Study of Glutinous and Non-Glutinous Rice (Oryza Sativa) Varieties on Their Antioxidant Compounds

Widiastuti Setyaningsih, Nikmatul Hidayah, Irfan Estiono Saputro, Miguel Palma Lovillo, and Carmelo García Barroso

Abstract—Cultivated rice (Oryza sativa) is a global cereal crop in Southeast Asia. It serves as staple food, thus has a major contribution to the calorie intake. Furthermore, a number of antioxidant substances have been identified in rice including phenolic compounds and melatonin. The concentration and composition of these antioxidant compounds were studied on glutinous and non-glutinous grains for both pigmented and non-pigmented rice varieties. Additionally, several medium-amylose rice grains of three Indonesian varieties (IR64, umbul-umbul and pandan wangi) were also evaluated. Finding indicates that the composition of phenolic compounds are noticeably different between glutinous and non-glutinous rice grains. The level of both melatonin and total phenolics in non-glutinous rice was higher than its glutinous variety. Hence, higher amylose content exhibits relatively higher amount of antioxidant compounds.

Keywords—Rice, amylose, glutinous, antioxidants

I. INTRODUCTION

Rice (Oryza sativa) is a pivotal cereal crop as subsists calories for Southeast Asian livelihoods. This grain is remarkably essential for the nutrition status of a large number of the population within this region. In addition to nutrition purposes, rice contains secondary metabolites that are possessing several antioxidant compounds namely melatonin [1] and phenolic compounds [2]. These compounds attracted a growing attention in recent years due to their health benefits, as their consumption has been linked to lowering the risk of diseases related with oxidative stress i.e. inhibitory effects on carcinogenesis and mutagenesis [3], [4]. Recent research has led to an increase in the production of antioxidant compounds in transgenic rice seeds and antioxidant-rich rice plants have been successfully generated. Functional rice products have also become more widely appreciated in the current market.

The phenolics compounds described in rice grains are phenolic acids [5] and its aldehydes [6]. The most common forms of phenolic acids in rice are derived from hydroxybenzoic (C₆–C₁; an aromatic ring linked to a carbon atom) and hydroxycinnamic (C₆–C₃; an aromatic ring linked to a three-carbon chain) acids. Along with these two major groups, aldehyde analogues such as protocatechuicaldehyde, p-hydroxybenzaldehyde [7] and vanillin [6], were also found in rice grains.

As well as phenolic compounds, the presence of melatonin [N-acetyl-3-(2-aminoethyl)-3-methoxyindole] in rice grain has been identified in a wide range of varieties [8]. However, both the level and composition of these compounds in different varieties of rice grains may diverge in phenolics and melatonin concentrations. This discrepancy reveals that one would also expect changes due to the differences on their type of starch that have been distinguished as glutinous (waxy) and non-glutinous variety.

Glutinous differs from the regular (non-glutinous) rice mainly in having low (<5%) or almost no amylose in its starch but basically high in amylopectin [9]. Amylose is essentially long linear chains composed of (1→4)-linked α-D-glucopyranosyl units with a few (1→6)-α-linkages branches whilst amylopectin has a higher molecular weight and much shorter chains of (1→4)-linked α-D-glucose units that are highly branched through additional (1→6)-α-linkages (Fig. 1). In general, the amylose content of rice starch varies from 0-2% in waxy (glutinous), 20-25% in normal or medium and up to 30% in high-amylose rice grains.

It has been reported higher production of antioxidant compounds from high-amylose genotypes versus the waxy (glutinous) ones for corn (Zea mays L.) grains [10], further work will be necessary to study the level and composition of antioxidants compounds in other foods, including rice. Hence, the objective of the study described here was to assess the levels of antioxidant compounds, including melatonin and phenolics on some rice varieties taking into account their amylose content.
II. MATERIALS AND METHODS

A. Materials and Chemicals

HPLC-grade methanol (MeOH), acetic acid, and ethyl acetate (EtOAc) were purchased from Merck (Darmstadt, Germany). Melatonin (MEL) standard, M-5250, was obtained from Sigma-Aldrich (St. Louis, MO, USA). Water was purified with a Milli-Q purification system (Millipore, Billerica, MA, USA). Phenolic compound standards of the highest available purity were used. Protocatechuic acid (PRO), protocatechuic aldehyde (PRA), vanillic acid (VAA), p-hydroxybenzoic acid (p-HBA), vanillin (VAN), p-hydroxybenzaldehyde (p-HB), ferulic acid (FER) and sinapic acid (SIN) were obtained from Fluka (Buchs, Switzerland). Guaiacol (GUA), p-Coumaric acid (p-COU), caffeic acid (CAF), chlorogenic acid (CHL), 5-hydroxymethyl-2-furaldehyde (HMF), and 5-methylfurfural (MF) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Ellagic acid (ELL) and iso-vanillic acid (IVA) were purchased from Sarsynthese (Merignac, France).

B. Rice Samples

Rice samples including non-pigmented and black pigmented of both glutinous and non-glutinous rice grains were obtained from an Asian market in Spain. Three varieties of Indonesian medium-amylose rice produced using conventional farming systems were obtained randomly from various smallholder rice mills in Central Java area (umbul-umbul, pandan wangi and IR64).

C. Sample Preparation

A rice sample (20 g) was placed in a plastic cylinder and rice grains were milled with an Ultraturrax homogenizer (IKA® T25 Digital, Germany) for 10 min prior to extraction. The milling process was stopped every 1 min to avoid excessive heating of the sample. The fine grain was then homogenized by stirring and then stored in a closed labelled bottle.

D. Extraction Technique

Phenolic compounds and melatonin extractions were performed in a Dionex ASE 200 extractor (Dionex, Sunnyvale, CA, USA) equipped with incorporating stainless steel extraction cells (11 mL volume) and collection vials (60 mL capacity). A cellulose filter was inserted at the outlet end of the extraction cell.

An accurately weighed sample of rice powder (2.5 g) and washed sea sand were loaded into the extraction cell. The Pressurized Liquid Extraction (PLE) condition for phenolic extractions was 60% (v/v) EtOAc in MeOH, static time of 10 min in 3 cycles at 190 °C under a pressure of 200atm, flushing 100% and purge for 60 s; while for melatonin extraction was 70% (v/v) EtOAc in MeOH, static time of 5 min in 2 cycles at 200 °C under a pressure of 200atm, flushing 50% and purge for 60 s. The resulting extract was dried under vacuum on a rotary evaporator. The residue was reconstituted with methanol and adjusted to a final volume of 5 mL. The liquid was then passed through a 0.45 μm nylon filter prior to injection on the HPLC-FD system.

E. Determination of Phenolic Compounds

High performance liquid chromatography (HPLC) analyses were carried out on Dionex HPLC system comprised a Dionex P680 HPLC Pump, Dionex ASI 101 Automated Sample Injector, Dionex PDA-100 Photodiode Array Detector, Dionex UCI-50 Universal Chromatography Interface, and Dionex TCC-100 Thermostatic Column Compartment. Separations were performed on a reversed phase RP 18 Lichrospher Column (LiChroCART 250 × 4 (5μm) Merck KgaA). Gradient elution was carried out at a flow rate of 1.0 mL min⁻¹. A PDA-100 Photodiode Array Detector was used for UV-Vis and the 3D mode was set at collection rate of 1.0 Hz, 3D wavelength scan range 250-600 nm, 3D bunch width 1 nm and band width 50 nm. The column compartment thermostat was set at 25°C. Injection volume was set to 25 μL.

A gradient elution program was used with two mobile phases: A (2% acetic acid and 5% MeOH in water) and B (2% acetic acid and 88% MeOH in water). The mobile phases were filtered through a 0.45 μm membrane filter (Millipore) and were degassed for 15 min prior to use. The gradient applied was as follows: (time, solvent B): 0 min, 0%; 10 min, 25%; 25 min, 40%; 30 min, 50%; 35 min, 50%. The identification of phenolic acids in the samples was achieved by spiking and by comparison of retention times and maximum UV-Vis absorptions with those of standards.

F. Determination of Melatonin

Chromatographic separations were carried out on an Alliance HPLC 2695 system, controlled by an Empower Pro 2002 data station (Waters, Milford, MA) and coupled with a fluorescence detector (Waters 474 Fluorescence Detector). The column separation and mobile phase preparation were similar with phenolics determination. The applied gradient was as follows: (time, solvent B): 0 min, 0%; 5 min, 35%; 12 min, 40%; 15 min, 40%; 20 min, 45%; 25 min, 50%. The established conditions for fluorescence detector were as follows: excitation wavelength was set at 290 nm, emission wavelength was set at 330 nm, sensitivity was set at gain 1000, attenuation was fixed at 16, and injection volume was set to 10 μL.
G. Determination of Amylose Content

Amylose content of the samples was determined by Iodometric method based on official methods of analysis of AOAC. In summary, 100 mg of sample were weighed and mixed with 1 mL 95% ethanol and 9 mL of 1N NaOH. The samples were diluted and the iodine solution was added. After 10 min incubation at room temperature, the absorbance at 625 nm was analysed with a spectrophotometer and the amylose content was calculated based on the standard curve. The samples were analysed in triplicate.

H. Performance of the Method

The analytical procedures for the chromatographic methods for melatonin and phenolic compounds were carried out according to the recommendations of ICH Guideline Q2 (R1) and suggestions in ISO 17025. Linearity, range, precision, detection and quantification limits of the method were established. Linearity was evaluated in order to express the ability of the method to obtain results that are directly proportional to the concentration of melatonin in two different ranges. Appropriate dilution from a stock solution of melatonin was carried out to give concentrations ranging from 0.75 to 15 µg L⁻¹ and 15 to 750 µg L⁻¹. A series of dilution from stock solution of phenolic compounds was carried out to give concentrations from 0.15 to 12 mg L⁻¹. Gnumeric 1.12.17 was used to generate the regression analysis. Calibration curves were obtained from these regression analyses and the melatonin and phenolic compounds in the extracts were quantified. The standard deviation (σ) obtained for the response and the slope (a) from the regression were then used to calculate the limit of detection (LOD) and limit of quantification (LOQ).

The precision of the method was evaluated by performing repeatability (intra-day) and intermediate precision (extra-day). Repeatability was assessed by ten independent analyses of the same samples on the same day while intermediate precision was determined by five independent analyses on three consecutive days. Precision was expressed as coefficient of variance (CV) of retention time and peak signal. The CV values for both repeatability and intermediate precision were less than 6% showing that the methods have adequate precisions.

The analytical properties for the determination of phenolic compounds and melatonin are listed in Table I.

I. Data Analysis

The experimental results in single- and two-factor experiments were analysed using Gnumeric. The Analysis of Variance (ANOVA) and Least Significant Difference (LSD) test were used to determine the significant differences (p < 0.05) between the means.

III. RESULT AND DISCUSSION

A. Phenolics in Glutinous and Non-glutinous Rice

In order to evaluate the phenolic levels and compositions in glutinous and non-glutinous rice, two clusters of rice varieties i.e. non-pigmented and black pigmented rice grains were extracted and analysed in duplicate (Table II). Result shows that the content of phenolic compounds in rice samples with higher amylose content (non-glutinous) were higher than in the glutinous samples for both clusters. The highest concentration of phenolics presented in pigmented-rice was 211.94±18.65 µg g⁻¹ owned by black non-glutinous rice then followed by its glutinous type (209.00±18.39 µg g⁻¹).

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Concentration range</th>
<th>Linear equation</th>
<th>R²</th>
<th>LOD</th>
<th>LOQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRO</td>
<td>0.15 – 12</td>
<td>y = 1.32x - 0.05</td>
<td>0.9997</td>
<td>0.23</td>
<td>0.77</td>
</tr>
<tr>
<td>PRA</td>
<td>0.15 – 12</td>
<td>y = 5.52x + 0.03</td>
<td>0.9999</td>
<td>0.17</td>
<td>0.56</td>
</tr>
<tr>
<td>p-HBA</td>
<td>0.15 – 12</td>
<td>y = 7.43x - 0.23</td>
<td>0.9997</td>
<td>0.22</td>
<td>0.74</td>
</tr>
<tr>
<td>p-HB</td>
<td>0.15 – 12</td>
<td>y = 3.44x - 0.07</td>
<td>0.9998</td>
<td>0.23</td>
<td>0.75</td>
</tr>
<tr>
<td>VAA</td>
<td>0.15 – 12</td>
<td>y = 1.34x + 0.06</td>
<td>0.9996</td>
<td>0.26</td>
<td>0.87</td>
</tr>
<tr>
<td>IVA</td>
<td>0.15 – 12</td>
<td>y = 1.37x + 0.08</td>
<td>0.9994</td>
<td>0.32</td>
<td>1.07</td>
</tr>
<tr>
<td>VAN</td>
<td>0.15 – 12</td>
<td>y = 1.85x - 0.02</td>
<td>0.9998</td>
<td>0.20</td>
<td>0.67</td>
</tr>
<tr>
<td>GUA</td>
<td>0.15 – 12</td>
<td>y = 0.35x + 0.03</td>
<td>0.9993</td>
<td>0.37</td>
<td>1.24</td>
</tr>
<tr>
<td>p-COU</td>
<td>0.15 – 12</td>
<td>y = 7.52x + 0.16</td>
<td>0.9998</td>
<td>0.18</td>
<td>0.61</td>
</tr>
<tr>
<td>FER</td>
<td>0.15 – 12</td>
<td>y = 4.96x - 0.27</td>
<td>0.9996</td>
<td>0.27</td>
<td>0.90</td>
</tr>
<tr>
<td>CAF</td>
<td>0.15 – 12</td>
<td>y = 3.74x - 1.05</td>
<td>0.9963</td>
<td>0.84</td>
<td>2.80</td>
</tr>
<tr>
<td>CHL</td>
<td>0.15 – 12</td>
<td>y = 10.56x - 3.85</td>
<td>0.9916</td>
<td>1.27</td>
<td>4.23</td>
</tr>
<tr>
<td>SIN</td>
<td>0.15 – 12</td>
<td>y = 4.72x - 0.29</td>
<td>0.9966</td>
<td>0.27</td>
<td>0.90</td>
</tr>
<tr>
<td>HMF</td>
<td>0.15 – 12</td>
<td>y = 24.87x - 1.95</td>
<td>0.9998</td>
<td>0.18</td>
<td>0.61</td>
</tr>
<tr>
<td>MF</td>
<td>0.15 – 12</td>
<td>y = 0.32x + 0.28</td>
<td>0.9954</td>
<td>0.94</td>
<td>3.12</td>
</tr>
<tr>
<td>ELL</td>
<td>0.15 – 10</td>
<td>y = 1.35x + 0.12</td>
<td>0.9827</td>
<td>1.59</td>
<td>4.83</td>
</tr>
<tr>
<td>Melatonin</td>
<td>0.75 to 15</td>
<td>y = 580.79x - 1806.8</td>
<td>0.9957</td>
<td>1.15</td>
<td>3.84</td>
</tr>
</tbody>
</table>

TABLE I. ANALYTICAL CHARACTERISTICS FOR THE DETERMINATION OF MELATONIN AND PHENOLIC COMPOUNDS

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The difference of phenolics content between black glutinous and black non-glutinous rice was not as impressive as if compared to the non-pigmented rice. Thus, in this particular case, phenolics concentration in rice appears to be strongly related to the pigment of rice in their bran. Both black pigmented glutinous and non-glutinous rice grains were produced without a bran removal process called polishing. Hence, as the phenolic compounds are mainly associated with the pericarp in the grain, the milling process to produce polished grain reduces the level of these compounds in the grain.

In contrary, the levels of phenolics in non-pigmented (polished) grains were prominently remarkable for different amylose content: 35.58±3.13 µg g⁻¹ for non-glutinous rice whilst 16.15±1.42 µg g⁻¹ for glutinous rice. The level of phenolics in non-glutinous was roughly two folds higher than its glutinous type. This finding agrees the result revealed by Chi et al. [11] who conducted a research related to phenolics analysis of Korean rice grain varieties. These researchers confirmed that polished non-glutinous rice (Ilpumbye) extract has a higher level of phenolics than the glutinous type (Hwasunchalbyo).

B. Melatonin in Glutinous and Non-glutinous Rice

The level of melatonin in rice with different amount of amylose content was studied on the same sample used in previous experiment of phenolics. However, unlike the result for phenolics, the difference in melatonin contents between black glutinous (182.04±2.79 µg Kg⁻¹) and non-glutinous rice (73.81±1.13 µg Kg⁻¹) was notably more significant. The level of melanin in black non-glutinous rice was more than double than in its glutinous type.

This variation is reasonable when taking into consideration the specific type of carbohydrates in rice support the synthesis of the neurotransmitter serotonin, which is later transformed into melatonin [12]. Thus, the presence of lower amounts of starch may not enhance the synthesis of melatonin.

C. Phenolics and Melatonin in Medium-Amylose Rice

Three rice varieties from conventional farming were used in this study. In addition to being considered as aromatic rice, together with umbul-umbul, pandan wanggi represents the local rice varieties of Indonesia. IR64 is a rice variety that was developed in a major advance in rice production as it provided higher yield potential, greater yield stability and more efficient management practices. Amylose content in these tested rice varieties was determined (Fig. 2). Subsequently, in order to study the effect of amylose content on the level of antioxidant compounds including phenolic and melatonin, a single-factor ANOVA has been used (p<0.05). ANOVA revealed that the amylose content has significant effect on the level of antioxidant compounds for both phenolics and melatonin. It is, therefore, Least Significant Differences (LSDs) for these factors were also estimated (Fig. 2).

The levels of both phenolics and melatonin for the three tested varieties were directly corresponded to the amount of amylose content wherein the higher the amylose content, the higher the amount of antioxidant compounds. This finding is consistent with that reported by Li et al.[10], who found that plant samples with high amylose contents exhibit better antioxidant activity than their glutinous genotypes. Despite significant research effort, the specific antioxidant enhanced by amylose has not been investigated further and phenolics as well as melatonin are considered as a potential molecule that has a high antioxidant activity [13].

IV. CONCLUSION

Antioxidant compounds in glutinous and non-glutinous rice were studied. The results suggest a positive correlation between the amylose content and the level of antioxidant compounds i.e. melatonin and phenolic compounds. However, particularly in pigmented variety, rice bran contributes greater effect on the level of phenolics in rice grain than the amylose content.

<table>
<thead>
<tr>
<th>TABLE II. PHENOLIC COMPOUNDS IN TESTED SAMPLES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cluster</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>Pigmented Rice</td>
</tr>
<tr>
<td>White Non-glutinous (Medium Amylose Content)</td>
</tr>
<tr>
<td>Non-pigmented Rice</td>
</tr>
<tr>
<td>White Glutinous (Low Amylose Content)</td>
</tr>
</tbody>
</table>

* ND: Not Detected (< LOD)
Fig. 2 Melatonin (1) and phenolics (2) levels in medium-amylose rice varieties. LSD: bars followed by the same letter are indicated as not significantly different \( (p = 0.05) \) conclusion.

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REFERENCES


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Professor García co-founded the Spanish Society for Wine Research (GIENOL) and was elected President of this Society in 1999. He is responsible for the postgraduate program in Agrifood Sciences, a common teaching program between UCA and the University of Cordoba (Spain) since 2006. In 2001 he became Chairman of the Andalusian Center for Wine Research, a research center at UCA sponsored by the local Andalusian Government.