white rice (16.39%) and brown rice (16.51%), respectively. This is in agreement with the American Heart Association (1988) guidelines, wherein it states that brown rice has higher percentage of insoluble dietary fiber compared to other rice samples. White rice on the other hand encompasses high amounts of soluble fiber (Davidson, 2007). According to Wolters (2011), by comparing crude and dietary fiber contents, it is ensured that the rice has low crude fiber content (as it dissolves all soluble fiber and some insoluble fiber).

In addition, an excessive amount of dietary fiber is not recommended as it can diminish the absorption of vital vitamins and essential minerals (Saibil, 1989).

Conclusions

Investigations conducted on six different rice varieties (local and imported, marketed in Penang region, Malaysia) did not show much differences in amino acids composition. Rice varieties analyzed had adequate amounts of essential minerals, with low levels of heavy metal contaminants. The fatty acid composition of rice varieties showed a wider range in relation to saturated and unsaturated fatty acid contents. Significant differences existed between the total, soluble and insoluble dietary fibers in the rice varieties studied. Overall, from the results generated, understanding the composition of local rice varieties
Table 4. Dietary fiber content in different rice varieties investigated in this study (n= 3 ± standard deviation)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Soluble Dietary Fiber (SDF) (%)</th>
<th>Insoluble Dietary Fiber (IDF) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>16.39 ± 0.07^a</td>
<td>4.17 ± 0.05^b</td>
</tr>
<tr>
<td>Brown</td>
<td>8.35 ± 0.04</td>
<td>16.51 ± 0.26</td>
</tr>
<tr>
<td>Bario</td>
<td>14.42 ± 0.02^a</td>
<td>27.09 ± 0.03^c</td>
</tr>
<tr>
<td>Black</td>
<td>8.17 ± 0.07</td>
<td>14.39 ± 0.07^d</td>
</tr>
<tr>
<td>Glutinous</td>
<td>15.91 ± 0.07^b</td>
<td>5.09 ± 0.08</td>
</tr>
<tr>
<td>Basmati</td>
<td>14.48 ± 0.05^a</td>
<td>4.14 ± 0.16^e</td>
</tr>
</tbody>
</table>

Same letter superscripted in the same column are not significantly different from each other at P ≤ 0.05.

is essential as it is expected to aid in providing details on the nutritional benefits for local populations as well as be of help to local industries while producing novel rice based healthy food products.

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