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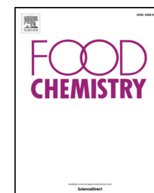

Comparative proteomics analysis reveals the effect of germination and selenium enrichment on the quality of brown rice during storage

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ABSTRACT

Selenium (Se) can delay the quality deterioration of rice during storage, however, the mechanisms remain unclear. In this study, changes in the quality of brown rice, germinated brown rice, and selenized germinated brown rice stored at 38 °C and 90% relative humidity with various vacuum levels for 120 or 150 days were investigated. Fatty acid value and carbonyl value were determined every 30 days. Comparative proteomics technology was applied to determine the mechanisms of germination and Se enrichment on the storage quality of rice. Approximately 142 abundance changed proteins were found, of which 37 proteins were identified. By functional classification, proteins involved in processes of carbohydrate metabolism, lipid metabolism, oxidation-reduction, and protein catabolism may contribute to the different storage qualities of three samples. This study provides novel insights into Se response in rice at the proteome level, which are expected to be beneficial for exploring Se response tracts in rice.

1. Introduction

Inorganic selenium (Se) is highly toxic to organisms, even at a very low concentration. Brown rice can accumulate inorganic Se from external media during germination and mainly transform into organic Se, which is distributed in Se-containing proteins (Liu & Gu, 2008). Organic Se species that enter the food chain via foods is thought to be the most bioavailable form for humans (Reeves et al., 2007), which can be obtained through foods. As a crucial nutrient in humans, Se was shown to reduce the risk of cancers (Rayman, 2008) at a beneficial dietary range (55–200 μg/g dry food) (Rayman, 2000). It has been estimated that up to 1 billion people worldwide have insufficient dietary intake of Se, which is a serious health threat (Wang, Wang, Ngai, & Wong, 2013). Rice grains are vital components of human diet and a major source of Se (Williams et al., 2009). However, Se concentration in rice grains is generally lower compared to that in other crops (Zhang, Shi, & Wang, 2006). Thus, Se deficiency status can be improved by optimizing Se levels in rice. Se-enriched germinated brown rice (Se-GBR) may meet the human demand for Se.

Additionally, Se is beneficial to plants (Liu, Shang, Feng, Zhang, & Wu, 2015) and plays positive roles in improving plant photooxidative stress tolerance (Pedrero, Madrid, Hartikainen, & Cámara, 2008). Plant growth, chlorophyll contents, and photosynthetic performance, as well as nitrogen contents, of tobacco plants were increased when plants were grown in media containing Se (Liu et al., 2015). Se actively maintains the high quality and delays the oxidative rancidity of rice during long-term storage at different temperatures (Li, Liu, & Chen, 2016). Most studies examined the importance of Se by evaluating physiological and biochemical properties, whereas Se metabolism and its regulatory mechanisms in stored rice grains remain unclear. Therefore, to understand the mechanisms underlying Se enrichment in rice grains during long-term storage under controlled conditions, future studies should examine proteome changes in rice grains.

Quantitative proteomics is an efficient approach in many fields of research (Sultan, Frisvad, Andersen, Svensson, & Finnie, 2017). As the workhorse of proteomic studies, two-dimensional gel electrophoresis (2-DE) with immobilized pH gradients (IPGs) reveals a map of intact proteins, which reflects changes at the protein expression level, isoforms, or post-translational modifications and enables the separation of complex mixtures of proteins according to their isoelectric point (pI) and molecular weight (Mw) (Jorrín-Novo et al., 2009). Mass spectrometry (MS) combined with liquid chromatography–mass spectrometry-based quantification methods, which can identify proteins with different pI and Mw, is commonly used to identify and quantify peptides in mixtures of varying complexity (Vissers, Langridge, & Aerts, 2007). Therefore, we employed a combination of these methods for

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complementary protein identification and achieve comprehensive proteome coverage.

In this study, the fatty acid value (FAV) and carbonyl value (CV) of brown rice (BR), germinated brown rice (GBR), and Se-GBR stored under controlled oxygen conditions were investigated, as rice is highly perishable and quality is decreased by oxidation and hydrolysis (Jaisut, Prachayawarakorn, Varanyanond, Tungtrakul, & Soponronnarit, 2009; Wang, Wang, Jing, & Zhang, 2012). Oxygen is the main factor adversely affecting oxidation reactions. In addition, a comprehensive proteomic study of germinated and Se responses in BR, GBR, and Se-GBR was carried out to determine the mechanisms of the effect of germination and Se enrichment on long-term stored rice.

2. Materials and methods

2.1. Samples and preparations

BR was hulled directly from rough rice of Lianjing 7. Se-GBR was obtained from BR, which was germinated in sodium selenite (60 μM) at 25 °C for 60 h and dried at 40 °C for 10 h. GBR was prepared using the same process with distilled water (Liu, Chen, Zhao, Gu, & Yang, 2011).

2.2. Storage and sampling

BR, GBR, and Se-GBR were stored at 38 °C and 90% relative humidity for 150 days. Vacuum levels were controlled at different levels (0, 0.5, 0.7, and 0.9 kg/cm^2, respectively). Samples were randomly selected from each groups every 30 days and stored at −60 °C until analysis. However, BR samples stored at 0 kg/cm^2 for 150 days were not studied, as they started to mildew after 120 days of storage.

2.3. Determination of oxidative stability

2.3.1. FAV determination

FAV was determined titrimetrically according to the AACC method 02-01A (2000) with slight modifications. Ten grams of sample were placed in 50 mL of petroleum ether for 10 min at 25 °C on an orbital shaker. After filtering, 20 mL of filtrate was added to 75 mL of ethanol: water (50:50, v/v). The mixture was titrated with KOH and 1% phenolphthalein was used as an indicator.

2.3.2. CV determination

The method for CV determination has been described in AOAC (1995). Extractable fat was determined in each rice sample on a Soxhlet apparatus by extraction with diethyl ether for 10 h, which was removed with a rotary vacuum evaporator. The dried residue was redissolved in 25 mL of benzene, which was refluxed for 1 h to dislodge carbonyl elements in the solvent. The detailed was described by Li et al. (2016).

2.4. Proteomic analysis of BR, GBR, and Se-GBR

2.4.1. Total protein extraction

Total proteins were extracted from BR, GBR, and Se-GBR by a modified phenol method as previously described (Hurkman & Tanaka, 1986). Rice material was ground in liquid nitrogen using a pre-chilled pestle and mortar. Approximately 10 mL extraction buffer (0.7 M sucrose, 0.5 M Tris-HCl pH 7.5, 50 m EDTA, 0.1 M KCl, and 2% DTT) and 10 mL saturated phenol solution were added. The mixtures were placed on a laboratory shaker for 10 min at 4 °C and centrifuged for 30 min (10,000 × g, 4 °C). The upper phenol phase was transferred to fresh test tubes. Approximately 10 mL of extraction buffer was added and the procedure described above was repeated. Re-centrifugation of the phenol phase mixture with 0.1 M ammonium acetate-methanol at −20 °C for 24 h was conducted for precipitation. The mixture was centrifuged for 30 min (10,000 × g, 4 °C) and the dry protein pellet was washed twice with 5 mL methanol. After centrifugation for 30 min (10,000 × g, 4 °C), the dry protein pellet washed twice with 5 mL acetone as described above. The pellet was freeze-dried and dissolved in rehydration buffer containing 7 M urea, 2 M thiourea, 2% CHAPS, 0.5% IPG buffer pH 4–7 NL (GE Healthcare, Little Chalfont, UK), and 80 mM DTT. The protein concentration reflecting extraction efficacy was quantified based on the Bradford method with bovine serum albumin as a standard (Bradford, 1976).

2.4.2. Protein electrophoretic separation

The proteomes extracted from rice samples were separated by 2-DE as described by Dunn (2004) with slight modifications. Each sample, containing equal amounts of protein dissolved in 200 μL of rehydration buffer, was loaded onto IPG strips with a non-linear pH gradient of 4–7 (GE Healthcare). The proteins were separated according to their pI using an IPGphor III Isoelectric Focusing device (GE Healthcare). Isoelectric focusing was conducted as follows: rehydration 0 V for 14 h; 500 V step for 1 h; gradient 1000 V for 1 h, gradient 8000 V for 3 h; 8000 V step for 5 h. All steps were performed at 20 °C. Prior to second dimension 2-DE, the IPG strips were incubated twice for 15 min on a laboratory rocker with equilibration buffer containing 6 M urea, 75 mM Tris pH 8.8, 30% (v/v) glycerol, 2% (v/v) SDS, 0.002% (w/v) bromophenol blue, 1% (w/v) DTT, and 2.5% (w/v) iodoacetamide. The strips were placed onto a 12.5% denaturing polyacrylamide gel. The second direction (SDS-PAGE) was conducted using the Ettan DALT six electrophoresis system (GE Healthcare) (Laemmli, 1970). The running program was as follows: 2 W/gel for 1 h, 17 W/gel for 4.5 h at 20 °C. To visualize separated proteins, gels were stained with Silver Blue as described by Domżalska et al. (2017). Gels were digitalized, annotated, and analyzed at 200 dpi using an Image Scanner III with Image Master 2D Platinum 7.0 software (GE Healthcare). For spot analysis, data were normalized by expressing abundance as relative volume (% vol) (Ahsan et al., 2008). Among proteins showing the greatest alterations in abundance among BR, GBR, and Se-GBR, those that reproducibly changed by at least 1.5-fold were analyzed by MALDI TOF/TOF.

2.4.3. Protein digestion and MALDI-TOF/TOF analysis

Spots containing proteins of interest were manually cut out and destained at 37 °C by washing with 25% and 50% (v/v) acetonitrile (ACN) in 25 mM NH_4HCO_3 (Shevchenko, Wilm, Vorm, & Mann, 1996). The target proteins were dehydrated in ACN and dried in a vacuum centrifuge for 10 min. Subsequently, they were incubated in trypsin solution (20 ng/μL in 25 mM NH_4HCO_3) for 30 min. Digestion was carried out for 14 h at 37 °C. Next, each peptide mixture was sonicated for 15 min and covered with freshly prepared extract solution. The extracts were evaporated to dryness from a mixture of 50% ACN and 0.1% trifluoroacetic acid, and then added onto the ground steel MALDI target plate. MALDI-TOF/TOF Autoflex speed™ (Bruker Daltonics, Billerica, MA, USA) was utilized to analysis peptide mass spectra. The mass spectrometer was conducted in standard data-dependent acquisition mode with the fragmentation of three of the most intensive precursor ions. The raw data were pre-processed with Data Analysis 4.0 software (Bruker Daltonics) and searched in the UniProt database to identify *Arabidopsis thaliana* homologs. Gene Ontology analysis was also performed to identify the functions and types of target proteins.

2.5. Statistical analysis

All date presented were calculated for three replicates and expressed as the means ± standard deviations. The results were analyzed for statistical significance by using Statistical Analysis System software 8.2 (SAS, Cary, NC, USA).

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