論文の要旨
Summary of the Dissertation

論文題目
Dissertation Title

"Development of Extracting Methods to Improve Nutritional Compounds in Germinated Brown Rice"

"発芽玄米に由来する栄養成分を向上する抽出方法の開発に関する研究**"**

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1. Background

The global demand for rice (*Oryza sativa* L.) with enhanced medicinal and nutritional benefits has been on the rise. Concerns about the association between excessive white rice consumption and the onset of type 2 diabetes and related diseases underscore the need for alternative options. Whole cereal grains, particularly brown rice and germinated brown rice (GBR), have shown potential in reducing diabetes risks due to their rich nutritional content and bioactive compounds. Previous studies suggest that subjecting GBR to abiotic stresses and varied germination conditions enhances its nutritional profile, including increased levels of bioactive compounds and antioxidant properties. While cooking has been reported to influence phenolic and momilactone levels in rice, the correlation remains unclear. Additionally, the optimal extraction methods for valuable bioactive compounds, especially momilactones, from rice have not been extensively explored.

2. Objectives

i. Isolate and identify potential phytochemicals and bioactive compounds from rice husks.
ii. Investigate the presence of phytochemicals and bioactive compounds in GBR, emphasizing their antioxidant, anti-skin aging, anti-diabetes, and anti-cancer properties under stress conditions.
iii. Enhance the extraction process by identifying the optimal combination of extraction method, solvent, and extraction parameters (temperature, time, and solvent-to-sample ratio) to achieve the highest yield of momilactone and phenolic compounds from GBR.

3. Structure of dissertation

Chapter 1. General introduction

Chapter 2. Isolation and quantification of momilactones, tricin, and *ρ*-coumaric acid from rice

Chapter 3. Salinity treatments promote the accumulations of momilactones and phenolic compounds in germinated brown rice

Chapter 4: Potential of germinated brown rice under salinity stress for antioxidant, anti-diabetic, and anti-skin aging activities

Chapter 5: Momilactones and phenolics in brown rice: enrichment, optimized extraction, and potentials for antioxidant and anti-diabetic activities

Chapter 6: General discussion

4. Materials and methods

Isolation and identification

The isolation process for MA, MB, *ρ*-coumaric acid, and tricin were followed the methods described in the previous study (Quan et al. 2019). The identification of isolated tricin, *ρ*-coumaric acid, MA, and MB was confirmed by ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) spectra.

Quantification of phytochemicals

Total phenolic content (TPC) of the GBR extracts was quantified based on the Folin-Ciocalteu method described by Mohammadabadi et al. (2022).

Total flavonoid content (TFC) was quantified following the aluminum chloride colorimetric method described in the research of Bueno-Costa et al. (2016).

The chemical analyses were conducted applying spectrophotometer, gas chromatography-mass spectrometry (GC-MS), and liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) methods.

Biological activity

Antioxidant activities of samples were determined via antiradical (DPPH and ABTS), reducing power, β-carotene bleaching assays were conducted following a method described by (Quan et al. 2019).

Enzymatic assays including α-amylase, α-glucosidase, and tyrosinase inhibitory effects of *A. Virginicus* extracts were evaluated following the method presented by Quan et al. (2019).

5. Results and discussion

In Chapter 2, we successfully isolated and identified momilactones A and B, tricin, and *ρ*-coumaric acid from rice husks. Subsequently, these compounds were identified and quantified in various rice grains, including white rice, brown rice, and germinated brown rice.

Moving on to Chapter 3, the germination of brown rice under different salinity conditions and periods revealed an optimized treatment (B2: 75 mM NaCl and 4-day germination), a first-time discovery, significantly enhancing the accumulation of valuable bioactive compounds, such as tricin, phenolics (*ρ*-coumaric acid, ferulic acid, cinnamic acid, and salicylic acid), momilactones A (MA), and B (MB) in germinated brown rice (GBR, Koshihikari var.). These findings suggest that the proposed germination conditions enhance the nutritional and therapeutic potential of brown rice, showcasing promising applications in functional foods and dietary strategies.

In Chapter 4, investigations into the impact of various exogenous treatments (temperature, salinity, incubation period) during the germination stage of brown rice unveiled noteworthy antioxidant activities and robust inhibitory effects against key enzymes.

For Chapter 5, the extraction process identified the optimal combination of extraction method, solvent, and extraction parameters (temperature, time, and solvent-to-sample ratio) to achieve the highest yield of momilactone and phenolic compounds from germinated brown rice. This pioneering study identified an optimized method for enriching and extracting momilactones A (MA) and B (MB) and phenolic compounds from germinated brown rice (GBR) and non-GBR of Koshihikari and Milky Queen varieties through the cooking process. Specifically, sample GKB4 (cooked Koshihikari GBR, extracted using 80% methanol with 2 h of sonication at RT) exhibited the highest contents of tricin, and phenolic compounds (caffeic acid, *ρ*-hydroxybenzoic acid, *ρ*-coumaric acid, ferulic acid, salicylic acid, and cinnamic acid), closely correlating with the strongest antioxidant activity. On the other hand, sample GKB9 (non-cooked Koshihikari GBR, extracted using 80% ethanol with 2 h of sonication at RT) showed the greatest quantities of MA and MB, consistent with its ability to inhibit α-amylase and α-glucosidase. GKB9, showing efficacy comparable to the diabetes drug acarbose, presents promising avenues for our research to enhance the nutritional value of underappreciated brown rice (BR), aiming to encourage its consumption and foster the development of health-improving rice-derived products.

A general discussion is presented in Chapter 6. Our thorough investigation into momilactones, phenolic compounds, and bioactive components across diverse rice grains, with a particular focus on germinated brown rice (GBR), has yielded valuable insights. The optimized germination conditions represent a pioneering approach to boost the nutritional and therapeutic potential of brown rice, indicating promising applications in the realm of functional foods. Furthermore, our innovative extraction methods, as demonstrated by GKB4 (cooked Koshihikari GBR extracted with 80% methanol and 2-h sonication), and GKB9 (non-cooked Koshihikari GBR extracted with 80% ethanol and 2 h-sonication), open exciting avenues for enhancing momilactones and phenolic compounds, providing opportunities to enhance the nutritional value of underappreciated brown rice and contribute to the development of health-promoting rice-derived products.