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Original Article

Metabolite profiling, antioxidant, and α-glucosidase inhibitory activities of germinated rice: nuclear-magnetic-resonance-based metabolomics study

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ABSTRACT

In an attempt to profile the metabolites of three different varieties of germinated rice, specifically black (GBR), red, and white rice, a ^{1}H-nuclear-magnetic-resonance-based metabolomics approach was conducted. Multivariate data analysis was applied to discriminate between the three different varieties using a partial least squares discriminant analysis (PLS-DA) model. The PLS model was used to evaluate the relationship between chemicals and biological activities of germinated rice. The PLS-DA score plot exhibited a noticeable separation between the three rice varieties into three clusters by PC1 and PC2. The PLS model indicated that α-linolenic acid, γ-oryzanol, α-tocopherol, γ-aminobutyric acid, 3-hydroxybutyric acid, fumaric acid, fatty acids, threonine, tryptophan, and vanillic acid were significantly correlated with the higher bioactivities demonstrated by GBR that was extracted in 100% ethanol. Subsequently, the proposed biosynthetic pathway analysis revealed that the increased quantities of secondary metabolites found in GBR may contribute to its nutritional value and health benefits.

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1. Introduction

Rice (*Oryza sativa* L.) is the most popular cereal crop, and more than half of the global population consumes it as a staple food. While white rice or common rice is consumed most often, there are also special rice varieties where the rice grain contains pigmented substances with colors such as red, purple, brown, or black. Recently, the germination process for cereal grains, in particular rice, has become a new research interest. Germinated rice (GR) is produced by soaking rice grains in water and allowing them to bud. During the germination process, endogenous hydrolytic enzymes are activated to break down starch, fibers, and proteins, which results in the modification of nutritional and physiological properties as well as textural characteristics [1–3]. Furthermore, GR has many beneficial pharmacological properties including use as antihyperlipidemic and antihypertensive agents and the capability of reducing the risk of some chronic diseases, such as diabetes, cancer, cardiovascular diseases, and Alzheimer's disease [4]. Moreover, pigmented rice has been reported to possess a potent antioxidant activity [5,6].

The benefits and values of consuming functional or nutraceutical foods on human health are prominent areas of research in the food science field. Several studies have reported that pigmented rice is a potent source of bioactive compounds, such as phenolic compounds, vitamins, and minerals [7–9]. Germinated rice is considered an important source of γ-aminobutyric acid (GABA), a nonprotein amino acid with significant biological activity. In addition, a previous study reported that γ-oryzanol, tocopherols, and tocotrienols are major bioactive compounds in GR [2]. These metabolites can differ from one variety to another. To date, some studies have profiled metabolites in GR using analytical methods [2–4]. Recent studies have demonstrated the successful isolation and elucidation of metabolites from rice using different analytical techniques [10–12]. Therefore, these findings necessitate the need for a comparison of the metabolite constituents in the other varieties of GR extract (GRE).

Nuclear magnetic resonance (NMR) is a powerful technique used to elucidate structural information and isolate the metabolites within complex systems. NMR spectrometry offers several advantages such as rapidity, reproducibility, and simplicity of sample preparation [13]. Recently, NMR techniques and multivariate data analysis (MVA) have been widely used for metabolite profiling and for the determination of differences between samples [14–17]. In addition, NMR spectrometry was also used to identify and characterize the composition of various types of plants, foods, and tissues [18–21].

In the present study, three varieties of GR were extracted using different solvents. The variability in the chemical composition and discrimination among the different GREs was elucidated using a combination of NMR-based metabolomics and compared with nongerminated rice (NGR). The correlation between metabolites and biological activities, including 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging and α-glucosidase inhibitory activities were also examined. The information gathered may help more efficiently in the use of GREs in medicinal products and foods.

2. Materials and methods

2.1. Chemicals

Absolute ethanol, sodium carbonate, Folin–Ciocalteu reagent, gallic acid, phosphate buffer, deuterated methanol-*d4* (CH_3OH-d_4), nondeuterated potassium dihydrogen phosphate (KH_2PO_4), deuterium oxide (D_2O), and trimethylsilyl propionic acid-*d4* sodium salt (TSP) were purchased from Merck (Darmstadt, Germany). The other chemicals, including p-nitrophenyl-α-D-glucopyranose (PNPG), glycine, α-glucosidase enzyme, and DPPH were supplied by Sigma–Aldrich (Germany).

2.2. Plant materials

Three seeds of paddy rice (*O. sativa* L.) varieties were obtained from the Organic Agriculture Project, Sukhothai Airport, Thailand. The paddy rice samples included *O. sativa* L. cv. Hom Mali 105 (WR), *O. sativa* L. cv. Hom Deang Sukhothai 1 (RR), and *O. sativa* L. cv. Hom Dam Sukhothai 2 (BR). The samples were packed in an aluminum foil bag and stored at 4°C in the refrigerator until use.

2.3. Germination process and sample preparation

Paddy rice samples (500 g) were soaked in water at room temperature at a ratio of 1:5 (w/v). The water was changed every 8 hours and decanted after 24 hours. Paddy rice samples were distributed on double layers of cotton cloth and placed in five plastic baskets to give the five biological replicates and allowed to germinate at room temperature for 7 days. GR samples were collected and dried for 24 hours in an oven at 45°C. Samples from each basket were randomly collected for the extraction with both solvent ratios. The dried samples were ground using a grinder to obtain a fine powder. The ground samples were packed in polyethylene bags and stored in a chiller at 4°C until use. The NGR was used as a control.

2.4. Extraction

Five grams of ground GR and NGR (from three varieties) were immersed in 50 mL 70% or 100% ethanol. The mixtures were sonicated in an ultrasonic bath at room temperature for 1 hour and then filtered through filter paper (Whatman No.1). The residues were re-extracted with 50 mL of the same solvent under the same conditions for sonication, and the solutions of each extract were combined. The extraction solvents were removed under vacuum using rotary evaporation at 40°C. The extracts were stored in a freezer at −20°C for further analysis.

2.5. DPPH radical scavenging

DPPH radical scavenging activity was investigated using a 96-well microplate as previously described [22]. A 50-μL aliquot of each of the tested samples was put into a well, followed by the addition of 100 μL DPPH (80 mg/L). The mixtures were then left in the dark to incubate for 30 minutes. The absorbance at 517 nm was measured using a microplate reader

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