Doctoral Dissertation

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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ABSTRACT

The global demand for rice (*Oryza sativa* L.) with enhanced medicinal and nutritional benefits has been increasing. Concerns regarding the link between excessive white rice consumption and the development of type 2 diabetes and related diseases highlight the necessity for alternative options. Whole cereal grains, specifically brown rice and germinated brown rice (GBR), show promise in reducing diabetes risks due to their abundant nutritional content and bioactive compounds. Previous studies indicate that subjecting GBR to abiotic stresses and diverse germination conditions enhances its nutritional profile, including elevated levels of bioactive compounds and antioxidant properties. The correlation between cooking and its impact on phenolic and momilactone levels in rice remains unclear. Moreover, the optimal extraction methods for valuable bioactive compounds, especially momilactones, from rice have not been extensively explored.

This investigation, conducted in a completely randomized design (CRD), aims to determine the effects of different salinity levels (75 and 150 mM) and germination periods (3, 4, and 5 days) on momilactone and phenolic accumulations in germinated brown rice (GBR) var. Koshihikari. The identification of bioactive compounds was confirmed using electrospray ionization-mass spectrometry (ESI-MS) and nuclear magnetic resonance (NMR) spectroscopy (^{1}H and ^{13}C). Momilactone A (MA) and momilactone B (MB) amounts were determined by ultra-performance liquid chromatography–electrospray ionization-mass spectrometry (UPLC–ESI-MS), while other compounds were quantified by spectrophotometry and high-performance liquid chromatography (HPLC). GBR under B2 treatment (75 mM salinity for 4 days) exhibited the greatest total phenolic and flavonoid contents (14.50 mg gallic acid and 11.06 mg rutin equivalents, respectively, per g dry weight). GBR treated with B2 also accumulated the highest quantities of MA, MB, *ρ*-coumaric, ferulic, cinnamic, salicylic acids, and tricin (18.94, 41.00, 93.77, 139.03, 46.05, 596.26, and 107.63 μg/g DW, respectively). These levels were consistent with the strongest antiradical activities in DPPH and ABTS assays (IC_{50} = 1.58 and 1.78 mg/mL, respectively). These findings have implications for promoting the value of GBR consumption and rice-based products that benefit human health.

Furthermore, exploration into the impact of various exogenous treatments (temperature, salinity, and incubation period) during the germination stage of brown rice revealed noteworthy antioxidant activities and robust inhibitory effects against key enzymes. The antioxidant

capacity of germinated brown rice (GBR) extracts was comprehensively evaluated through reducing power (RP) and β-carotene bleaching (βC) assays. Among nine samples, GBR5 (75 mM NaCl and 4 day-germination period) stood out with the lowest IC_{50} value in RP (2.07 mg/mL) and the highest βC assay value (94.56% LPI), showcasing its potential to counteract oxidative stress. In all three tests, GBR5 was the sample that exhibited the strongest inhibitory properties. The inhibitory effects on α-amylase (0.69 mg/mL), α-glucosidase (0.15 mg/mL), and tyrosinase (0.44 mg/mL) revealed that GBR5 had low IC_{50} values, making it a viable option for treating diseases pertaining to skin pigmentation and carbohydrate metabolism. GBR extracts, particularly GBR5, outperformed the control group, highlighting their potential health benefits. GC-MS analysis revealed variations in compound composition, with palmitic acid being the most abundant in GBR5. The study emphasizes the importance of selecting the appropriate GBR sample and recommends further research into specific bioactive compounds to fully comprehend the health-promoting qualities of GBR, especially GBR5, which holds potential as functional foods with medicinal implications.

Moreover, this study also represents the first attempt to enrich MA, MB, and phenolic compounds in GBR and non-GBR var. Koshihikari and Milky Queen through the cooking process. Extraction methods for these compounds were optimized by applying various conditions, including solvents (80% methanol and 80% ethanol), heat (80 °C), and sonication (2 h). Momilactone and phenolic quantities were determined by ultra-performance liquid chromatography–electrospray ionization mass spectrometry (UPLC–ESI-MS) and high-performance liquid chromatography (HPLC), respectively. Cooked Koshihikari GBR extract using 80% methanol and sonication (GKB4) revealed the highest amounts of tricin, caffeic acid, *ρ*-hydroxybenzoic acid, *ρ*-coumaric acid, ferulic acid, salicylic acid, and cinnamic acid (1.71, 1.01, 0.62, 0.45, 0.94, 2.50, and 0.37 mg/g DW, respectively), consistent with the strongest antiradical activities in DPPH and ABTS assays (IC_{50} = 1.47 and 1.70 mg/mL, respectively). Non-cooked GBR Koshihikari extract using 80% ethanol and sonication (GKB9) exhibited the highest MA and MB contents (147.73 and 118.8 μg/g DW, respectively). Notably, GKB9 showed potent inhibition of α-amylase and α-glucosidase (IC_{50} = 0.48 and 0.15 mg/mL, respectively), compared with the anti-diabetic drug acarbose (IC_{50} = 0.26 and 2.48 mg/mL, respectively). The findings hold significant implications for developing phenolics and momilactone-enriched brown rice with health-beneficial properties. 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.

In conclusion, this comprehensive investigation into momilactones, phenolic compounds, and bioactive components in diverse rice grains, particularly GBR, offers valuable insights. The optimized germination conditions and extraction methods open exciting avenues for enhancing the nutritional and therapeutic potential of brown rice, paving the way for the development of functional foods with medicinal implications.

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CHAPTER I.

GENERAL INTRODUCTION

1.1. Background

Rice, a globally consumed staple, presents itself as an intriguing grain harboring an array of bioactive compounds that augment its nutritional value (Fukagawa & Ziska, 2019). Contributing to approximately 25% of the world's energy intake, rice stands as the second most crucial cereal crop, cultivated in over 114 nations and serving as a dietary mainstay for half of the global population, with an astonishing annual production of 645 million tons (Phillips et al., 2024). Notably, Asian farmers, constituting nearly 90% of the total, play a predominant role in this extensive cultivation (Fukagawa & Ziska, 2019).

The outermost layer of the rice kernel, referred to as rice bran, encompasses the pericarp, aleurone, sub aleurone layer, and germ (Sharif, 2014). This component manifests substantial concentrations of essential nutrients, including protein, fat, and dietary fiber, further contributing to the overall nutritional richness of rice (Sharif, 2014). In contemporary times, rice serves not only as a crucial staple in our diets but has also been shown to offer certain health advantages when consumed by humans. Interestingly, even though brown rice (BR) has a richer nutritional and bioactive content in its bran and embryo, it isn't as widely preferred as white rice (WR) (Fukagawa & Ziska, 2019; Sharif, 2014). Brown rice (BR) contains approximately 2% of the overall dietary fiber and acts as a crucial provider of γ-oryzanol, vitamin E, minerals, phenolic compounds, phytosterols, and phytic acid. Consequently, the adoption of brown rice as a nutritional and functional food has become a recent phenomenon. Nevertheless, owing to the dense composition of its outer bran layer, brown rice often presents a firmer texture, posing challenges in processing and rendering it less digestible in comparison to white rice. In 1995, Japan introduced germinated brown rice (GBR) as an initiative to boost the consumption of brown rice (Patil & Khan, 2011). This method involves the process of germination, aiming to enhance the quality of brown rice by increasing moisture uptake by the outer grain, leading to a softer texture. Additionally, this process enriches the bioactive components by activating residual enzymes in the rice, further improving its nutritional profile.

The allelopathic compounds known as momilactones are essential to the rice plant's defense systems against biotic stresses (Xuan et al., 2016). Momilactones A (MA) and B (MB)

were initially indicated as potent allelochemicals and phytoalexins in rice and moss (Fukuta et al., 2007; Kato et al. 1973). Recently, momilactones have been identified as valuable compounds possessing various beneficial properties, including antioxidant (Quan et al., 2019a), antidiabetic (Quan et al., 2019a, 2019b), anti-obesity (Quan et al., 2019c), anti-skin aging (Quan et al., 2019a), antimicrobial (Fukuta et al., 2007), anti-inflammatory (Cho et al., 2015), and anticancer (leukemia (Park et al., 2014), lymphoma (Lee et al., 2008), myeloma (Anh et al., 2022), colon (Kim et al., 2007) and breast cancers (Joung et al., 2008)) properties. Tricin is a multipurpose flavonoid that presents potential therapeutic applications as it navigates the complex metabolic pathways (Xuan et al., 2016). Tricin, a significant flavonoid, can be extracted from different parts of the rice plant, including grains, leaves, brans, and husks. Studies have indicated that tricin possesses antioxidant (Quan et al., 2019a), anti-skin aging (Quan et al., 2019a), and anticancer (Cai et al., 2004; Shalini et al., 2012, 2016) in numerous studies. In the meanwhile, one of the secondary metabolites that add to rice's distinct chemical profile is *ρ*-coumaric acid, a member of the hydroxycinnamic acid family (Tehami et al., 2023). Rice predominantly contains phenolic acids like *ρ*-coumaric acid, which have been acknowledged for their bioactive effects, such as antioxidant, anti-inflammatory, and anticancer properties (Wongsa et al., 2021).

The extraction process, akin to a careful excavation, aims to liberate momilactones, tricin, and *ρ*-coumaric acid from their botanical confines. Chromatographic techniques emerge as the guiding compass, guiding scientists through the intricate landscape of organic compounds present in both the rice grain and husks (Hasan et al., 2023). Quantification becomes the subsequent challenge, demanding a meticulous approach comparable to discerning the subtle flavours in a culinary masterpiece. High-performance liquid chromatography (HPLC) and mass spectrometry serve as the scientific instruments of choice, allowing researchers to quantify the concentration of each compound with a precision akin to a master chef measuring ingredients (Cáceres et al., 2017). As the results unfold, a comprehensive narrative of the chemical profile within both the rice grain and husks begins to emerge. The isolation and quantification of momilactones, tricin, and *ρ*-coumaric acid offer not only a glimpse into the intricacies of rice metabolism but also open avenues for applications in agriculture, food science, and pharmacology (Anh et al., 2023; Hasan et al., 2023). The grains and husks, once considered mere components of a dietary staple, reveal themselves as reservoirs of bioactive compounds, inviting further exploration and appreciation for the hidden treasures within our daily sustenance (Quan, et al., 2019). The purpose of this work is to use chromatographic

techniques to carefully extract momilactones, tricin, and ρ-coumaric acid from rice husks (*Oryza sativa* L. var. Koshihikari). The concentration of every chemical will then be precisely measured using mass spectrometry and high-performance liquid chromatography (HPLC). This work sheds light on rice metabolism and has potential implications in pharmacology, food science, and agriculture by analyzing the chemical composition of rice husks and grains. The comprehensive understanding of these bioactive compounds in rice opens avenues for further exploration and appreciation of the hidden treasures within our daily sustenance.

The transformative properties of germinated brown rice (GBR), highlighting its alternative features while preserving essential nutritional values. The quality of GBR is improved by heightened water absorption, leading to a softer texture, and enzymatic processes during seed germination alter bioactive substances (Ding et al., 2016; Ng et al., 2013), contributing to its proliferation of constituents like γ-aminobutyric acid (GABA) (Cho & Lim, 2016; Nascimento et al., 2020), vitamins, and amino acids (Trachoo et al., 2006; Choi, 2006). Previous studies suggest that subjecting GBR to abiotic stresses and diverse germination conditions can enhance its nutritional profile and accumulate bioactive compounds and antioxidants. Specifically, soaking and germination periods stimulate sprout growth and increase contents of total phenolics, total flavonoids, and GABA in GBR (Islam et al., 2022; Munarko et al., 2021; Owolabi et al., 2019). Abiotic stresses such as salt and cold conditions further improve GABA, polyphenols, and antioxidant activity in GBR (Choe et al., 2021; Nascimento et al., 2020).This approach presents a promising strategy to elevate the consumption value of brown rice. Additionally, the introduction explores the significance of secondary metabolites in rice, such as phenolics, terpenes, and lactones, which play crucial roles in nutrition and physiological processes. Compounds like tricin, phenolic acids, and diterpene lactones contribute to antioxidant (Quan et al., 2019a), anti-inflammatory (Wongsa et al., 2021), anti-skin aging (Quan et al., 2019a), anticancer (Cai et al., 2004; Shalini et al., 2012, 2016), and other bioactive properties (Quan et al., 2019b). The study emphasizes the importance of antioxidant properties in rice consumption, employing popular assays like DPPH and ABTS to evaluate antioxidant capacity (Floegel et al., 2011; Olszowy & Dawidowicz, 2018). Lastly, the focus shifts to Koshihikari, a renowned Japonica rice cultivar, detailing its physical attributes and widespread popularity (Kobayashi et al., 2018). Despite its popularity, the by-products, including brans and husks, have been historically underutilized, prompting the research objective to isolate bioactive compounds from Koshihikari rice husks, with a focus on phenolics and momilactones (Quan et al., 2019a, 2019b, 2019c). The investigation also aims

to evaluate differences in these compounds within Koshihikari brown rice seeds exposed to various salt treatments and germination durations, exploring the correlation between bioactive compounds and antioxidant capacity under salt conditions.

Researchers, over the past decade, have delved into the potential of GBR to not only elevate rice quality but also harness therapeutic benefits. GBR emerges as a top choice for consumers seeking multifunctional bioactive components effective in managing conditions like diabetes, high cholesterol, and hypertension (Cho & Lim, 2016). Studies indicated a positive correlation between GBR consumption and addressing cardiovascular diseases, metabolic syndrome, and type 2 diabetes (Izadi & Azadbakht, 2015). The role of GBR extends to combating oxidative stress, a risk factor for various diseases, including cancer (Valavanidis et al., 2013; Valko et al., 2006), neurodegenerative disease (Wojtunik-Kulesza et al., 2016), diabetes (Barclay et al., 2008), premature aging (Getoff, 2007), and arthritis (Kuwabara et al., 2003; Rajendran et al., 2014). GBR, rich in antioxidants and phenolic acids, becomes a noteworthy dietary source in this context. The intricate interplay of agriculture and health unfolds as researchers explore the potential of salinity stress to paradoxically enhance GBR's bioactive properties. As we peel back the layers of this nutritional enigma, the study at hand seeks to assess GBR's response to various stresses during germination, evaluating biological activities such as antioxidant, anti-diabetic, and anti-skin aging properties. This dynamic intersection of agriculture and health positions germinated brown rice as a promising avenue to address contemporary health challenges.

The intricate world of rice unfolds with a focus on secondary compounds, particularly tricin−a vital flavonoid found in various rice organs. Tricin has showcased its versatility with potential antioxidant (Quan et al., 2019a), anti-skin aging (Quan et al., 2019a), and anticancer activities (Delbaere et al., 2010; Floegel et al., 2011; Quan et al., 2019c; Wongsa et al., 2021). Phenolic acids, including caffeic acid, *ρ*-hydroxybenzoic acid, *ρ*-coumaric acid, ferulic acid, salicylic acid, and cinnamic acid, abound in rice, particularly concentrated in the bran layer (Wu et al., 2023), making brown rice (BR) a richer source compared to white rice (do Nascimento et al., 2022; Gujral et al., 2012). GBR takes this richness to another level, boasting twice the quantity of these phenolic compounds compared to BR. Momilactones A (MA) and B (MB), valuable diterpene lactones from rice, further contribute to the medicinal properties of rice (Anh et al., 2023). BR, a repository of bioactive compounds, has shown potential during the germination process under saline conditions (Hasan et al., 2023). Nevertheless, the influence of cooking on the levels of phenolic and momilactone compounds remains unclear,

prompting the need for a comprehensive evaluation. The study dives into the cooking process's impact on enriching phenolics and momilactones in both GBR and non-GBR, optimizing extraction procedures with various conditions such as solvents, temperatures, and sonication. With a focus on 80% methanol and 80% ethanol as solvents, temperatures at 25 °C and 80 °C, and a 2-hour sonication process, the study aims to unravel the potential antioxidant and antidiabetic properties of rice samples fortified with these valuable compounds. This exploration promises to shed light on the intricate interplay between cooking, extraction methods, and the bioactive potential of rice compounds, offering insights into the nutritional benefits of this staple food.

The primary objective of this investigation is to advance our comprehension of the chemical composition of rice, specifically focusing on bioactive compounds such as tricin, phenolic acids, momilactones, and *ρ*-coumaric acid. Employing meticulously optimized extraction methodologies and rigorous quantification procedures, we aspire to elucidate the quantitative abundance of these compounds within both rice husks and grains, thereby elucidating potential applications in pharmacology, food science, and agriculture. Additionally, the study endeavours to scrutinize the impact of culinary processes, specifically cooking, on the bioactive constitution of rice. Furthermore, our aim is to evaluate the antioxidant and antidiabetic capabilities present in rice samples enhanced with phenolics and momilactones. The attainment of these scientific objectives is anticipated to furnish valuable insights into the nuanced nutritional advantages offered by rice, transcending its conventional role as a dietary staple.

1.1.1. Rice

Rice isn't just a dietary staple; it's a powerhouse of bioactivities that contribute to both nutrition and health (Wada et al., 2022). Recent years have seen an increasing focus on understanding the diverse bioactive compounds present in rice and their potential benefits. From antioxidants and anti-inflammatory agents to essential vitamins and minerals, rice offers more than just carbohydrates (Sen et al., 2020). Global rice output has been on the rise, meeting the demands of a growing population. Various countries have employed innovative techniques to enhance yield and nutritional content. Biofortification programs, for instance, aim to increase the levels of essential nutrients in rice varieties, addressing nutritional deficiencies in communities that heavily rely on this grain (Senguttuvel et al., 2023). The intersection of rice production and bioactivities opens doors to sustainable agriculture practices that not only boost crop quantity but also enhance the nutritional quality of the final product (Dorairaj & Govender,

2023). As research progresses, the intricate relationship between rice's bioactive components and its production methods is becoming a focal point for ensuring not just quantity but the quality of the world's rice supply (Mohidem et al., 2022).

1.1.2. Brown Rice (BR)

Brown rice, often hailed as a nutritional powerhouse, is the whole, unrefined grain of rice with only the outermost layer, the hull, removed (Kim et al., 2011). Unlike white rice, which undergoes milling that strips away the bran and germ layers, brown rice retains its natural goodness, offering a plethora of bioactivities (Kim et al., 2011). Rich in fiber, vitamins, and minerals, brown rice contributes to digestive health and provides a sustained release of energy (Wu et al., 2023). The bran layer of brown rice contains antioxidants, such as tocopherols and tocotrienols, which combat oxidative stress and inflammation in the body (Tan et al., 2023). Additionally, it houses essential fatty acids and phenolic compounds, contributing to its potential anti-cancer and heart-protective properties (Tan et al., 2023). Brown rice's bioactive components extend to its role in blood sugar regulation (Ravichanthiran et al., 2018). The fiber content, combined with magnesium and other micronutrients, may help modulate blood glucose levels, making it a favourable choice for those managing diabetes or aiming for overall metabolic health (Ravichanthiran et al., 2018). As a wholesome alternative to refined grains, brown rice stands as a testament to the symbiosis between nature's nutritional bounty and our well-being, offering not just sustenance but a spectrum of bioactivities that promote a healthier lifestyle.

1.1.3. Germinated Brown Rice (GBR)

Germinated brown rice, a whole grain rice variety that undergoes the natural process of germination, has gained considerable attention for its enriched bioactive potential and potential health benefits (Cho & Lim, 2016; Hasan et al., 2023; Ravichanthiran et al., 2018). Unlike its non-germinated counterpart, germinated brown rice retains its bran and germ layers, which are abundant sources of essential nutrients and bioactive compounds. Through the germination process, dormant enzymes become activated, leading to biochemical changes that enhance the nutritional profile of brown rice (Ma et al., 2023). The rich composition of bioactive compounds in germinated brown rice, including antioxidants, polyphenols, tocotrienols, and enzymes, presents numerous therapeutic applications and health advantages (Chaiyasut et al., 2017; Demeekul et al., 2021; Ghose et al., 2013; Goufo & Trindade, 2014; Hasan et al., 2023; Ma et al., 2023; Rahim et al., 2022; Seechamnanturakit et al., 2018; Waliat et al., 2023; Xia et

al., 2019; Zahra & Jabeen, 2020). These compounds work synergistically to exert various physiological effects, such as reducing oxidative stress, improving cardiovascular health, enhancing glycaemic control, supporting gut health, and potentially protecting against neurodegenerative diseases (Mattioli et al., 2020; Mittal et al., 2023; Rahman et al., 2022; Razak et al., 2023; Sharifi-Rad et al., 2020). The impact of germination on brown rice results in what is known as "germinated brown rice" or "sprouted brown rice." This type of rice offers several benefits, including increased nutritional value, as the breakdown of complex compounds makes vitamins, minerals, and antioxidants more easily absorbed by the body. Germination is the process by which a plant embryo inside a seed begins to grow, triggered by specific environmental conditions such as moisture, oxygen, and suitable temperature (El-Maarouf-Bouteau & Bailly, 2008; Haj Sghaier et al., 2022; Khaeim et al., 2022; Nonogaki, 2006). During germination of brown rice, enzymes become activated, breaking down complex compounds into simpler forms that serve as nutrients for the emerging plant (Ali & Elozeiri, 2017; do Nascimento et al., 2022; Nemzer & Al-Taher, 2023; Sukegawa et al., 2021). The rice kernel absorbs water, swells, and softens its outer layers, allowing the young shoot and root to emerge. As the process continues, growth hormones stimulate cell division and elongation, leading to the development of the new plant. By understanding the nutritional and health implications of germinated brown rice, we can unlock its full potential as a functional food and advocate for its inclusion in a balanced and diverse diet. As consumers seek wholesome and nutrient-rich food choices, germinated brown rice presents a promising option for promoting overall well-being and reducing the burden of chronic diseases.

1.1.4. Bioactive Compounds of Germinated Brown Rice

Germinated brown rice is enriched with various bioactive compounds that contribute to its enhanced nutritional profile and potential health benefits. The germination process triggers the activation of enzymes and metabolic pathways, leading to the accumulation of these bioactive compounds. Here are some of the key bioactive compounds found in germinated brown rice:

1.1.4.1. Polyphenols

Germinated brown rice contains a diverse range of polyphenolic compounds, including phenolic acids (e.g., ferulic acid, caffeic acid) and flavonoids (e.g., quercetin, kaempferol) (Pandey et al., 2009; Hasan et al., 2023; Ravichanthiran et al., 2018). Polyphenols are potent antioxidants that aid in neutralizing detrimental free radicals and alleviating oxidative stress in

the body (Goufo & Trindade, 2014; Kruk et al., 2022; Wisetkomolmat et al., 2023; Zhang & Tsao, 2016).

1.1.4.2. Momilactones A and B

Momilactones A and B, two structurally distinct diterpenoids isolated from rice plants (*Oryza sativa*), have garnered substantial attention in the scientific community due to their intriguing biological activities (Zhao et al., 2018). The structural elucidation of momilactones A and B revealed intricate diterpenoid skeletons with unique features. Momilactone A, characterized by a cyclooctane ring system, and momilactone B, possessing a rearranged tricyclic skeleton, present captivating challenges and opportunities for synthetic and biosynthetic studies. One of the most notable biological activities of momilactones is their allelopathic effects (Xuan et al., 2016). Studies have consistently demonstrated the inhibitory impact of these compounds on the germination and growth of neighboring plants, positioning them as potential natural herbicides. Investigations into the mode of action have suggested interference with key physiological processes, including disruption of cell division and elongation. Momilactones also exhibit anti-fungal properties, showcasing their potential as natural fungicides (Anh et al., 2023). The inhibition of various fungal pathogens by momilactones points towards their role in plant defense mechanisms. Additionally, anti-bacterial activities have been reported, broadening the spectrum of potential applications in agriculture. The biosynthetic pathways of momilactones have been a subject of intensive research. Enzymatic processes leading to the formation of these compounds have been elucidated, paving the way for metabolic engineering strategies aimed at enhancing their production or introducing allelopathic traits into other crops (De La Peña & Sattely, 2021). Despite their bioactivities, challenges remain in terms of practical applications. Questions regarding the environmental fate, persistence, and ecological consequences of momilactones in agricultural systems require further investigation. Additionally, efforts are underway to develop formulations and delivery mechanisms that ensure effective and controlled release of momilactones in the field. Momilactones have recently been recognized as valuable compounds with a range of beneficial properties, including antioxidant (Quan et al., 2019), antidiabetic (Quan et al., 2019b, 2019c) anti-obesity (Quan et al., 2019b) , anti-skin aging (Quan et al., 2019a) antimicrobial (Fukuta et al., 2007), anti-inflammatory (Cho et al. 2015), and anticancer (leukemia, (Park et al., 2014), lymphoma (Lee et al., 2008), myeloma (Anh et al., 2022), colon (Kim et al., 2007) and breast cancers (Kim et al. 2007; Lee et al. 2008; Park et al. 2014; Anh et al. 2022) properties. Momilactones A and B represent a captivating class of

natural products with diverse biological activities, ranging from allelopathy to anti-fungal and anti-bacterial effects. As our understanding of their chemistry and mode of action deepens, the potential for harnessing these compounds in sustainable agriculture becomes increasingly promising.

1.1.4.3. Antioxidants

Germinated brown rice is a good source of antioxidants, such as tocotrienols and gamma-oryzanol (do Nascimento et al., 2022; Hasan et al., 2023; Waliat et al., 2023). Tocotrienols are a type of vitamin E with stronger antioxidant activity compared to tocopherols, the more common form of vitamin E (Sen et al., 2006). The antioxidants and phenolic compounds in germinated brown rice, such as gamma-oryzanol and tocotrienols, have been associated with improving cardiovascular health (Chung et al., 2016). They can help lower LDL cholesterol levels, increase HDL cholesterol (the "good" cholesterol), and reduce oxidative stress, collectively supporting heart health and reducing the risk of cardiovascular diseases (Ramazani et al., 2020). These compounds scavenge and neutralize free radicals, reducing oxidative stress and preventing cellular damage. By protecting cells from oxidative damage, germinated brown rice may help mitigate the risk of chronic diseases, such as cardiovascular diseases, cancer, and neurodegenerative disorders (Tyagi et al., 2021).

1.1.4.4. Phytohormones

Germinated brown rice contains phytohormones, such as gibberellins and auxins (Ganie et al., 2022). These compounds play essential roles in regulating plant growth and development. Some studies suggest that these phytohormones may have health-promoting effects in humans, but further research is needed to fully understand their impact (Mukherjee et al., 2022).

1.1.4.5. Enzymes

During germination, enzymes like amylases and proteases become active and contribute to the breakdown of complex carbohydrates and proteins into simpler forms. This enzymatic activity increases the availability and digestibility of nutrients in germinated brown rice (do Nascimento et al., 2022; Guzmán-Ortiz et al., 2019). The enzymatic breakdown of complex carbohydrates during germination increases the availability of simple sugars, potentially leading to a more gradual and controlled release of glucose into the bloodstream (Chiu & Taylor, 2011). This improved glycemic response may benefit individuals with diabetes or those aiming to maintain stable blood sugar levels.

1.1.4.6. Dietary Fiber

Germinated brown rice retains its natural fiber content, which is predominantly found in the bran layer. Dietary fiber is essential for promoting healthy digestion, preventing constipation, and supporting gut health (Nirmala Prasadi & Joye, 2020). The dietary fiber content in germinated brown rice supports a healthy gut microbiome by acting as a prebiotic, promoting the growth of beneficial gut bacteria (Lin et al., 2019). Additionally, the enzymatic breakdown of complex carbohydrates may make the rice easier to digest and gentler on the gastrointestinal system.

1.1.4.7. Phenolic Lipids

Germinated brown rice contains phenolic lipids, which are unique compounds with both phenolic and lipid components (Cho & Lim, 2016). These compounds have demonstrated antioxidant and anti-inflammatory properties, potentially contributing to the health benefits of germinated brown rice (Ravichanthiran et al., 2018).

1.1.4.8. Phytic Acid

Although phytic acid is considered an antinutrient due to its ability to bind to minerals and reduce their absorption, germination leads to a partial breakdown of phytic acid. This can enhance the bioavailability of minerals like zinc, iron, and calcium in germinated brown rice and contribute to better nutrient absorption and utilization in the body (Nkhata et al., 2018). The combination of these bioactive compounds in germinated brown rice gives it a potential edge over regular brown rice in terms of nutritional value and health benefits. Regular consumption of germinated brown rice as part of a balanced diet may offer various advantages, such as improved antioxidant status, enhanced nutrient absorption, better glycemic control, and reduced risk of chronic diseases (Chinma et al., 2023; Imam et al., 2012). However, it is essential to note that the concentration of these bioactive compounds can vary depending on factors such as germination conditions and rice variety. As with any food, variety and moderation are key to obtain the best benefits from germinated brown rice.

1.1.4.9. Metabolic Regulation

Germinated brown rice's bioactive compounds may interact with metabolic pathways, influencing cellular signalling and gene expression. These interactions can have implications for metabolism and energy regulation in the body. It is important to note that while the mechanisms of action of germinated brown rice's bioactive compounds have been studied in preclinical and animal models, more research is needed to fully understand their effects in

humans. Additionally, individual responses to these compounds may vary based on genetic factors, diet, and overall health status. Nevertheless, the accumulating evidence supports the potential health benefits of consuming germinated brown rice as part of a balanced and nutritious diet.

Germinated brown rice holds significant promise as a nutritious and health-promoting food option. Addressing the challenges and investing in further research and development can unlock its full potential and pave the way for its integration into health-conscious diets and therapeutic applications. As consumer awareness and scientific knowledge continue to grow, germinated brown rice may become an essential component in promoting overall health and disease prevention.

1.2. Research Objectives

- To isolate, identify, and quantify momilactones, phenolics, and flavonoids from rice.
- To explore and enrich momilactones, phenolics, and flavonoids in brown rice.
- To examine the fluctuations in bioactive compounds within Koshihikari brown rice seeds exposed to diverse salt treatments and different germination periods.
- To explore the impact of different exogenous treatments (temperature, salinity, and germination periods) on the antioxidant, anti-diabetic, and anti-skin aging potentials of BR during the germination stage.
- To optimize extraction methods for achieving the highest yield of momilactones, phenolics, and flavonoids from brown rice.

CHAPTER II.

ISOLATION AND QUANTIFICATION OF MOMILACTONES, TRICIN, AND ρ-COUMARIC ACID FROM RICE (*ORYZA SATIVA* L. VAR. KOSHIHIKARI)

2.1. Introduction

Rice, not only a staple food consumed all over the world, but it's also a fascinating grain with a variety of bioactive chemicals that add to its nutritional worth (Fukagawa & Ziska, 2019). Rice accounts for about 25% of global energy intake (Phillips et al., 2024). As the world's second most important cereal crop, rice is grown in at least 114 nations and is a staple food for half of the world's population, with an astounding global production of 645 million tons. Nearly 90% of all farmers are Asian, and they make the largest contribution (Fukagawa & Ziska, 2019). The pericarp, aleurone, sub aleurone layer, and germ make up the outermost layer of the rice kernel, also known as rice bran (Sharif MK, 2014). It has substantial concentrations of vital nutrients, including as protein, fat, and dietary fiber (Sharif MK, 2014).

The allelopathic compounds known as momilactones are essential to the rice plant's defence systems against biotic stresses (Xuan et al., 2016). Initially, Momilactones A (MA) and B (MB) were identified as powerful allelochemicals and phytoalexins in both rice and moss (Fukuta et al., 2007; Kato et al. 1973). Recently, momilactones have been reported as valuable compounds with multiple beneficial properties, consisting of antioxidant (Quan et al., 2019a), antidiabetic (Quan et al., 2019a, 2019c), anti-obesity (Quan et al., 2019c), anti-skin aging (Quan et al., 2019a), antimicrobial (Fukuta et al., 2007), anti-inflammatory (Cho et al., 2015), and anticancer (leukemia (Park et al., 2014), lymphoma (Lee et al., 2008), myeloma (Anh et al., 2022), colon (Kim et al., 2007) and breast cancers (Joung et al., 2008)) properties. Tricin is a multipurpose flavonoid that presents potential therapeutic applications as it navigates the complex metabolic pathways (Xuan et al., 2016). Tricin, an important flavonoid, can be isolated from various rice plant organs (grains, leaves, brans, and husks). Tricin has been reported to have potentials for antioxidants (Quan et al., 2019a), anti-skin aging (Quan et al., 2019a), and anticancer (Cai et al., 2004; Shalini et al., 2012, 2016) in numerous studies. In the meanwhile, one of the secondary metabolites that add to rice's distinct chemical profile is ρ-coumaric acid, a member of the hydroxycinnamic acid family (Tehami et al., 2023). In rice,

more prevalent are phenolic acids such as ρ-coumaric acid, which have been recognized for their bioactive properties, including antioxidant, anti-inflammatory, and anticancer activities (Wongsa et al., 2021).

The extraction process, akin to a careful excavation, aims to liberate momilactones, tricin, and ρ-coumaric acid from their botanical confines. Chromatographic techniques emerge as the guiding compass, guiding scientists through the intricate landscape of organic compounds present in both the rice grain and husks (Hasan et al., 2023). Quantification becomes the subsequent challenge, demanding a meticulous approach comparable to discerning the subtle flavors in a culinary masterpiece. High-performance liquid chromatography (HPLC) and mass spectrometry serve as the scientific instruments of choice, allowing researchers to quantify the concentration of each compound with a precision akin to a master chef measuring ingredients (Cáceres et al., 2017). As the results unfold, a comprehensive narrative of the chemical profile within both the rice grain and husks begins to emerge. The isolation and quantification of momilactones, tricin, and ρ-coumaric acid offer not only a glimpse into the intricacies of rice metabolism but also open avenues for applications in agriculture, food science, and pharmacology (Anh et al., 2023; Hasan et al., 2023). The grains and husks, once considered mere components of a dietary staple, reveal themselves as reservoirs of bioactive compounds, inviting further exploration and appreciation for the hidden treasures within our daily sustenance (Quan et al., 2019).

The purpose of this work is to use chromatographic techniques to carefully extract momilactones, tricin, and ρ-coumaric acid from rice husks (*Oryza sativa* L. var. Koshihikari). The concentration of every chemical will then be precisely measured using mass spectrometry and high-performance liquid chromatography (HPLC). This work sheds light on rice metabolism and has potential implications in pharmacology, food science, and agriculture by analyzing the chemical composition of rice husks and grains. The comprehensive understanding of these bioactive compounds in rice opens avenues for further exploration and appreciation of the hidden treasures within our daily sustenance.

2.2. Materials and Methods

2.2.1. Materials

Rice husks, sourced from rice mills near Hiroshima University's Higashihiroshima Campus in Japan, were collected, specifically choosing mature and healthy rice grains for milling to ensure husk quality. Subsequently, the obtained husks underwent a thorough cleaning

process to eliminate dust and impurities. These husk samples were then dried, preserved, and assigned a voucher specimen (KOS-MOMI 19HJ) at the laboratory of Plant Physiology and Biochemistry, Graduate School of Advanced Science and Engineering, Hiroshima University, Japan. Additionally, brown rice of the Koshihikari variety was acquired from a Japan Agriculture (JA) shop in Hiroshima, Japan, for the purpose of preparing germinated brown rice (GBR).

To conduct the extraction and isolation procedures, methanol, hexane, and ethyl acetate were acquired from Junsei Chemical Co., Ltd. in Tokyo, Japan, and silica gel was purchased from Sigma-Aldrich in St. Louis, MO, USA. The isolated compounds were dissolved using deuterated dimethyl sulfoxide (DMSO-d6) and deuterated chloroform ($CDCl_3$), both obtained from Sigma-Aldrich in St. Louis, MO, USA. Formic acid, trifluoroacetic acid, acetonitrile, methanol plus, and distilled water utilized in HPLC and UPLC analyses were procured from Sigma-Aldrich (St. Louis, MO, USA), EMD Millipore Corporation (Billerica, MA, USA), Fisher Chemical (Hampton, VA, USA), Kanto Chemical Co., Inc. (Tokyo, Japan), and Nacalai Tesque (Kyoto, Japan), respectively.

2.2.2. Preparation for Germination

With a few changes, the germination process was created using the technique outlined by (Cáceres et al., 2017). First, in order to eradicate surface bacteria and fungus without harming internal organs, 100 g of brown rice was soaked in 0.1% NaOCl at a 1:2 (w/v) ratio for 30 minutes (Cáceres et al., 2017). In order to get rid of any remaining NaClO, it was also cleaned five times using fresh tap water and dried for five minutes. To prevent bacterial and fungal infestations, distilled water was used to wash the seeds every four hours.

2.2.3. Extracted Phytochemicals from GBR

Following germination, GBR was drained for five minutes and thrice cleaned with distilled water. After that, the samples were dried for seven days at 40 °C in an oven. 50 g of powdered WR, BR, and GBR were extracted by immersing it in 80% methanol for a week, twice, at room temperature. Following 10 minutes of centrifugation at 10,000 rpm and 4 °C, the extractions were filtered. To obtain methanol crude extract, all methanolic extracts were then evaporated at 50 °C. Lastly, stock solutions of 20 mg/mL were obtained by dissolving the crude extracts in methanol for upcoming studies.

2.2.4. Isolation of Tricin, ρ-Coumaric Acid, and Momilactones A (MA) and B (MB)

The procedures for isolating tricin, *ρ*-coumaric acid, and momilactones A (MA) and B (MB) mirrored the methodologies outlined in the earlier research conducted by Quan et al. (2019a). Briefly, 30 kg of rice husks underwent a drying process in an oven at 50 °C for six days, followed by extraction with 100% MeOH for two weeks at room temperature. The resulting MeOH crude extract was then combined with an appropriate quantity of distilled water and sequentially partitioned with hexane and EtOAc. Subsequently, the obtained EtOAc extract underwent column chromatography, utilizing silica gel as the stationary phase and a hexane:EtOAc (v/v) mixture as the mobile phase. Momilactones A (MA) and B (MB) were isolated from the eluate of hexane:EtOAc (8:2, v/v), while tricin and *ρ*-coumaric acid were purified from the eluate of hexane:EtOAc (7:3, v/v).

2.2.5. Confirmation of Isolated Tricin, ρ-Coumaric Acid, and Momilactones A (MA) and B (MB) by ^{1}H- and ^{13}C-Nuclear Magnetic Resonance (NMR) and Electrospray Ionization-Mass-Spectrometry (ESI-MS)

The confirmation of the identity of isolated tricin, *ρ*-coumaric acid, MA, and MB was achieved through ^{1}H- and ^{1}3C-nuclear magnetic resonance (NMR) spectra. Specifically, the ^{1}H- and ^{13}C NMR spectra of *ρ*-coumaric acid (in DMSO-d6) were obtained using an NMR spectrometer (Bruker Ascend 400, BRUKER BioSpin, Fällanden, Switzerland) at 400 and 101 MHz, respectively. On the other hand, the ^{1}H- and ^{13}C NMR spectra of tricin (in DMSO-d6) and MA and MB (in $CDCl_3$) were recorded using an NMR spectrometer (JNM-ECA600, JEOL Ltd., Tokyo, Japan) at 600 and 151 MHz, respectively. Coupling constants (J) and chemical shifts (δ) were expressed in Hz and parts per million (ppm), respectively. The abbreviations s, d, t, q, dd, and dt denote the resonance multiplicities singlet, doublet, triplet, quartet, doublet of doublets, and doublet of triplets, respectively.

Tricin, *ρ*-coumaric acid, MA, and MB were confirmed through electrospray ionization-mass spectrometry (ESI-MS) (LTQ Orbitrap XL, Thermo Fisher Scientific, Waltham, MA, USA). The compounds, at a concentration of 10 μg/mL, were dissolved in a MeOH:ACN mixture (8:2, v/v) and introduced into the ESI system (positive ion mode) via an auto-sampler with a 3 μL injection volume. The flow rate was maintained at 0.2 mL/min. ESI conditions included ion source and capillary voltages set at 4.5 kV and 50 V, respectively. The tube lens offset was 80 V, capillary temperature at 330 °C, and nitrogen served as the gas carrier with sheath and aux flow rates of 50 arb and 10 arb, respectively. Mass spectra were recorded at a

resolution of 60,000 over a scan range of 100–2000 m/z. Identification of MS/MS spectra involved referencing the PubChem online database (National Center for Biotechnology Information, U.S. National Library of Medicine, Bethesda, MD, USA) and relevant literature.

2.2.6. Identification and Quantification of Momilactones A (MA) and B (MB) in GBR by Ultra-Performance Liquid Chromatography–Electrospray Ionization-Mass Spectrometry (UPLC–ESI–MS)

Using ultra-performance liquid chromatography-electrospray ionization-mass spectrometry (UPLC–ESI–MS), MA and MB in GBR samples were identified and measured. The LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) and the electrospray ionization (ESI) source were the two main components of the UPLC-ESI-MS system. 3.0 µL of GBR sample (in MeOH) was introduced into an Acquity UPLC® BEH C18 column (1.7 µm, 50 × 2.1 mm i.d.) at 25 °C using an autosampler (Vanquish autosampler, Thermo Fisher Scientific, Waltham, MA, USA). Solvents A and B, which were 0.1% trifluoroacetic acid in water and 0.1% trifluoroacetic acid in acetonitrile, respectively, were used in a mobile phase gradient. The gradient was created using the process reported by Anh et al. (2022). A positive Fourier transform mass spectrometer (FTMS) mode with 60,000 resolution and a scan range of 100–1000 m/z was used for the MS analysis. The calibration curves for MA and MB were made using different standard concentrations of MA and MB (0.5, 1, 5, and 10 µg/mL). MA and MB amounts were calculated using standard curves by applying the peak areas of MA and MB found in each sample.

2.2.7. Identification and Quantification of Tricin, and ρ-Coumaric Acid by High-Performance Liquid Chromatography (HPLC)

The components of the HPLC system were a detector (UV-4075 UV/VIS, Jasco, Tokyo, Japan), a controller (LC-Net II/ADC, Jasco, Japan), and a pump (PU-4180 RHPLC, Jasco, Tokyo, Japan). The stationary phase was an XBridge BEH Shield RP18 column (130 Å, 5 µm, 2.1 × 100 mm, Waters Cooperation, Milford, MA, USA). The mobile phases, solvents A (0.1% formic acid in water) and B (acetonitrile), were added and fixed using the same gradient procedure as described by Anh et al. (2022). At room temperature, each procedure was carried out for 35 minutes. For tricin and *ρ*-coumaric acid, matching peaks scanned at 350 nm and 280 nm, respectively, served as sample identification markers. These chemicals were measured using the peak area.

2.2.8. Statistical Analysis

Every experiment was carried out using three replications and a completely randomized design (CRD). One-way ANOVA was used in the analyses using Minitab software (Minitab 16.2.3, Minitab Inc., State College, PA, USA). The results (n = 3) were shown as means ± standard deviations (SD).

2.3. Results and Discussion

2.3.1. Confirmation of Isolated Tricin, ρ-Coumaric Acid, and Momilactones A (MA) and B (MB)

Tricin, *ρ*-coumaric acid, and momilactones A (MA) and B (MB) obtained in isolation underwent identification and confirmation through electrospray ionization-mass spectrometry (ESI-MS) as well as ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) techniques. Figure 2.1 displays the mass spectra of these compounds.

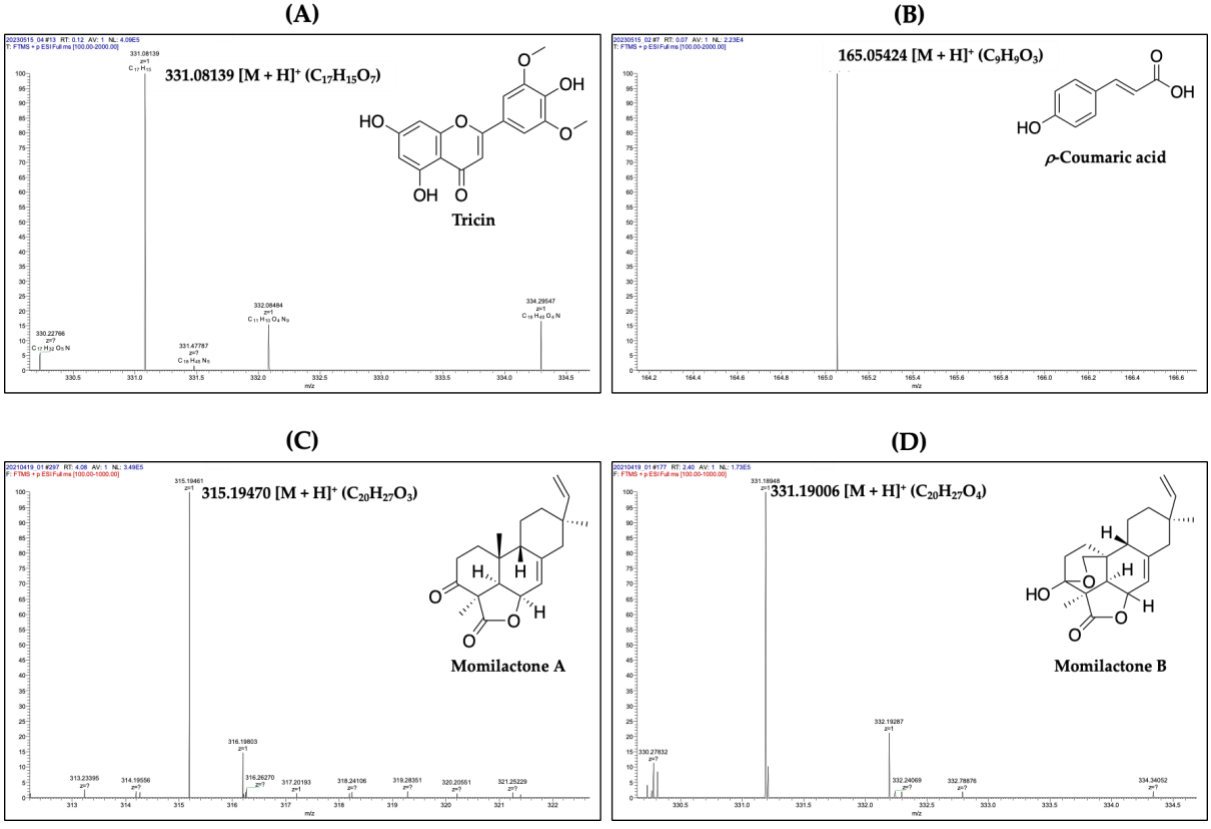

Figure 2.1. Mass-spectra of isolated (A) tricin, (B) *ρ*-coumaric acid, (C) momilactone A (MA), and (D) momilactone B (MB) in this study by ESI-MS.

Tricin: ESI-MS (m/z): 331.08139 $[M + H]^+$ ($C_{17}H_{15}O_7$) (Figure 2.1). The mass spectrum of tricin was analyzed in comparison with the data presented by Quan et al. (2019).

The ^{1}H NMR (600 MHz, DMSO-d6) δ 12.90 (^{1}H, s, 5-OH), 10.84 (d, J = 124.2, 7-OH), 9.26 (s, ^{1}H, 4-OH), 7.26 (s, H-60 and H-20), 6.92 (s, H-3), 6.49 (d, J = 2.1, H-8), 6.13 (d, J = 2.1, H-6), 3.82 (s, 2OCH$_3$), 3.26 (s, 220H), 2.43 (dt, J = 3.6 and 1.8, 171H) (Figure 2.2a). The ^{13}C NMR (151 MHz, DMSO-d6) δ 182.35 (C-4), 164.66 (C-2), 164.19 (C-7), 161.94 (C-5), 157.87 (C-9), 148.72 (C-30 and C-50), 140.38 (C-40), 56.90 (2OCH$_3$), 40.05 (dp, J = 42.0, 21.0 Hz) (Figure 2.2a). The NMR findings of tricin are aligned with the reference data provided in a study conducted by Wang et al. (2018).

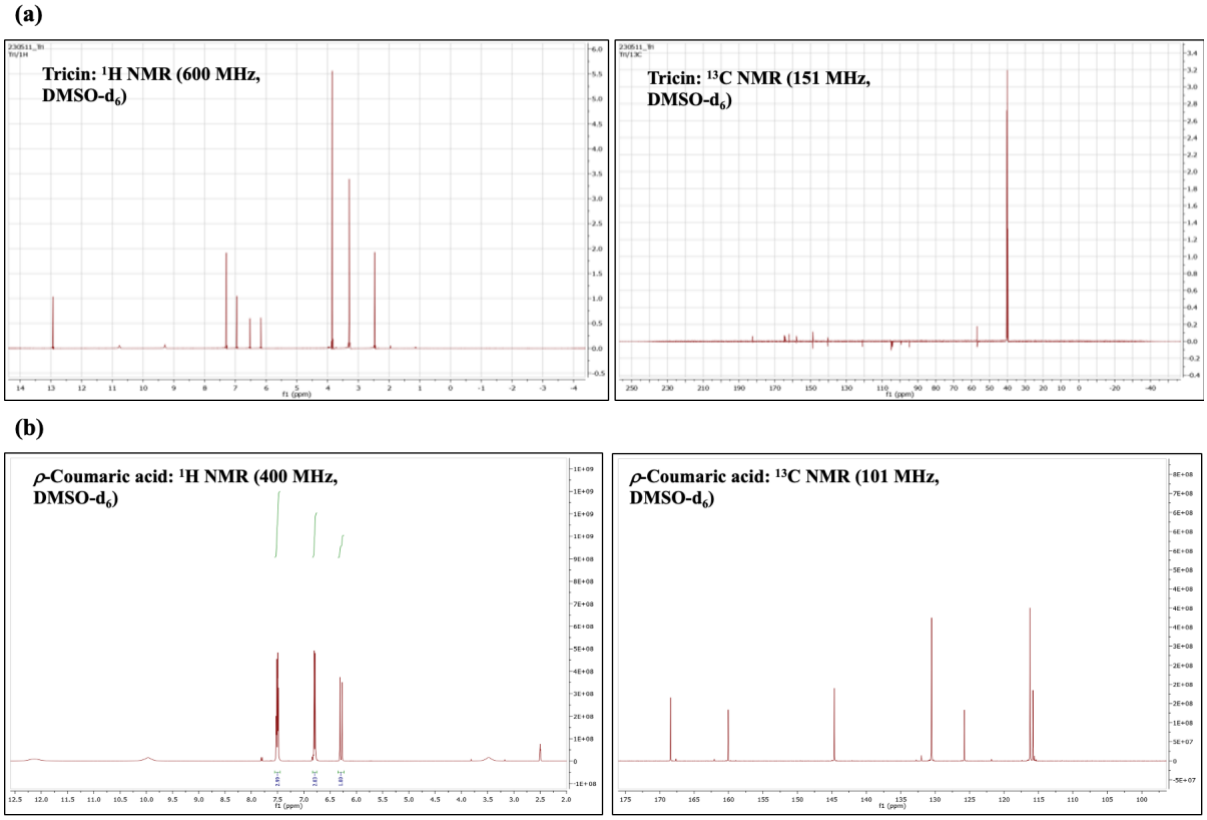

Figure 2.2. ^{1}H- and ^{13}C-NMR spectrum of isolated (a) tricin and (b) *ρ*-coumaric acid in this study.

***ρ*-Coumaric acid: ESI-MS (m/z):** 165.05424 $[M + H]^{+}$ ($C_9H_9O_3$) (Figure 2.1). The ^{1}H NMR (400 MHz, DMSO-d6) δ 7.51 (dd, J = 16.0 and 8.6, H-7, H-2, H-6), 6.79 (d, J = 8.6, H-3, H-5), and 6.29 (d, J = 16.0, H-8) (Figure 2.2b). The ^{13}C NMR (101 MHz, DMSO-d6) δ 168.42 (COOH), 160.05 (C-4), 144.64 (C-7), 130.53 (C-2, C-6), 125.75 (C-1), 116.22 (C-3, C-5), and 115.80 (C-8) (Figure 2.2b). The NMR and ESI-MS results are entirely similar to those in the literature Quan et al. (2019).

MA: ESI-MS (m/z): 315.19470 $[M + H]^{+}$ ($C_{20}H_{27}O_3$) (Figure 2.1). The confirmation of the mass spectrum of MA relied on reference data as reported by Quan et al. (2019). The ^{1}H-

NMR (600 MHz, $CDCl_3$) δ 5.87 (s, 1H), 5.86 (s, 1H), 5.85 (s, 1H), 5.83 (d, J = 17.0, 11.0, H-15), 5.71 (d, J = 5.0, H-7), 5.00 (d, J = 1.0, 1H), 4.97 (d, J = 1.0, 1H), 4.95 (d, J = 1.0, 1H), 4.93 (d, J = 1.0, 1H), 4.84 (t, J = 5.1, H-6), 4.10–4.06 (m, 1H), 3.97 (s, 1H), 3.95 (s, 1H), 3.30 (s, 1H), 3.28 (s, 1H), 3.21 (s, 1H), 3.18 (s, 1H), 2.67–2.56 (m, H-2), 2.32 (d, J = 5.1, H-5), 2.21 (d, J = 12.0, 2H-14), 2.08–2.04 (m, 1H), 1.93–1.86 (m, 1H), 1.79 (dd, J = 12.9, 3.9, 1H), 1.77–1.72 (m, H-9, H-11α), 1.63–1.55 (m, H2-1β, H2-12), 1.53 (s, H-18), 1.00 (s, H-20), 0.89 (s, H-17) (Figure 2.3a). The ^{13}C NMR (151 MHz, $CDCl_3$) δ 205.24 (C-3), 174.37 (C-19), 149.03 (C-8), 148.10 (C-15), 114.12 (C-7), 110.25 (C-16), 73.23 (C-6), 53.64 (C-4), 50.26 (C-9), 47.60 (C-14), 46.54 (C-5), 40.20 (C-13), 37.31 (C-12), 34.95 (C-1), 32.53 (C-10), 31.29 (C-2), 24.06 (C-11), 22.03 (C-20), 21.87 (C-17), 21.54 (C-18) (Figure 2.3a). The NMR spectrum aligns with the data presented in the report by Quan et al. (2019).

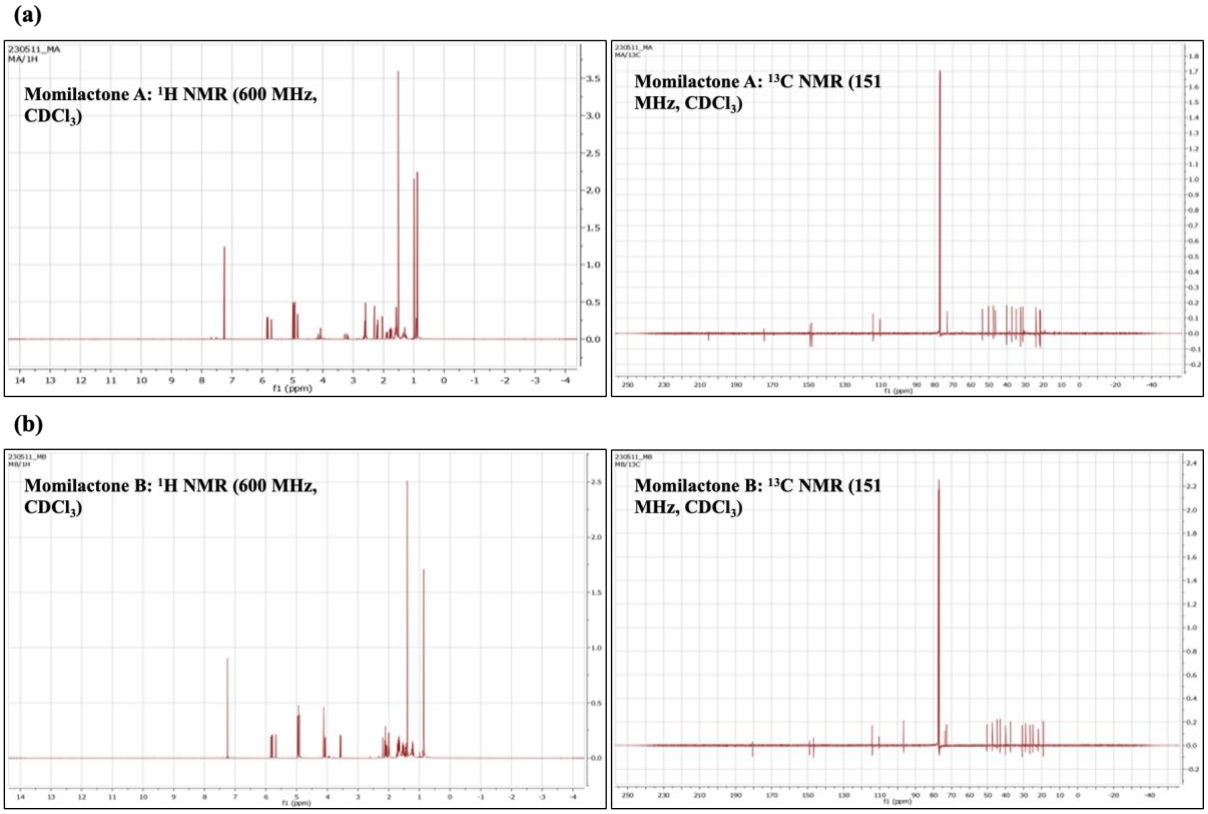

Figure 2.3. ^1H- and ^{13}C-NMR spectrum of isolated (a) MA and (b) MB in this study.

MB: ESI-MS (m/z): 331.19006 $[M + H]^+$ ($C_{20}H_{27}O_4$) (Figure 2.1). The results obtained were validated by comparing them to the findings in the previous report by Goufo & Trindade et al. (2014). The ^1H NMR (600 MHz, $CDCl_3$) δ 5.82 (dd, J = 17.5, 10.7, H-15), 5.69 (d, J = 4.8, H-7), 4.98–4.92 (m, 1H), 4.13 (s, 1H), 4.08 (dd, J = 9.2, 3.4, 1H), 3.58 (dd, J = 9.2, 2.1, 1H), 2.20 (dd, J = 6.8, 2.0, H-5), 2.14–2.07 (m, H-2, H-14), 2.04–1.98 (m, 1H), 1.75–1.64 (m,

H-9, H-11α), 1.59–1.57 (m, 1H), 1.57–1.51 (m, H-1β, H-12), 1.50 (d, J = 4.2, 1H), 1.48–1.43 (m, 1H), 1.41 (s, H-18), 1.26–1.19 (m, 1H), 0.87 (s, H-17) (Figure 2.3b). The ^{13}C NMR (151 MHz, CDCl3) δ 180.53 (C-19), 148.91 (C-15), 146.76 (C-8), 114.09 (C-7), 110.30 (C-16), 96.67 (C-3), 73.81 (C-6), 72.79 (C-20), 50.41 (C-4), 47.49 (C-14), 44.76 (C-9), 43.05 (C-5), 40.06 (C-13), 37.29 (C-12), 30.81 (C-10), 28.89 (C-1), 26.51 (C-2), 24.86 (C-11), 21.94 (C-17), and 19.06 (C-18) (Figure 2.3b). The NMR data were examined in relation to the findings published by Quan et al. (2019).

2.3.2. Quantities of Momilactones A(MA) and B (MB), Tricin, and ρ-Coumaric Acid (μg/g DW) in Rice (Oryza sativa var. Koshihikari)

The findings show that the amounts of the examined chemicals varied significantly across the various rice samples in Table 2.1. Momilactone A (MA) was found in white rice (WR) at a low concentration of 0.01 μg/g DW, but momilactone B (MB) was found in slightly higher concentrations of 0.09 μg/g DW. There were 0.89 μg/g DW of tricin and 1.73 μg/g DW of *ρ*-coumaric acid, respectively. On the other hand, momilactone concentrations were greater in brown rice (BR), with MA and MB values of 0.98 and 0.63 μg/g DW, respectively. Notably, brown rice had significantly higher quantities of tricin and *ρ*-coumaric acid, measuring 34.84 and 37.06 μg/g DW, respectively. Germinated brown rice (GBR), has the highest quantities of all the chemicals examined. Momilactones A and B demonstrated noteworthy rises, with respective levels of 7.33 and 18.68 μg/g DW, respectively. Moreover, tricin and *ρ*-coumaric acid showed impressive concentrations: 44.43 μg/g DW and 46.43 μg/g DW, respectively.

Table 2.1. Quantities of momilactones A(MA) and B (MB), tricin, and *ρ*-coumaric acid (μg/g DW) in rice (*Oryza sativa* var. Koshihikari).

Sample	MA	MB	Tricin	*ρ*-Coumaric acid
WR	0.01±0.005	0.09±0.003	0.89±0.022	1.73±0.151
BR	0.98±0.024	0.63±0.152	34.84±0.734	37.06±0.502
GBR	7.33±0.392	18.68±0.891	44.43±4.921	46.43±3.374

Data are expressed as means ± SD (standard deviation). WR: White rice; BR: Brown rice; GBR: Germinated brown rice; MA: momilactone A; MB: momilactone B; DW: dry weight.

The physiological and biochemical alterations that take place throughout the germination process can be the reason for the observed differences in momilactones, tricin, and *ρ*-coumaric

acid concentrations amongst various rice samples. In particular, germinated brown rice showed a significant build-up of bioactive compounds, indicating that it could be a good source of these chemicals with a variety of health-promoting qualities. Overall, the findings highlight how processing and germination, among other factors, can affect the chemical profile of rice, which is dynamic. The results demonstrate the potential of rice, particularly germinated brown rice, as a functional food with increased bioactive component content and have implications for both nutritional and medicinal uses.

2.4. Conclusions

In this study, the biochemical compositions of rice grains are elucidated through the identification, and quantification of momilactones A (MA) and B (MB), tricin, and ρ-coumaric acid across three discrete rice samples: refined white rice, unpolished brown rice, and sprouted brown rice. The results show that brown rice (BR) has larger quantities of tricin and ρ-coumaric acid, along with increased levels of MA and MB. However, for the first time germinated brown rice (GBR) surpasses both WR and BR in all examined compounds, with remarkable concentrations of MA, and MB, as well as tricin, and ρ-coumaric acid. These results highlight how processing, especially germination, affects rice's chemical makeup and point to GBR as a possible source of bioactive chemicals. The observed differences between the various rice samples highlight how the rice chemical profile is dynamic and subject to changes brought about by biochemical and physiological processes during germination. All things considered, these findings not only advance our knowledge of rice metabolism but also raise the possibility that rice-particularly germinated brown rice-may serve as a functional food full of bioactive ingredients with uses in both medicine and nutrition.

CHAPTER III.

SALINITY TREATMENTS PROMOTE THE ACCUMULATIONS OF MOMILACTONES AND PHENOLIC COMPOUNDS IN GERMINATED BROWN RICE

3.1. Introduction

Rice (*Oryza sativa* L.) contributes approximately 20% of the world's dietary energy, a proportion that is relatively higher than that of wheat (19%) and maize (5%) (Cho & Lim, 2016). Rice is rich in diverse secondary metabolites such as phenolic acids, flavonoids, terpenoids, steroids, and alkaloids (Wang et al., 2018). Nowadays, rice not only plays a vital role as an indispensable food source but has also been demonstrated to possess certain health benefits for human consumption. Notably, despite brown rice (BR) possessing a higher nutritional and bioactive composition in its bran and embryo, it is less popular than white rice (WR) (Cho & Lim, 2016; Ravichanthiran et al., 2018). BR includes around 2% of the total dietary fiber and serves as a vital source of γ-oryzanol, vitamin E, minerals, phenolic compounds, phytosterols, and phytic acid. Therefore, the utilization of brown rice as a nutritional and functional food has emerged as a recent trend. However, due to the compact structure of its outer bran layer, brown rice tends to have a firmer texture, making it more challenging to process and less digestible compared to white rice.

As an unavoidable outcome, germinated brown rice (GBR) has demonstrated effective alternative characteristics while retaining its inherent nutritional value. The quality of GBR is improved through heightened water absorption in the outer kernel, leading to a softened texture. Furthermore, enzymatic activities during seed germination alter bioactive substances through interactions between proteins and carbohydrates in the grain endosperm (Ding et al., 2016; Ng et al., 2013). Accordingly, GBR has been reported to have a proliferation of bio-functional constituents such as γ-aminobutyric acid (GABA) (Cho & Lim, 2016; Nascimento et al., 2020), vitamins, and amino acids (Trachoo et al., 2006), as well as a reduction of sugar (Choi, 2006), compared to non-GBR. Conversely, past research has suggested that exposing germinated brown rice (GBR) to abiotic stresses and diverse germination conditions can result in enhanced nutritional profiles and increased accumulation of bioactive compounds, along with improved antioxidant properties. Varied soaking and germination durations demonstrated promotive

effects on sprout growth and raised levels of total phenolics, total flavonoids, and GABA in germinated brown rice (Islam et al., 2022; Munarko et al., 2021; Owolabi et al., 2019). Meanwhile, abiotic stresses such as salt and cold conditions may improve the contents of GABA, polyphenols, and antioxidant activity (Choe et al., 2021; Nascimento et al., 2020). Hence, employing abiotic stresses and varied germination conditions for germinated brown rice (GBR) emerges as a promising approach to enhance the consumption value of brown rice. In a recent investigation, Choe et al. (2021) observed that germinated brown rice (GBR) exhibited an increased content of polyphenols and flavonoids, aligning with enhanced antioxidant activity during treatments involving calcium chloride ($CaCl_2$). However, none of the researchers specifically examined the impact of salinity treatment on the accumulation of bioactive compounds and antioxidant capacity in GBR.

In rice, even though they are present in relatively limited amounts, secondary metabolites like phenolics, terpenes, and lactones play crucial roles in influencing both nutritional value and essential physiological processes. These processes encompass metabolism, synthesis, and responses to environmental factors. For instance, tricin, a significant flavonoid, can be extracted from various parts of the rice plant, including grains, leaves, brans, and husks. Tricin has been reported to have potentials for antioxidants (Quan, et al., 2019a), anti-skin aging (Quan et al., 2019a), and anticancer (Cai et al., 2004; Shalini et al., 2012, 2016) in numerous studies. Additionally, rice contains more abundant phenolic acids, including *ρ*-coumaric, ferulic, cinnamic, and salicylic acids, which are acknowledged for their bioactive properties, encompassing antioxidant, anti-inflammatory, and anticancer activities (Wongsa et al., 2021). Notably, these phenolic and flavonoid compounds are accumulated with dominant contents in the bran layer (Ravichanthiran et al., 2018), so they are generally found in greater amounts in BR compared to WR (Ghasemzadeh et al., 2018; Gong et al., 2017). Moreover, the quantities of these phenolic compounds in GBR are up to twice higher than those in BR (Ravichanthiran et al., 2018). On the other hand, momilactones A (MA) and B (MB) have been acknowledged as valuable diterpene lactones derived from rice, which have recently exhibited antioxidant (Quan et al., 2019a), anticancer (leukemia(Park et al., 2014), lymphoma (Lee et al., 2008), and colon cancer (S. J. Kim et al., 2007), anti-diabetes (Quan et al., 2019b, 2019c), anti-obesity (Quan et al., 2019c), and anti-skin aging (Quan et al., 2019a) properties. Though MA and MB have shown high potential for medicinal and pharmaceutical purposes, their contents in GBR have never been elucidated (Quan et al., 2019b). In addition to the mentioned beneficial compounds, the antioxidant property is a crucial factor determining the value of rice

consumption (Goufo & Trindade, 2014). Notably, the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assays are widely used and convenient methods for assessing the antioxidant capacity of rice samples (Floegel et al., 2011). The ABTS assay relies on the generation of a blue/green ABTS•+ radical cation, while the DPPH assay involves the reduction of the purple-colored DPPH• radical to 1,1-diphenyl-2-picrylhydrazine (Floegel et al., 2011).Furthermore, ABTS and DPPH radicals differ in molecular weight, stability, affinity, solubility, absorption ability, and pH requirements (Floegel et al., 2011; Olszowy & Dawidowicz, 2018). Generally, both DPPH and ABTS assays have their respective advantages and limitations, and their combined application provides a more comprehensive understanding of the antioxidant properties of target products.

Koshihikari, a renowned Japonica model rice cultivar widely cultivated in Japan, is characterized by small, plump grains that are relatively lightweight and possess a rounded shape (Kobayashi et al., 2018). They exhibit a light brown or tan color and a smooth, glossy texture (Kobayashi et al., 2018). Correspondingly, the rice husks are typically thin, light, and exhibit a pale brown color (Kobayashi et al., 2018). Thanks to its favorable physical attributes and established aroma and taste, Koshihikari rice has gained immense popularity and preference among consumers (Kobayashi et al., 2018). However, the predominant cultivation of this variety contributes significantly to the annual production of rice by-products, including brans and husks, which historically have been underutilized or wasted (Quan et al., 2019a, 2019c). Conversely, scientific investigations have uncovered valuable bioactive compounds within these by-products, demonstrating substantial health-promoting effects (Quan et al., 2019a, 2019b, 2019c). Consequently, the primary aim of this research endeavor was to obtain Koshihikari rice husks for isolating bioactive compounds, with a specific focus on phenolics and momilactones. Additionally, the study sought to evaluate the variations in these compounds within Koshihikari brown rice (BR) seeds subjected to different salt treatments (0, 75, and 150 mM) and varying germination durations (3, 4, and 5 days). Furthermore, an investigation was conducted to explore the correlation between the levels of bioactive compounds and the antioxidant capacity exhibited during exposure to salt conditions.

3.2. Materials and Methods

3.2.1. Materials

Brown rice of the Koshihikari was purchased from a Japan Agriculture (JA) shop in Hiroshima, Japan, to prepare germinated brown rice (GBR). For extraction processes, methanol,

hexane, and ethyl acetate were purchased from Junsei Chemical Co., Ltd. (Tokyo, Japan). Standards comprising ferulic acid, cinnamic acid, and salicylic acid and chemicals including sodium acetate (CH_3COONa), sodium carbonate (Na_2CO_3), sodium hypochlorite (NaClO), aluminum chloride ($AlCl_3$), Folin–Ciocalteu's reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), potassium persulfate ($K_2S_2O_8$), and 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were acquired from Kanto Chemical Co., Inc. (Tokyo, Japan). Formic acid, trifluoroacetic acid, acetonitrile, methanol plus, and distilled water used for HPLC and UPLC analyses were obtained from Sigma- Aldrich (St. Louis, MO, USA), EMD Millipore Corporation (Billerica, MA, USA), Fisher Chemical (Hampton, VA, USA), Kanto Chemical Co., Inc. (Tokyo, Japan), and Nacalai Tesque (Kyoto, Japan), respectively.

3.2.2. Germination and Treatments

The germination process was conducted based on the procedure outlined by Cáceres et al. (2017), with several adjustments. Nine treatments were implemented during the germination phase under specific experimental conditions, including a soaking time of 36 hours, a temperature of 30 °C, and varying salt (NaCl) concentrations, namely 0, 75, and 150 mM. Details of all treatments can be found in Table 3.1. The treatments were designed using a completely randomized design (CRD). For analysis, three replications within each treatment were randomly selected, with each replication consisting of 100 individuals. Germination was carried out in darkness for durations of 3, 4, and 5 days for each treatment. Initially, 100 grams of brown rice was individually measured for each of the nine plastic pots. The rice underwent a 30-minute soaking period in 0.1% NaOCl at a ratio of 1:2 (w/v) to eliminate surface bacteria and fungi without causing harm to the internal organs, following the method outlined by Cáceres et al. (2017). Moreover, the rice was subjected to five washes with clean tap water and dried for 5 minutes to eliminate any remaining NaClO residue. Subsequently, aqueous solutions of NaCl at concentrations of 75 mM and 150 mM were prepared using distilled water for the various treatments. All rice samples were immersed in the saline solution with a grain-to-solution ratio of 1:2 (w/v) and placed in an incubator for varying durations at 30 °C (Table 3.1). After the soaking period, the seeds were rinsed with distilled water to eliminate the salinity. The trays containing brown rice seeds were then placed in an incubator at 30 °C for germination in the dark, spanning periods of 3, 4, and 5 days (Table 3.1). The closed system maintained a relative humidity of approximately 65%. To prevent bacterial and fungal invasions, the seeds underwent washing with distilled water every four hours.

Table 3.1. Description of different treatments

Treatments Code	NaCl Concentration (mM)	Germination Time (Day)
A1	0	
A2	75	3
A3	150	
B1	0	
B2	75	4
B3	150	
C1	0	
C2	75	5
C3	150	

3.2.3. Extracted Phytochemicals from GBR

Following germination, germinated brown rice (GBR) underwent two washes with distilled water and was allowed to drain for 5 minutes. The samples were then dried in an oven at 40 °C for a period of 7 days. For the extraction process, 50 grams of GBR powder were immersed in 80% methanol for one week, with two replications, at room temperature. The extractions underwent filtration after centrifugation (10,000 rpm) for 10 minutes at 4 °C. Subsequently, all methanolic extracts were evaporated at 50 °C to yield methanol crude extract. Finally, the crude extracts were dissolved in methanol to create stock solutions with a concentration of 20 mg/mL for subsequent experiments.

3.2.4. Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) in GBR Extracts

The quantification of the total phenolic content (TPC) in the extracts of germinated brown rice (GBR) was carried out using the Folin–Ciocalteu method described by Mohammadabadi et al. (2022) with several modifications. Briefly, a mixture comprising the germinated brown rice (GBR) sample, 10% Folin–Ciocalteu's reagent, and 7.5% Na_2CO_3 in volumes of 20 µL, 100 µL, and 80 µL, respectively, was prepared and allowed to incubate for

30 minutes at 25 °C in darkness. The results were then scanned at 765 nm. The quantification of total flavonoid content (TFC) followed the aluminum chloride colorimetric method detailed in the study conducted by Bueno-Costa et al. (2016). More specifically, a total volume of 100 µL of a mixture (1:1, v/v) containing the germinated brown rice (GBR) sample and 2% $AlCl_3$ was incubated for 15 minutes at 25 °C in darkness. The absorbance was then measured at 430 nm. Calibration curves for total phenolic content (TPC) (0.0052x + 0.0645, r2 = 0.9969) and total flavonoid content (TFC) (0.009x + 0.0644; r2 = 0.9998) were established using standards of gallic acid and rutin, with concentrations ranging from 6.25 to 100 µg/mL. These calibration curves were employed to estimate TPC and TFC in GBR samples. Both TPC and TFC were expressed in milligrams of gallic acid equivalent (GAE) and rutin equivalent (RE), respectively, per one gram of sample dry weight (DW).

3.2.5. Antioxidant Activities of GBR

The assessment of radical scavenging activities in germinated brown rice (GBR) extracts was conducted using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) assays, following the procedures outlined by Anh et al. (2021). For the DPPH assay, a mixture consisting of the germinated brown rice (GBR) sample, DPPH working solution (0.5 mM), and acetate buffer (0.1 mM, pH 5.5) in volumes of 80 µL, 40 µL, and 80 µL, respectively, was incubated for 20 minutes at 25 °C in darkness. In the ABTS assay, a combination (1:9, v/v) of the GBR sample and ABTS working solution (200 µL) was incubated for 30 minutes at 25 °C in darkness. The radical scavenging activities (%) were determined by assessing the reduced absorbance at 517 nm and 734 nm for the DPPH and ABTS assays, respectively, in comparison to the control (MeOH).

$$\text{Radical scavenging activity (\%)} = (Ac - (As - Ab)/Ac) \times 100 \quad (3.1)$$

where Ac is the absorbance of the control (MeOH), As is the absorbance of the sample, and Ab is the absorbance of the blank (without radical solution).

3.2.6. Identification and Quantification of Momilactones A (MA) and B (MB) in GBR by Ultra-Performance Liquid Chromatography–Electrospray Ionization–Mass Spectrometry (UPLC–ESI-MS)

Momilactone A (MA) and Momilactone B (MB) in germinated brown rice (GBR) samples were identified and quantified through ultra-performance liquid chromatography–electrospray ionization-mass spectrometry (UPLC–ESI-MS). The UPLC–ESI-MS system comprised a mass spectrometer (LTQ Orbitrap XL, Thermo Fisher Scientific, Waltham, MA,

USA) and an electrospray ionization (ESI) source. A volume of 3.0 µL of the GBR sample (in MeOH) was injected using an autosampler (Vanquish autosampler, Thermo Fisher Scientific, Waltham, MA, USA) into a column (1.7 µm, 50 × 2.1 mm i.d.) (Acquity UPLC® BEH C18, Waters Cooperation, Milford, MA, USA) maintained at 25 °C. A mobile phase gradient was employed, with solvents A and B being 0.1% trifluoroacetic acid in water and 0.1% trifluoroacetic acid in acetonitrile, respectively. The gradient program followed the procedure outlined by Anh et al. (2022). MS analysis was performed using a positive Fourier transform mass spectrometer (FTMS) mode with a resolution of 60,000 and a scan range of 100–1000 m/z. Calibration curves for MA and MB were established using various standard concentrations (0.5, 1, 5, and 10 µg/mL). Utilizing these standard curves, the quantities of MA and MB were determined by applying the peak areas detected in each sample.

3.2.7. Identification and Quantification of Tricin, ρ-Coumaric Acid, Ferulic Acid, Cinnamic Acid, and Salicylic Acid by High-Performance Liquid Chromatography (HPLC)

The detection and quantification of tricin, *ρ*-coumaric acid, ferulic acid, cinnamic acid, and salicylic acid through high-performance liquid chromatography (HPLC) analyses were compared with the standards established using the method outlined by Anh et al. (2022). In brief, the HPLC system included a pump (PU-4180 RHPLC, Jasco, Tokyo, Japan), a controller (LC-Net II/ADC, Jasco, Japan), and a detector (UV-4075 UV/VIS, Jasco, Tokyo, Japan). The stationary phase employed was a column (130 Å, 5 µm, 2.1 × 100 mm) (XBridge BEH Shield RP18, Waters Cooperation, Milford, MA, USA). Mobile phases consisted of solvent A (0.1% formic acid in water) and solvent B (acetonitrile), and these were utilized following the same gradient program detailed by Anh et al. (2022). Each procedure lasted for 35 minutes at room temperature. Each sample was distinguished by its respective peak, scanned at 350 nm for tricin and 280 nm for *ρ*-coumaric acid, ferulic acid, cinnamic acid, and salicylic acid. Quantification of these compounds was based on the peak area.

3.2.8. Statistical Analysis

All experiments were carried out following a completely randomized design (CRD) with three replications. The analyses were executed using Minitab software (Minitab 16.2.3, Minitab Inc., State College, PA, USA) through one-way and two-way ANOVA. The results were expressed as means ± standard deviations (SD) (n = 3). Pearson's correlation coefficients among various parameters were also determined using the same software.

3.3. Results and Discussion

3.3.1. Total Phenolic (TPC) and Flavonoid (TFC) Contents

Fundamentally, the biological activity of natural products is governed by their chemical composition (Anh et al., 2021). Moreover, there has been rapid progress in methods for identifying and quantifying natural compounds (Anh et al., 2023). As a result, delving into the phytochemical profiles of targeted products becomes imperative for studies exploring their potential bioactivity. In our research, the initial assessment cantered on compound groups such as phenolics and flavonoids, known for their pharmaceutical and medicinal properties, including antioxidant, antibacterial, anticancer, cardioprotective, immune system-promoting, anti-inflammatory, and skin-protective effects (Tungmunnithum et al., 2018).The total phenolic (TPC) and flavonoid (TFC) contents of GBR are illustrated in Figure 3.1.

Significant variations were observed in the total phenolic contents (TPCs) and total flavonoid contents (TFCs) among different treatments. Notably, the treatments B2 and C2 exhibited the highest TPCs, measuring 14.50 and 14.36 mg GAE/g DW, respectively. In contrast, the lowest TPC was recorded in treatment A2 at 6.17 mg GAE/g DW. On the other hand, the highest TFC (11.06 mg RE/g DW) was identified in treatment B2, while the lowest TFC (2.54 mg RE/g DW) was observed in treatment A2. Prior research has suggested an increase in both TPC and TFC in rice seedlings exposed to salinity effects (Huong et al., 2020; Minh et al., 2016; Xuan et al., 2022), which might be due to the upregulation of genes encoding the major biosynthetic enzymes (e.g., phenylalanine ammonia lyase and chalcone synthase) in plant responses to biotic stresses (Anh et al., 2021, 2022; Sharma et al., 2016). In this study, the total phenolic content (TPC) and total flavonoid content (TFC) exhibited a notable increase in treatments subjected to 75 mM salinity. However, a significant decrease was observed when the salinity level was elevated to an extreme of 150 mM. Our results indicate that a moderate salinity level of 75 mM, coupled with a 4-day germination period, represents the most suitable conditions for enhancing TPC and TFC in germinated brown rice (GBR).

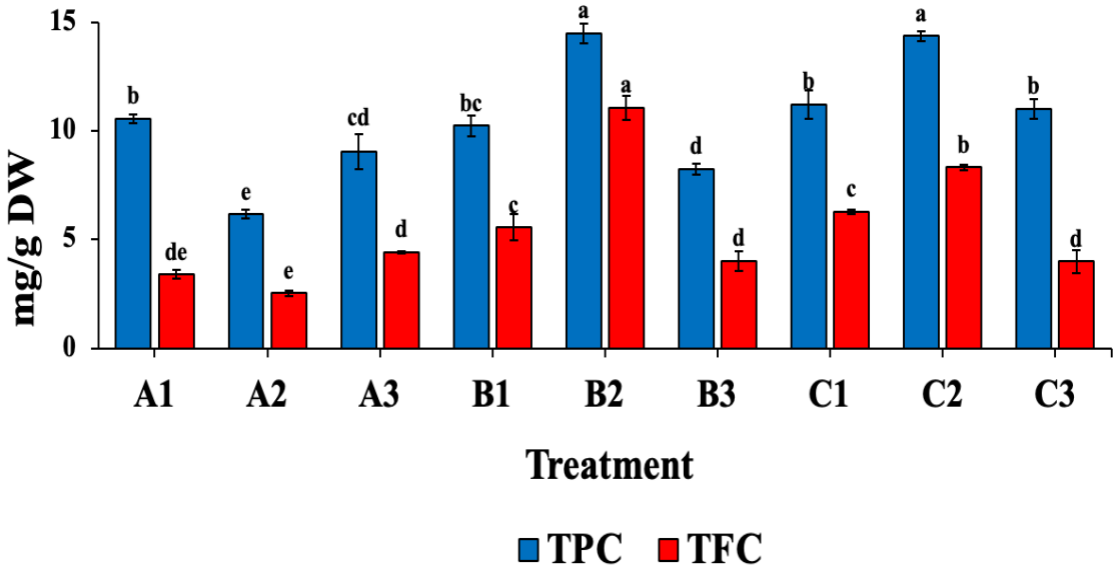

Figure 3.1. Total phenolic (TPC) and total flavonoid (TFC) contents of GBR extracts.

TPC and TFC outcomes are expressed as mg gallic acid equivalent per g dry weight (mg GAE/g DW) and mg rutin equivalent per g dry weight (mg RE/g DW), respectively. Whiskers enclosed in a column express the standard deviation (SD). Different letters attached to a column (same color) indicate significant differences at $p < 0.05$. Table 3.1 provides a comprehensive overview of the sample codes, offering a detailed description to enhance clarity and understanding.

Considering the analysis of total phenolic content (TPC), the Folin–Ciocalteu method is widely employed due to its quick and cost-effective nature, facilitating comparative analyses between samples (Prior et al., 2005). However, this method has limitations in accurately quantifying TPC as it reacts with other components such as amino acids, peptides, and reducing sugars (Prior et al., 2005). For a more precise determination of TPC, an enhanced approach involving solid-phase extraction using Sep-Pak C18 column cartridges is necessary to purify the extract and eliminate unwanted components (Mohammadabadi et al., 2022). While the Folin–Ciocalteu method serves as a valuable screening tool for assessing the relative phenolic content in various samples, it may not provide precise quantification of individual phenolic compounds (Prior et al., 2005). Therefore, in further exploration, we employed HPLC analysis to discern the profiles of specific phenolics present in germinated brown rice in this study.

3.3.2. Contents of Tricin, ρ-Coumaric Acid, Ferulic Acid, Cinnamic Acid, and Salicylic Acid in GBR

Tricin, *ρ*-coumaric acid, ferulic acid, cinnamic acid, and salicylic acid serve multifaceted roles that contribute to human health, encompassing antioxidant, anticancer, and anti-chronic diseases properties (Shahidi & Ambigaipalan, 2015). In this study, the identification of these phenolic compounds is presented in Supplementary Figure S2, and their quantification is detailed in Table 3.2. Notably, in treatment B2 (75 mM salinity, and 4-day germination), tricin, *ρ*-coumaric acid, ferulic acid, salicylic acid, and cinnamic acid exhibited increasing quantities, measuring 107.63, 93.77, 139.03, 46.05, and 596.26 μg/g DW, respectively. The heightened levels of *ρ*-coumaric, salicylic, and ferulic acids align with a previous study involving rice seedlings subjected to salinity (100 mM) (Xuan et al., 2022). However, the amounts of cinnamic acid and tricin decreased in that study, which contrasts with our findings (Xuan et al., 2022). This discrepancy could be attributed to variations in genetic diversity among the tested rice varieties. Different rice varieties, including tolerant and susceptible cultivars, exhibit diverse mechanisms in phenolic accumulation to cope with stress conditions (Anh et al., 2022; Huong et al., 2020; Minh et al., 2016; Xuan et al., 2022). On the contrary, our study revealed a significant decrease in the quantities of these phenolics in germinated brown rice when exposed to extreme salinity levels (150 mM). This suggests that the optimal conditions for the enhancement of bioactive phenolics in germinated brown rice involve a salinity of 75 mM maintained for 4 days during the germination process.

Table 3.2. Quantities of momilactones, tricin, *ρ*-coumaric acid, ferulic acid, cinnamic acid, and salicylic acid (μg/g DW) in GBR

Treatment Code	MA	MB	Tricin	*ρ*-Coumaric Acid	Ferulic Acid	Cinnamic Acid	Salicylic Acid
A1	7.33 ± 0.39 ^{c}	18.68 ± 0.89 ^{c}	44.43 ± 8.92 ^{cd}	46.43 ± 3.37 ^{de}	64.92 ± 3.34 ^{bc}	28.16 ± 0.64 ^{b}	290.27 ± 68.05 ^{bc}
A2	2.92 ± 0.06 ^{ef}	9.30 ± 0.09 ^{e}	41.12 ± 5.57 ^{cde}	39.88 ± 0.63 ^{ef}	53.23 ± 1.88 ^{c}	21.86 ± 1.09 ^{c}	349.04 ± 83.3 ^{b}
A3	1.93 ± 0.09 ^{f}	7.27 ± 0.12 ^{f}	29.55 ± 2.04 ^{ef}	36.29 ± 1.66 ^{f}	49.05 ± 2.72 ^{c}	22.58 ± 1.10 ^{c}	194.16 ± 14.77 ^{cd}
B1	5.68 ± 1.38 ^{cd}	18.88 ± 0.57 ^{c}	33.93 ± 1.38 ^{def}	61.77 ± 1.96 ^{b}	57.22 ± 7.63 ^{bc}	11.82 ± 0.82 ^{d}	88.49 ± 22.89 ^{de}
B2	18.94 ± 0.47 ^{a}	41.00 ± 0.51 ^{a}	107.63 ± 6.75 ^{a}	93.77 ± 4.35 ^{a}	139.03 ± 5.16 ^{a}	46.05 ± 0.88 ^{a}	596.26 ± 1.14 ^{a}
B3	4.19 ± 0.03 ^{de}	12.70 ± 0.75 ^{d}	25.51 ± 0.94 ^{f}	44.99 ± 1.44 ^{def}	61.98 ± 2.36 ^{bc}	22.01 ± 0.52 ^{c}	52.86 ± 3.2 ^{e}
C1	4.90 ± 0.17 ^{de}	11.97 ± 0.05 ^{d}	49.54 ± 0.34 ^{c}	44.48 ± 2.90 ^{def}	52.55 ± 4.77 ^{c}	-	-
C2	10.17 ± 0.49 ^{b}	24.79 ± 0.55 ^{b}	65.13 ± 3.06 ^{b}	59.95 ± 5.51 ^{bc}	76.55 ± 9.07 ^{bc}	-	-
C3	1.70 ± 0.0 1^{f}	7.20 ± 0.29 ^{f}	31.67 ± 0.59 ^{ef}	51.52 ± 3.19 ^{cd}	60.81 ± 5.97 ^{bc}	-	-
ANOVA							
Period	***	***	***	***	***	***	***
Treatment	***	***	***	***	***	***	***
Period × Treatment	***	***	***	***	***	***	***

Data are expressed as means ± SD (standard deviation). Different superscript letters (^{a,b,c,d,e,f}) in a column indicate significant differences at $p < 0.05$; *** denotes a significant difference at $p < 0.001$. MA: momilactone A; MB: momilactone B; DW: dry weight; -: not detected; Table 3.1 provides a comprehensive overview of the sample codes, offering a detailed description to enhance clarity and understanding.

3.3.3. Contents of Momilactones A (MA) and B (MB) in GBR

Momilactones A (MA) and B (MB) are recognized as valuable bioactive compounds derived from rice, offering a range of health-related benefits, including antioxidant (Quan et al., 2019a), anti- cancer (leukemia (Park et al., 2014), lymphoma (Lee et al., 2008), and colon cancer (Kim et al., 2007), anti-diabetes (Quan et al., 2019b, 2019c), anti-obesity (Quan et al., 2019c), and anti-skin aging (Kato et al. 1973; Quan et al., 2019a) activities. In recent times, utilizing an enhanced method for sample preparation and quantification has enabled the detection of momilactones with heightened sensitivity in various parts of the rice plant, including leaves, roots, husks, and more (Kato et al. 1973; Quan et al., 2019b, 2019d). Nonetheless, harnessing momilactones from rice sources continues to encounter numerous constraints, primarily stemming from the scarcity of commercial availability and challenges in the isolation process (Kato et al. 1973; Quan et al., 2019b). A few reports about momilactone isolation and purification have been published, and in those studies, a minor amount of momilactones can be isolated from rice sources (Ahmad et al., 2019; Chung et al., 2005; Kato et al. 1973; Quan et al., 2019d). Additionally, the published artificial syntheses of MA were also challenging since they included multiple complicated steps, required high costs, resulted in low yields (40–50%), and were environmentally unfriendly (Germain & Deslongchamps, 2002).Conversely, the synthetic procedures for MB have not been documented. Indeed, owing to their restricted accessibility, investigations into momilactones have been relatively scarce and underdeveloped over the past fifty years. Recently, only two studies focused on optimizing the extraction conditions of MA and MB from rice husks (Ahmad et al., 2019; Minh et al., 2018), while no research has been conducted to enhance the momilactone contents of rice grains to increase their consumption value. Therefore, this study, for the inaugural time, delved into the impacts of various conditions (salinity and germination periods) on the buildup of MA and MB in germinated brown rice (GBR). In Supplementary Figure S3, the presence of MA and MB in GBR is confirmed by comparing their retention times and mass spectra with those of the standards. Numerous studies indicated that the antioxidant, anti-diabetic, and anticancer potentials of MB were greater than those of MA (Anh et al., 2022; Fukuta et al., 2007; Quan et al., 2019b). However, Chung et al. (2006) announced that the endogenous quantity of MA was generally higher than that of MB in different 99 rice varieties. In contrast to previous reports, our findings demonstrate that Koshihikari GBR exhibited a greater amount of MB than MA in all treatments (Table 3.2). Significantly, the highest accumulation of MA (18.94 μg/g DW) and MB (41.00 μg/g DW) was recorded in the B2 treatment. The amounts of MA and MB in GBR

under B2 were significantly higher than those of preceding studies, in which MA and MB quantities ranged from 2.07 to 16.44 μg/g DW and 1.06 to 12.73 μg/g DW, respectively (Quan et al., 2019a, 2019b, 2019c). The increased accumulation of MA and MB in GBR in B2 treatment may be caused by the elevated expression of related genes to momilactone biosynthesis, including OsCPS4, OsKSL4, CYP99A3, OsMAS, and OsMAS2 (Anh et al., 2022). However, under a high salinity level of 150 mM, the accumulations of both MA and MB in GBR experienced a notable decrease compared to the non-saline treatment. These findings indicate that a moderate salt concentration of 75 mM, coupled with a 4-day germination period, represents the optimal conditions for enhancing the contents of MA and MB, potentially contributing to the pharmaceutical and medicinal values of GBR.

3.3.4. Antioxidant Activity of GBR by the DPPH and ABTS Radical Scavenging Assays

In human physiology, oxidative stress is intricately linked with inflammation, recognized as a pivotal process in the progression of numerous chronic diseases such as diabetes, aging, and cancer (Ambade & Mandrekar, 2012; Tucker et al., 2015). Specifically, inflammation can worsen oxidative stress and vice versa (Ambade & Mandrekar, 2012; Tucker et al., 2015). Several experimental observations have substantiated the existence and influence of oxidative stress in various chronic diseases, contributing to elevated rates of morbidity and mortality (Ambade & Mandrekar, 2012; Tucker et al., 2015). In light of this, evaluating the antioxidant properties of the samples becomes a crucial aspect of our study. As depicted in Figure 3, alterations in salinity levels and germination periods appear to enhance the antioxidative activity of GBR. Notably, among all treatments, B2 exhibited the highest antiradical activities against DPPH and ABTS (IC_{50} = 1.58 and 1.78 mg/mL, respectively) compared to the others. Numerous studies have consistently affirmed that varying germination conditions and exposure to salt stress contribute to the improved antioxidant capacity of GBR (Lin et al., 2015; Nascimento et al., 2020; Ti et al., 2014). This outcome could stem from the increased expression of pertinent enzymes like superoxide dismutase, catalase, glutathione peroxidase, and ascorbate peroxidase in rice when subjected to abiotic stresses (Xuan & Khang, 2018). Therefore, the impact of salinity could potentially play a role in augmenting the antioxidant capabilities of GBR in this study. However, it is crucial to exercise caution in determining the salt concentration, as excessive levels may result in diminished antioxidant capacities of GBR (Falcinelli et al., 2017). Consistent with the findings of Falcinelli et al. (2017), our study demonstrates a notable reduction in the antioxidant activity of GBR at a high NaCl

concentration of 150 mM. Conversely, a moderate salinity level of 75 mM appears to be the most conducive condition for enhancing the antioxidant activity of GBR.

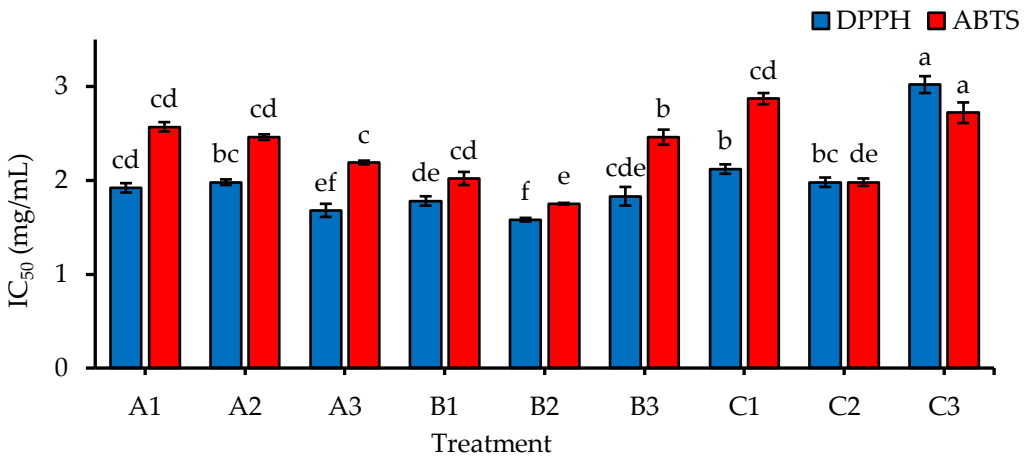

Figure 3.2. Antioxidant activities of GBR extracts.

IC_{50} is the required concentration (mg/mL) for scavenging 50% of radicals. Whiskers enclosed in a column express the standard deviation (SD). Different letters attached to a column (same color) indicate significant differences at $p < 0.05$. DPPH: 2,2-diphenyl-1-picrylhydrazyl assay; ABTS: 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid assay; Table 3.1 provides a comprehensive overview of the sample codes, offering a detailed description to enhance clarity and understanding.

3.3.5. Correlation between Antioxidant Activities and Phytochemicals of GBR

Table 3.3 presents Pearson's correlation coefficients between antioxidant activities and phytochemicals. The simultaneous accumulation of MA, MB, tricin, *ρ*-coumaric acid, ferulic acid, cinnamic acid, and salicylic acid was observed, exhibiting a strong correlation with the antioxidant activities of germinated brown rice (GBR). Extensive previous studies have reported on the antioxidant abilities of tricin, *ρ*-coumaric acid, ferulic acid, cinnamic acid, and salicylic acids (Soong & Barlow, 2004). In particular, these compounds can enhance antioxidant capacities by neutralizing free radicals through the donation of hydrogen ions (Soong & Barlow, 2004). Conversely, while the antioxidant activities of MA and MB have been mentioned in several publications (Fukuta et al., 2007; Quan et al., 2019a), their underlying mechanisms remain unclear. In another consideration, Anh et al. (2022) hypothesized that MA and MB might not directly contribute to the antioxidant responses of rice against adverse stresses, but they might play a role in signalling the production of antioxidant compounds such as phenolics.

Table 3.3. Pearson's correlation coefficients between phytochemicals and antioxidant activities of GBR

	MA	MB	*ρ*-Cou	Tri	Fer	Sal	Cin	DPPH	ABTS	TFC
MB	0.984 ***									
***ρ*-Cou**	0.888 ***	0.915 ***								
Tri	0.940 ***	0.898 ***	0.837 ***							
Fer	0.908 ***	0.901 ***	0.905 ***	0.886 ***						
Sal	0.610 **	0.572 **	0.497 *	0.613 **	0.653 **					
Cin	0.548 **	0.526 **	0.419 *	0.458 *	0.594 **	0.900 ***				
DPPH	0.510 **	0.540 **	0.355 *	0.397 *	0.431 *	0.577 **	0.722 **			
ABTS	0.053	0.043	0.063	0.003	0.259	0.18	0.347 *	0.041		
TFC	0.860 ***	0.858 ***	0.851 ***	0.864 ***	0.809 ***	0.304 *	0.216	0.410 *	−0.07	
TPC	0.743 **	0.744 **	0.733 **	0.727 **	0.670 **	0.068	−0.029	0.09	−0.113	0.861 ***

*, **, and *** indicate significances at $p < 0.05$, 0.01, and 0.001, respectively; MA: momilactone A; MB: momilactone B; *ρ*-Cou: *ρ*-coumaric acid; Tri: tricin; Fer: ferulic acid; Sal: salicylic acid; Cin: cinnamic acid; TPC: total phenolic content; TFC: total flavonoid content. ABTS: 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) assay; DPPH: 2,2-diphenyl-1-picrylhydrazyl assay.

Therefore, in this study, the increased levels of MA and MB in germinated brown rice (GBR) exposed to moderate salinity (75 mM NaCl) and a 4-day germination period could potentially result in the augmentation of tricin, *ρ*-coumaric, ferulic, cinnamic, and salicylic acids. This, in turn, may contribute to the heightened antioxidant capacity of GBR, although further validation is necessary.

Additionally, due to the well-established correlation between oxidative stress and chronic diseases (Ambade & Mandrekar, 2012; Tucker et al., 2015), extensive research has been undertaken to explore the use of antioxidant substances for the treatment of such disorders (Biswas, 2016). However, the failures have been documented through clinical evaluations, which might be attributed to the single use of antioxidant agents to target specific diseases (Biswas, 2016). Furthermore, interactions among compounds may hold greater significance than individual ones, leading to enhanced therapeutic efficiency (Anh et al., 2021; Quan et al., 2019a, 2019d). Hence, the concurrent increase in bioactive compounds and antioxidant activity observed in germinated brown rice (GBR) in this study could potentially generate a synergistic effect with positive implications for human health. Our findings might advocate for the enhancement of rice consumption values and the advancement of pharmaceuticals, functional foods, and supplements. For instance, GBR subjected to B2 treatment (75 mM salinity for 4 days) could be utilized in the production of a fermented functional beverage like kombucha, known for its growing popularity due to its health benefits (Quan et al., 2022). Alternatively, when contemplating the effects of human digestion on the intended products, future studies should extensively investigate their bioaccessibility and bioavailability throughout the digestive process (Un et al., 2022).

3.4. Conclusions

For the first time, this study has identified an optimized treatment (B2: 75 mM NaCl and 4-day germination) that significantly boosts the accumulation of valuable bioactive compounds, including phenolics, momilactones A (MA), and momilactones B (MB), in germinated brown rice (GBR) of the Koshihikari variety. Notably, GBR treated with B2 exhibited the highest levels of total phenolics and total flavonoids. Additionally, the quantification results revealed that GBR under B2 treatment accumulated the highest quantities of MA, MB, tricin, *ρ*-coumaric acid, ferulic acid, cinnamic acid, and salicylic acid. The B2 treatment also markedly improved the antioxidant activities of GBR, as demonstrated in antiradical assays (DPPH and ABTS). Given that brown rice has been less favoured, resulting in improper utilization or waste, the findings of our study offer promising prospects for enhancing the nutritional value of brown

rice and promoting the development of rice-derived products that contribute to human well-being. As a result, this research aims to encourage the consumption of brown rice by highlighting its intrinsic value and potential health benefits. Furthermore, these findings are anticipated to contribute to the achievement of the Sustainable Development Goals (SDGs) by enhancing overall well-being, eradicating poverty, and ensuring global food security, particularly in countries dependent on rice cultivation.

3.5. Supplementary Data

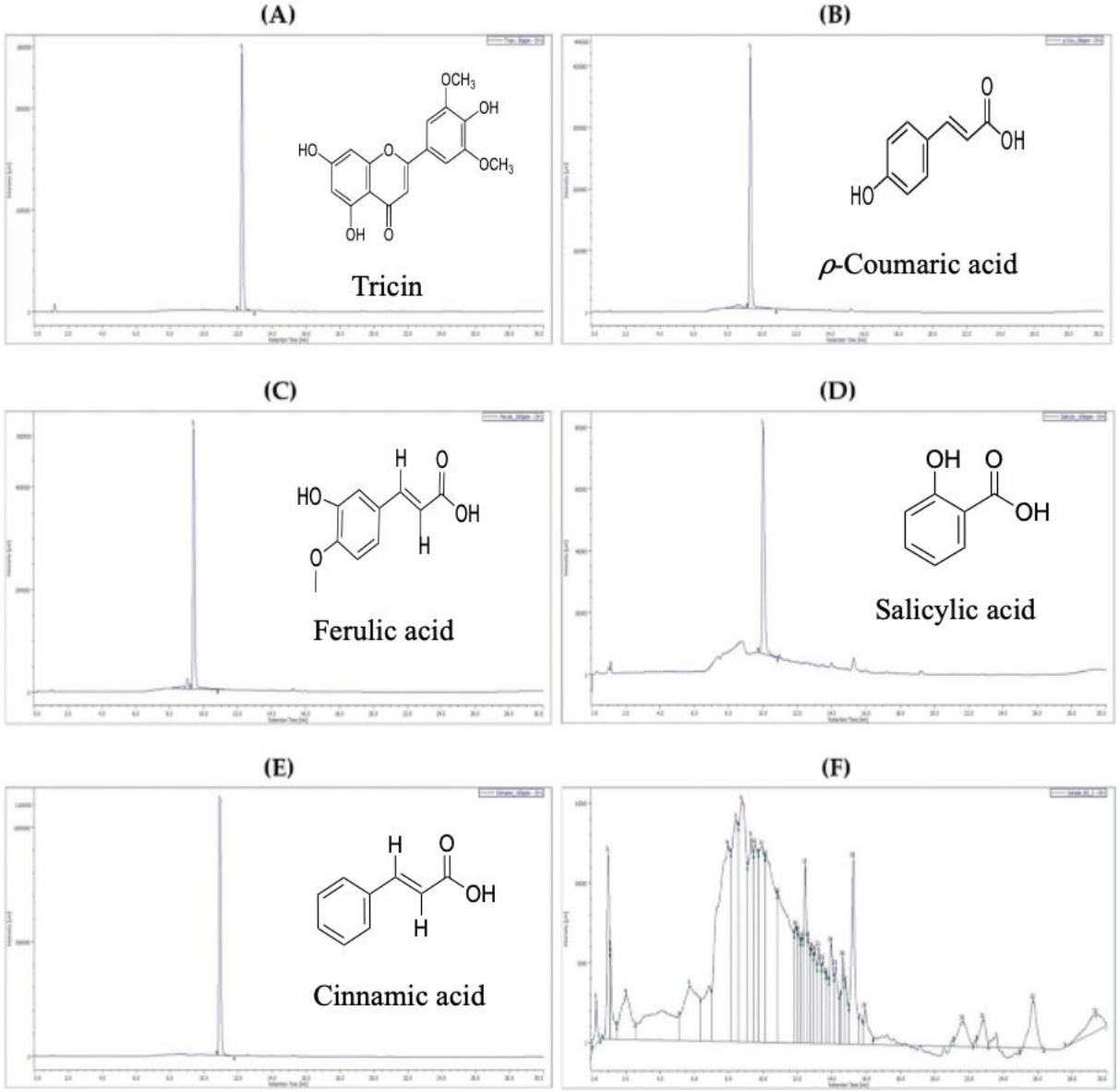

Figure S1: High-performance liquid chromatography (HPLC) chromatograms of standards (A) tricin; (B) *ρ*-coumaric acid; (C) ferulic acid; (D) salicylic acid; (E) cinnamic acid, and (F) detected phenolic compounds in B2 (75 mM NaCl and 4 day-germination).

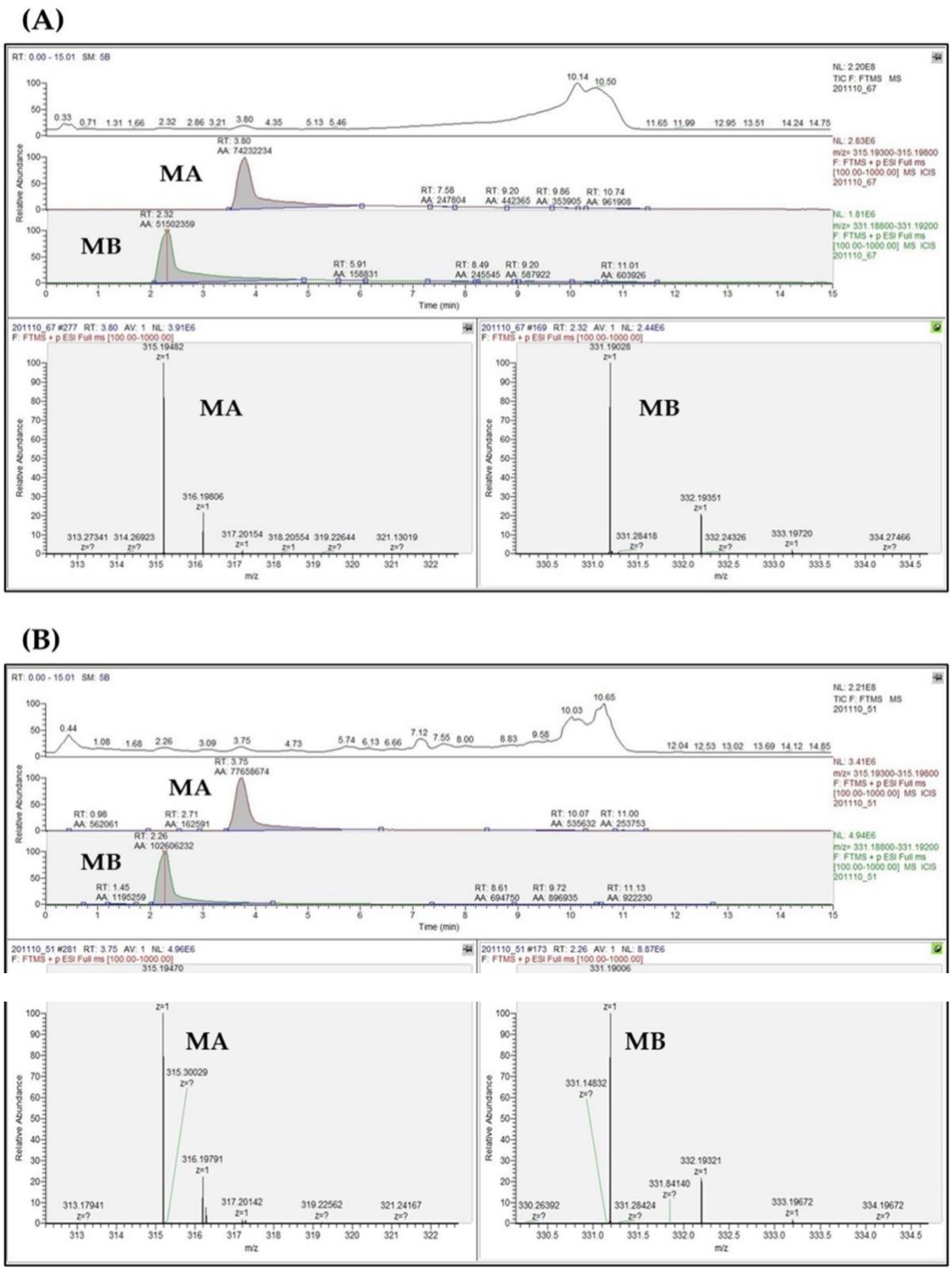

Figure S2: UPLC–ESI-MS chromatograms of (A) standard momilactones A (MA) and B (MB), and (B) MA and MB detected in GBR in B2 treatment (75 mM NaCl and 4-day germination).

CHAPTER IV.

POTENTIALS OF GERMINATED BROWN RICE UNDER SALINITY STRESS FOR ANTIOXIDANT, ANTI-DIABETIC, AND ANTI-SKIN AGING ACTIVITIES

4.1. Introduction

Rice accounts for approximately 20% of the world's dietary energy, surpassing the contributions of wheat (19%) and maize (5%) in a similar context (Cho & Lim, 2016). In Asia, the environmental conditions have spurred rice paddy production to approximately 662.9 million tons in 2012, marking a 27% increase from the 2002 levels (FAO, 2013). Asian populations exhibit a higher preference for rice compared to other continents (Cho & Lim, 2016b). Despite being a widely consumed staple in Asia, white rice is implicated in beriberi (vitamin B1 deficiency disease), primarily due to nutritional imbalances. In contrast, brown rice, containing more nutritional and bioactive components in its bran and embryo, is less consumed (Cho & Lim, 2016). Brown rice inherently contains around 2% dietary fiber of total solids and crucial components like gamma-oryzanol, vitamin E, minerals, phenolic compounds, phytosterols, and phytic acid.

Germinated brown rice, characterized by the sprouting of the rice kernel, presents an intriguing canvas for investigation (Patil & Khan, 2011). Germination introduces new bio-functional components in germinated brown rice (GBR), including the noteworthy gamma-aminobutyric acid (GABA) (Cho & Lim, 2016). To promote the consumption of brown rice, germinated brown rice (GBR) was introduced in Japan in 1995 (Patil & Khan, 2011). The process of germination enhances the quality of brown rice by increasing water absorption on the outer kernel, softening its texture, and activating residual enzymes to enrich bioactive components. Germination, as emphasized by (Ding et al., 2016; Ng et al., 2013), leads to a wide range of bioactive compounds. The variation in these compounds is attributed to enzymatic activity, particularly in the interaction between proteins and carbohydrates in the grain endosperm. Nascimento et al. (2020) suggested utilizing germinated grains like BR as an alternative approach to develop rice varieties with enhanced characteristics. This enhancement is achieved by inducing germination in BR through exposure to cold and salt stresses, as demonstrated in their study, which showed increased GABA production, improved starch

digestibility, and other favorable parameters in brown rice (BR). Germination is influenced by various factors, both intrinsic (cultivar, rice milling, storage conditions) and external (temperature, humidity, air, light, pH) (Cho & Lim, 2016). For the optimal germination of brown rice, Capanzana & Buckle, (1997) recommend soaking for 24 hours at 25 °C followed by a 3-day germination period with atmospheric air at 30 °C. Additionally, germination boosts nutrient levels, such as Vitamin B and total protein contents (Trachoo et al., 2006). Choi et al. (2006) observed significantly higher amounts of fructose, reducing sugar, and GABA-3.4 times, 2.75 times, and 797 times, respectively-when germination occurred within 24 hours compared to non-GBR.

Over the past decade, researchers have shown significant interest in exploring the potential of GBR to enhance rice quality and leverage its therapeutic benefits. Consequently, GBR has become the top choice for consumers, thanks to its multifunctional bioactive components that prove effective in managing conditions such as diabetes, high cholesterol, and hypertension (Cho & Lim, 2016b). Additionally, research indicates a positive correlation between the prevalence of cardiovascular disease, metabolic syndrome, and type 2 diabetes in developing countries (Izadi & Azadbakht, 2015). To address type 2 diabetes, a potential strategy involves inhibiting key enzymes in glucose formation, with α-amylase playing a crucial role in breaking down starch (Barclay et al., 2008). Regarding skin issues, inhibiting the key enzyme tyrosinase in melanin formation can effectively prevent skin hyperpigmentation symptoms (Demirseren et al., 2014). Imam et al. (2012) have even demonstrated that individuals with diabetes can experience benefits by switching to GBR from WR.

An excess production of reactive oxygen species (ROS), including free radicals like hydrogen peroxide, hypochlorous acid, superoxide anion, singlet oxygen, lipid peroxides, hypochlorite, and hydroxyl radical, leads to oxidative stress-an influential risk factor for various diseases such as cancer (Valavanidis et al., 2013; Valko et al., 2006), diabetes, and arthrosclerosis (Rajendran et al., 2014). Conditions like arthritis (Kuwabara et al., 2003), neurodegenerative diseases (Wojtunik-Kulesza et al., 2016), and premature aging (Getoff, 2007) (Getoff, 2007) are also linked to oxidative stress. Antioxidants play a crucial role in combating oxidative stress, and brown rice (BR) is a noteworthy dietary source containing various phenolic acids responsible for antioxidant activities (Ravichanthiran et al., 2018). Germination with water soaking has been identified as a method to enhance antioxidant and bio-functional compounds in brown rice. A recent experiment suggests that germination in a

closed vessel at a higher temperature (36 °C) with a long soaking time (72 h) is optimal for increasing the abundance and quantity of bioactive molecules (Lin et al., 2019).

In this dynamic intersection of agriculture and health, the potential of germinated brown rice takes centre stage, offering a promising avenue for addressing contemporary health challenges. As we unravel the layers of this nutritional enigma, we embark on a journey to understand how salinity stress, often considered a hindrance, might paradoxically enhance the bioactive properties of germinated brown rice. Therefore, this study was conducted to assess GBR after applying several stresses during germination through evaluation of biological activities such as antioxidant, anti-diabetics and anti-skin aging activities for the first time.

4.2. Materials and Methods

4.2.1. Materials

To make germinated brown rice (GBR), Koshihikari brown rice was bought at a Japan Agriculture (JA) store in Hiroshima, Japan. Methanol, hexane, and ethyl acetate were acquired for the extraction procedures from Junsei Chemical Co., Ltd. (Tokyo, Japan). Chemicals such as sodium acetate (CH_3COONa), sodium carbonate (Na_2CO_3), sodium hypochlorite (NaClO), aluminum chloride ($AlCl_3$), potassium persulfate ($K_2S_2O_8$), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were obtained from Kanto Chemical Co., Inc. (Tokyo, Japan) and included in the standards for ferulic acid, cinnamic acid, and salicylic acid. For the HPLC and UPLC analyses, Sigma-Aldrich (St. Louis, MO, USA), EMD Millipore Corporation (Billerica, MA, USA), Fisher Chemical (Hampton, VA, USA), Kanto Chemical Co., Inc. (Tokyo, Japan), and Nacalai Tesque (Kyoto, Japan) provided the formic acid, trifluoroacetic acid, acetonitrile, methanol plus, and distilled water, respectively.

4.2.2. Treatment and Germination

The germination process was conducted in accordance with the procedure outlined by Cáceres et al. (2017), incorporating several modifications. During the germination phase, nine treatments were administered. The experimental parameters included a 36-hour soaking period, a temperature of 30 °C, and varying amounts of salt (NaCl) ranging from 0 to 150 mM. Table 4.1 lists all of the experimental treatments. The experimental treatments were formulated following a completely randomized design (CRD). In the analysis phase, three replications were randomly chosen for each treatment, and each replication comprised a cohort of 100 individuals. For all treatments, germination took place in the dark for three, four, and five days.

First, nine plastic pots weighing 100 g each of brown rice were measured. In order to eradicate surface bacteria and fungus without harming internal organs, the rice was immersed in 0.1% NaOCl at a 1:2 (w/v) ratio for 30 minutes (Cáceres et al., 2017). Additionally, it underwent five times washes with fresh tap water and was dried for 5 minutes to eliminate any remaining NaClO. Subsequently, aqueous solutions of NaCl at concentrations of 75 mM and 150 mM were created using distilled water for distinct treatments. All rice samples were immersed in the salinity solution (grain to solution ratio of 1:2 w/v) and maintained in an incubator for varying durations at 30°C (Table 4.1).

Table 4.1. Description of treatments

Treatments code	**NaCl Conc. (mM)**	**Temperature (°C)**	**Soaking period (hour)**	**Germination period (day)**
GBR1	0	30	36	3
GBR2	75			
GBR3	150			
GBR4	0			4
GBR5	75			
GBR6	150			
GBR7	0			5
GBR8	75			
GBR9	150			
Control	Non-germinated brown rice (non-GBR)			

After the soaking phase, the seeds underwent a distilled water rinse to eliminate salinity. The trays holding brown rice seeds were then positioned in a dark incubator at 30 °C for 3, 4, and 5 days for the germination process (Table 4.1). A closed system maintained a relative humidity of approximately 65%. To prevent bacterial and fungal invasions, the seeds were washed with distilled water every four hours.

4.2.3. Preparation of Samples

Following germination, GBR underwent three rounds of washing with distilled water and was left to drain for few minutes. Subsequently, the samples were oven-dried for 7 days at 40 °C. For the extraction process, 50 grams of germinated brown rice powder were soaked in 90% methanol for 7 days with two repetitions at room temperature. The resulting extractions were filtered post-centrifugation (15,000 rpm) for 10 minutes at 4 °C. Following this, the methanolic extracts were evaporated at 45 °C to obtain the methanol crude extract. Subsequently, the crude extracts were dissolved in methanol to form stock solutions with a concentration of 40 mg/mL for further experiments.

4.2.4. Antioxidant Assays

4.2.4.1. Reducing Power Assay

The assessment of reducing capacity was conducted following the procedure outlined by Quan et al. (2019d). Initially, 100 µL of extracts was combined with 250 µL of 0.2 M phosphate buffer (pH 6.6), and 250 µL of potassium ferricyanide (1%, w/v). Subsequently, the mixture was incubated at a temperature of 50 °C for 30 minutes. Following this, 250 µL of trichloroacetic acid (10%, v/v) was introduced to halt the reaction, and the solution was centrifuged at 4000 rpm for 10 minutes. Finally, the supernatant (100 µL) was diluted with distilled water (100 µL), and 20 µL of ferric chloride ($FeCl_3$) solution (0.1%, w/v) was added. After thorough shaking, the absorbance was measured at 700 nm using a microplate reader (MultiskanTM Microplate Spectrophotometer, Thermo Fisher Scientific, Osaka, Japan). BHT served as the positive control, and the IC_{50} value, indicating reducing power, was calculated as described earlier, with a lower IC_{50} value signifying higher reducing power.

4.2.4.2. β-Carotene Bleaching Assay

To evaluate the antioxidant activity of the extracts, the β-carotene bleaching activity was measured using a β-carotene linoleate bleaching system following the procedure outlined by Tuyen et al., (2017) with slight adjustments. In summary, a mixture of 100 µL β-carotene (200 µg/mL in chloroform), 20 µL linoleic acid, and 200 mg Tween-40 was created in a round-bottom flask. The chloroform was evaporated from this mixture at 40 °C, followed by the slow addition of 50 mL oxygenated water to form a stable emulsion through vigorous shaking. Prior to each experiment, a fresh emulsion was prepared. Subsequently, 25 µL of the sample or control (1000 µg/mL in methanol) and 200 µL of the emulsion solution were combined in each well of a 96-well plate. The solution underwent incubation at 45 °C, and the absorbance was

measured at 492 nm. Extracts were weighed at 15-minute intervals for a total duration of 180 minutes. The inhibition of lipid peroxidation (LPI) was measured using the following equation:

$$\text{Lipid peroxidation inhibition (\%)} = A_{180}/A_0 \times 100 \quad (4.1)$$

Here, A_{180} represents the absorbance recorded after 180 minutes of incubation, and A_0 denotes the absorbance measured at the initial zero-minute mark for the test sample. Methanol served as the negative control, whereas BHT functioned as the positive control. A greater LPI value signifies stronger antioxidant activity.

4.2.5. Enzymatic Assays

4.2.5.1. α–Amylase Inhibition Assay

The inhibitory activity against α-amylase was assessed using the method of starch-iodine, as described by Quan et al. (2019c), with minor adjustments. GBR extracts were dissolved in 0.2 M phosphate buffer saline (pH 6.9) along with α-amylase solution (2 mg/mL) derived from porcine pancreas (type VI-B, Sigma-Aldrich, St. Louis, MO, USA). Dissolvable starch (0.5%) solutions and iodine (0.25 mM) were prepared in deionized water. Initially, 20 µL of α-amylase were diluted and subjected to incubation with the GBR extract sample at 37 °C for a duration of 9 minutes. Subsequently, 30 µL of starch (0.5%) solution was added and incubated for an additional 7 minutes at 37 °C. Following this, 20 µL of 1M HCl and 100 µL of iodine solution were introduced to initiate the reaction. The resulting mixture was analyzed at a wavelength of 565 nm using a microplate reader. The IC_{50} value for GBR's impact on α-amylase was established through the calculation of inhibition percentage using the following formula:

$$\text{(\%) inhibition} = (A - C) / (B - C) \times 100 \quad (4.2)$$

Here, A represents the absorbance of the reaction in the presence of the sample, B is the absorbance of the reaction without the enzyme, and C is the absorbance of the reaction in the absence of the enzyme. Acarbose served as the positive reference.

4.2.5.2. α-Glucosidase Inhibition

The inhibitory effect of GBR extracts on α-glucosidase activity was determined through a method described by Johnson et al. (2011) with minor modifications. Initially, 20 µL of GBR extract in 40 µL of 0.1 M potassium phosphate buffer (pH 7) were pre-mixed with 20 µL of 0.5 U/mL α-glucosidase enzyme (derived from Saccharomyces cerevisiae, Sigma Aldrich, St Louis, MO, USA). Following a 5-minute incubation at room temperature, a 20 µL portion of 5 mM p-nitrophenyl-α-D-glucopyranoside (pNPG) substrate in the buffer was introduced. The

resulting mixture underwent an additional 10-minute incubation at room temperature. Ultimately, the reaction was terminated by adding 100 µL of Na_2CO_3, and the resulting mixture was measured at a wavelength of 405 nm using a microplate reader. The inhibition percentage and IC_{50} value were computed using the specified formula:

$$\% \text{ inhibition} = (1 - A_s/A_c) \times 100 \quad (4.3)$$

where As is the absorbance of the reaction with sample or standard inhibitor (acarbose) and Ac is the absorbance of the reaction with DMSO as a negative control.

4.2.5.3. Tyrosinase Inhibition

The procedure employed solutions of potassium phosphate buffer (20 mM, pH = 6.8), tyrosinase (500 units/mL in buffer), and L-tyrosine substrate (2 mM in distilled water). Samples or controls were prepared in DMSO. Initially, 20 µL of the sample or control and 20 µL of the enzyme solution were mixed in 120 µL of buffer. The mixture underwent incubation at 25 °C for 5 minutes. Subsequently, the enzymatic reaction commenced with the addition of 50 µL of L-tyrosine solution. After a 10-minute incubation under the same conditions, the absorbance of the final solution was measured at 470 nm (Quan et al., 2019). DMSO served as the negative control, while kojic acid was utilized as the standard inhibitor.

$$\% \text{ inhibition} = (C - S)/C \times 100 \quad (4.4)$$

Where C represents the absorbance of the reaction with DMSO after subtracting the control's blank (absence of enzymatic reaction on the substrate), and S denotes the absorbance of the reaction with the sample or inhibitor after subtracting the sample's blank (absence of enzymatic reaction on the substrate). IC_{50} values of samples against tyrosinase were determined using the same method employed for the anti-radical assay.

4.2.6. Identification of Phytochemical Components by GC-MS

The GC-MS system (JMS-T100 GCV, JEOL Ltd., Tokyo, Japan) was utilized to examine the phytochemical constituents of the methyl extract obtained from GBR. This system featured a DB-5MS column (30 m × 0.25 mm I.D. × 0.25 µm film thickness) provided by Agilent Technologies, J&W Scientific Products, Folsom, United States. The sample, prepared at a concentration of 1 mg/mL, underwent injection via an autosampler within the system. Helium functioned as the carrier gas, maintaining a split ratio of 5:1. The temperature profile of the GC oven encompassed an initial temperature of 50 °C without holding, followed by a gradual temperature increase of 10 °C per minute for 30 minutes, and a subsequent 20-minute

maintenance period. The injector and detector temperatures were set at 300 °C and 320 °C, respectively. Mass scanning across the range of 29 to 800 amu was employed to capture the mass spectra. The analysis yielded information for each identified compound, encompassing chromatogram results, linear retention index (LRI), mass spectrum (electron ionization), and Kovats index (KI). JEOL Ltd. Software version 2.65a, Tokyo, Japan, was utilized for the control of the GC-MS system and data peak analysis (Andriana et al., 2019).

4.3. Results and Discussion

4.3.1. Antioxidant Activity by the Reducing Power and β-Carotene Bleaching Assay in GBR

The antioxidant capacity of extracts from Germinated Brown Rice (GBR) was thoroughly assessed using both reducing power (RP) and β-carotene bleaching (βC) assays, providing insights into the varied antioxidant capabilities of various GBR samples. The distinctive trends in reducing power and β-carotene bleaching inhibition among the GBR extracts are revealed through the IC_{50} values presented in Table 4.2.

GBR5 emerges as a standout performer in both assays, showcasing the lowest IC_{50} value in the RP assay and the highest βC assay value. The remarkable reducing power of GBR5 (2.07 mg/mL) implies its potential to donate electrons and counteract oxidative stress. Simultaneously, the high β-carotene bleaching inhibition (94.56% LPI) suggests the presence of bioactive compounds capable of protecting against lipid peroxidation. GBR4 also exhibits noteworthy antioxidant activity in both assays, emphasizing its potential as a natural source of antioxidants. Conversely, GBR3 lags behind in reducing power and β-carotene bleaching, indicating variations in the antioxidant profiles of different GBR samples. These differences could stem from variations in germination conditions, rice varieties, or other factors influencing the bioactive composition of GBR. For the purpose of assessing the effectiveness of GBR extracts, a comparison with the control group and BHT is essential. In both tests, GBR5 outperforms the control group, suggesting that it may be a more potent natural antioxidant. But BHT's lowering power is still higher, indicating that synthetic antioxidants might still be advantageous in some particular situations. Notably, GBR extracts—especially GBR5—show competitive suppression of β-carotene bleaching that is on par with BHT. This discovery highlights GBR's potential as a natural substitute for artificial antioxidants, with potentially positive effects on nutrition and health.

Table 4.2. Antioxidant activity IC_{50} value (mg/mL) of GBR extracts

Treatment	RP assay	βC assay (% LPI)
GBR1	6.04 ± 0.16^{cd}	68.95 ± 2.20^{cd}
GBR2	6.15 ± 0.12^{cd}	80.94 ± 1.74^{ef}
GBR3	9.54 ± 0.24^{f}	73.38 ± 1.29^{bcd}
GBR4	5.28 ± 0.12^{c}	76.80 ± 1.74^{f}
GBR5	2.07 ± 0.07^{a}	94.56 ± 4.35^{a}
GBR6	4.38 ± 0.118^{b}	72.80 ± 1.77^{bcd}
GBR7	6.78 ± 0.07^{cd}	58.45 ± 2.73^{f}
GBR8	6.43 ± 0.43^{cd}	86.96 ± 1.29^{de}
GBR9	6.47 ± 0.08^{cd}	71.07 ± 1.61^{bcd}
Control	7.22 ± 0.03^{e}	87.63 ± 2.15^{b}
BHT	0.05±0.01	90.00±1.13

Data express means ± SD (standard deviation); Different superscript letters (^{a,b,c,d,e,f}) in a column indicate significant differences at $p < 0.05$; RP, reducing power; βC, β-carotene bleaching; BHT: butylated hydroxytoluene; IC_{50} means half-maximal inhibitory concentration.

In conclusion, the antioxidant activity of GBR extracts varies significantly among samples, with GBR5 standing out as a promising source of natural antioxidants. The observed variations emphasize the importance of selecting the appropriate GBR sample for deriving optimal antioxidant benefits. Further research into the specific bioactive compounds responsible for the observed effects will provide valuable insights into the potential health benefits of GBR consumption and its role as a functional food.

4.3.2. α-Amylase, α-Glucosidase and Tyrosinase Inhibitory Activity of GBR

An extensive analysis of the inhibitory effects of GBR extracts against major enzymes involved in the digestion of carbohydrates (α-amylase, and α-glucosidase) and the formation

of melanin (tyrosinase) sheds light on the potential health advantages of GBR. The IC_{50} values in Table 4.3. highlight the wide range of bioactivity of these extracts and show clear differences amongst GBR samples.

Table 4.3. α-Amylase, α-glucosidase and tyrosinase inhibitory activity IC_{50} value (mg/mL) of GBR extracts

Treatment	α-Amylase	α-Glucosidase	Tyrosinase
GBR1	2.15 ± 0.04^{bc}	2.01 ± 0.91^{c}	ND
GBR2	2.15 ± 0.06^{bc}	1.20 ± 0.91^{bc}	3.21 ± 0.05^{c}
GBR3	1.28 ± 0.05^{b}	ND	2.68 ± 0.07^{bc}
GBR4	1.15 ± 0.02^{b}	1.15 ± 0.80^{b}	ND
GBR5	0.69 ± 0.03^{a}	0.15 ± 0.01^{a}	0.44 ± 0.01^{a}
GBR6	1.22 ± 0.06^{b}	1.93 ± 0.36^{bc}	0.85 ± 0.02^{b}
GBR7	1.19 ± 0.03^{b}	ND	ND
GBR8	2.01 ± 0.03^{bc}	1.34 ± 0.52^{bc}	1.84 ± 0.05^{c}
GBR9	2.14 ± 0.02^{bc}	ND	3.87 ± 0.06^{c}
Control	4.21 ± 0.50^{d}	ND	ND
Acarbose	0.26±0.08	2.48±0.13	0.02 ± 0.00

Data express means ± SD (standard devivation); ND, not determined. Different superscript letters (^{a,b,c,d}) in a column indicate significant difference at $p < 0.05$; IC_{50} means half-maximal inhibitory concentration.

In all three tests, GBR5 is the sample that stands out the most and exhibits the strongest inhibitory properties. The assays for α-amylase (0.69 mg/mL), α-glucosidase (0.15 mg/mL), and tyrosinase (0.44 mg/mL) reveal that GBR5 has low IC_{50} values, making it a viable option for treating diseases pertaining to skin pigmentation and carbohydrate metabolism. This shows that GBR5 contains bioactive substances that significantly inhibit these enzymes, perhaps adding to its therapeutic potential. Notable inhibitory properties are also shown by GBR4 and

GBR6, suggesting that these proteins may be used to modify the actions of enzymes. The observed discrepancies between GBR samples could be explained by variances in the germination procedure, types of rice, or particular bioactive substances found in each sample. A more thorough understanding of the variables determining the inhibitory effects of GBR extracts may be possible with additional research into the compositional variations among them. The potential health benefits of GBR in enzyme inhibition are highlighted by the comparison with the control group. In all three tests, GBR5, in particular, performs better than the control group, demonstrating its effectiveness in reducing the breakdown of carbohydrates and the production of melanin. This supports the idea that GBR contains bioactive chemicals that can modulate important physiological processes and is a natural dietary component. Moreover, the analogy with the well-known pharmaceutical drug Acarbose highlights the competitive inhibitory properties of GBR extracts, particularly GBR5. Given that GBR extracts can equal or even outperform Acarbose in its effects, there may be a place for GBR as a natural treatment option for disorders involving the metabolism of carbohydrates. This highlights the importance of GBR in conventional diets and provides opportunities for the development of functional foods with potential therapeutic applications.

Finally, the inhibitory actions demonstrated by GBR extracts against α-amylase, α-glucosidase, and tyrosinase underscore their potential as functional foods with medicinal implications for ailments associated with skin pigmentation and carbohydrate metabolism. Particularly GBR5, which continuously performs well, calls for more research into the bioactive components of GBR to fully comprehend the health-promoting qualities of GBR. The observed differences amongst GBR samples highlight the necessity of focused research into certain substances that cause these inhibitory effects.

4.3.3. GC-MS results for GBR

The GC-MS analysis of treatment GBR4 (75 mM NaCl, 30 °C, and 36 h) detected four predominant compounds, while treatment GBR5 (150 mM NaCl, 30 °C, and 36 h) revealed five major compounds, as outlined in Table 4.4. Notably, in treatment GBR5, palmitic acid emerged as the most abundant compound, constituting 27.60% of the peak area, surpassing its presence in sample GBR4. The determination of Kovats index (KI) and linear retention index (LRI) involved comparisons with existing literature. The quantification results indicated palmitic acid levels of 4.56 mg per g of GBR dry weight for treatment GBR4 and 5.48 mg per g for treatment GBR5.

Table 4.4. Dominant phytochemicals in methanolic extract from GBR identified by GC-MS

Treatment	No	Identified Compounds	Chemical Formula	Chemical Classification	Retention Time	Area (%)	Content mg/g DW
GBR4	1	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	Fatty acid ester	16.7	20.75	-
	2	Palmitic acid	$C_{16}H_{32}O_2$	Fatty acid	17.02	19.5	4.56
	3	9,12-Octadecadienoic acid, methyl ester, (E,E)-	$C_{19}H_{34}O_2$	Fatty acid methyl ester	18.32	19.75	-
	4	9-Octadecenoic acid (Z)-, methyl ester	$C_{19}H_{36}O_2$	Fatty acid ester	18.38	12.17	-
GBR5	1	Pentanoic acid	$C_5H_{10}O_2$	Fatty acid	14.93	1.75	-
	2	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	Fatty acid ester	16.69	7.53	-
	3	Palmitic acid	$C_{16}H_{32}O_2$	Fatty acid	17.02	27.6	5.48
	4	9-Dodecenoic acid, methyl ester, (E)-	$C_{13}H_{24}O_2$	Fatty acid methyl ester	18.38	5.28	-
	5	Linoelaidic acid	$C_{18}H_{32}O_2$	Fatty acid	18.65	11.27	-

RT, retention time; LRI, linear retention index; KI, Kovats index; DW, dry weight; -, not determined.

4.5. Conclusions

This study explored the impact of specific stress conditions namely, 75 mM NaCl salinity and 4-day germination periods on germinated brown rice. The outcomes unveiled notable antioxidant activities and robust inhibitory effects against key enzymes in the samples subjected to these conditions. Furthermore, quantification results underscored the presence of palmitic acid in GBR samples, indicating concentrations of 3.38 and 4.47 mg/g of GBR dry weight in treatments with 0 mM and 75 mM salinity, both accompanied by 4-day germination periods. These findings collectively suggest that the applied germination conditions amplify the nutritional and therapeutic potential of brown rice, presenting promising opportunities for integration into functional foods and dietary strategies.

CHAPTER V.

MOMILACTONES AND PHENOLICS IN BROWN RICE: ENRICHMENT, OPTIMIZED EXTRACTION, AND POTENTIALS FOR ANTIOXIDANT AND ANTI-DIABETIC ACTIVITIES

5.1. Introduction

Nowadays, consumers are increasingly inclined towards pigmented rice varieties due to their abundance in bioactive constituents, such as antioxidants and anti-inflammatory elements, as pointed out by Alves et al. (2016). Brown rice (BR), in particular, not only acts as a nutritional powerhouse but also possesses a remarkable array of bioactive compounds contributing to human health benefits (Ravichanthiran et al., 2018; Sukegawa et al., 2021; Wu et al., 2023). BR is rich in phenolics and flavonoids, demonstrating potential properties that combat oxidative stress and inflammation in the body (Gong et al., 2017; Tyagi et al., 2022). Research indicates that these compounds might contribute to lowering the risk of chronic diseases and fostering general well-being (Barber et al., 2020). The primary source of these bioactive compounds is the outer layers of the grain, which are preserved in brown rice, in contrast to white rice where processing removes them (Verardo et al., 2016). Therefore, choosing brown rice (BR) as a nutritious and adaptable food choice has gained popularity recently. However, due to the dense composition of its outer bran layer, BR often has a firmer texture, making it more challenging to cook and less easily digestible compared to white rice. As a result, germinated brown rice (GBR) has emerged as a viable alternative, offering additional benefits while preserving its nutritional value. The quality of BR is enhanced through increased water absorption in the outer kernel, resulting in a softer texture. Moreover, during the germination stage, enzymatic processes lead to the modification of bioactive compounds through interactions among carbohydrates and proteins in the endosperm (Alves et al., 2016; Gong et al., 2017). Consequently, germinated brown rice (GBR) is recognized for having elevated levels of bio-functional components like γ-aminobutyric acid (GABA) (Ravichanthiran et al., 2018; Tyagi et al., 2022), along with increased quantities of vitamins and amino acids (Verardo et al., 2016), while also exhibiting reduced sugar content (Barber et al., 2020) in comparison with non-GBR. Earlier studies have demonstrated that subjecting GBR

to abiotic stressors and diverse conditions can enhance its nutritional characteristics and lead to the accumulation of bioactive substances and antioxidants. In a previous investigation, GBR treated with 75 mM salinity for 4 days exhibited the highest levels of momilactones and phenolic compounds, aligning with its robust antioxidant activity (Hasan et al., 2023). Therefore, the utilization of abiotic stressors and varied germination conditions represents a promising approach to enhance the nutritional value of brown rice (BR).

Secondary compounds in rice serve crucial roles in terms of both nutritional value and physiological processes, including metabolism, synthesis, and responses to the environment. Of these, tricin, a vital flavonoid, can be detected from different rice organs (e.g., leaves, husks, brans, and grains). Studies have demonstrated tricin's potential for antioxidant (Quan et al., 2019a), for anti-skin aging (Quan et al., 2019a), and as an anticancer activity (Floegel et al., 2011; Quan et al., 2019c; Wongsa et al., 2021). Conversely, phenolic acids, such as caffeic acid, *ρ*-hydroxybenzoic acid, *ρ*-coumaric acid, ferulic acid, salicylic acid, and cinnamic acids are more prevalent in rice and are recognized for their bioactive characteristics, including antioxidant, anti-inflammatory, and anticancer properties (Delbaere et al., 2010). It is noteworthy that these phenolic compounds are primarily concentrated in the bran layer (Wu et al., 2023), making them generally more abundant in BR than in white rice (do Nascimento et al., 2022; Gujral et al., 2012). Furthermore, the quantities of these phenolic compounds in GBR can be twice as high as those found in BR (Wu et al., 2023). In addition, momilactones A (MA) and B (MB) have been acknowledged as valuable diterpene lactones from rice (*Oryza lineage*) (Anh et al., 2023), which exhibit various medicinal properties, including antioxidant (Quan et al., 2019a), anticancer (Kim et al., 2007; Lee et al., 2008; Park et al., 2014), anti-diabetic (Quan et al., 2019a, 2019c), anti-obesity (Quan et al., 2019c), and anti-skin aging (Quan et al., 2019a) potentials.

Brown rice (BR) has showcased its capability to harbor a plethora of valuable bioactive compounds, including phenolics and momilactones (Cho & Lim, 2016; Hasan et al., 2023; Ravichanthiran et al., 2018). In a prior study, it was established that these beneficial compounds experience an enrichment during the germination of brown rice under saline conditions for a span of 4 days (Hasan et al., 2023). However, the influence of cooking on the quantities of phenolics and momilactones and their interrelation has not been definitively clarified. Studies have suggested that the act of cooking plays a role in modulating the concentrations of phenolics and flavonoids in rice, emphasizing the importance of assessing the phytochemical content and related bioactivities in cooked rice (Ti et al., 2015). Alternatively, efficient

extraction techniques for valuable bioactive compounds, particularly momilactones derived from rice, represent a crucial avenue for unlocking their biological advantages. Despite being identified in rice some time ago, the optimization of momilactone extraction methods has seen limited progress. The choice of solvents (such as water, methanol, or ethanol) is a critical factor in ensuring the effective retrieval of the desired compounds during the extraction process. Ahmad et al. (2019) highlighted that the concentrations of MA and MB were higher in extracts utilizing a blend of methanol and water when compared to alternative solvents. Additionally, sonication, a non-thermal processing technique employing high-frequency sound waves to disrupt plant cell walls, creates conducive environments for the liberation of phytochemicals. Studies have shown that sonication-assisted extraction can enhance the availability of antioxidants, MA, MB, phenolic acids, and flavonoids in rice (Bonto et al., 2021; Cui et al., 2010; Zubair et al., 2018). Minh et al. (2018) observed that extracting rice husk at 100 °C elevated the yield of both MA and MB. Notably, MB was obtained with a higher concentration than MA, even though MA is generally more abundant in rice husk than MB.

The aforementioned reasons inspired us to conduct research with the goal of enhancing the levels of phenolics and momilactones in both germinated brown rice (GBR) and non-GBR through a cooking process. The extraction method for these compounds was also fine-tuned by exploring different conditions, including various solvents (80% methanol and 80% ethanol), temperatures (25 °C and 80 °C), and sonication duration (2 hours). Additionally, we examined the antioxidant and antidiabetic potentials of rice samples enriched with phenolics and momilactones.

5.2. Materials and Methods

5.2.1. Materials and Treatments

Brown rice (BR, *Oryza sativa*) grains sourced from two rice varieties, Koshihikari and Milky Queen, were procured from the Japan Agriculture (JA) shop in Hiroshima, Japan. Extraction solvents, methanol, and ethanol were obtained from Junsei Chemical Co., Ltd. (Tokyo, Japan). Standards such as tricin, caffeic acid, *ρ*-hydroxybenzoic acid, *ρ*-coumaric acid, ferulic acid, salicylic acid, and cinnamic acid, along with sodium acetate (CH_3COONa), sodium carbonate (Na_2CO_3), acetonitrile, aluminum chloride ($AlCl_3$), methanol plus, Folin–Ciocalteu reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), potassium persulfate ($K_2S_2O_8$), 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), and sodium hypochlorite (NaClO), were sourced from Kanto Chemical Co., Inc. (Tokyo, Japan). Dimethyl sulfoxide (DMSO) was

obtained from Sigma-Aldrich (St. Louis, MO, USA). Formic acid and distilled water were purchased from Fisher Chemical (Hampton, VA, USA) and EMD Millipore Corporation (Billerica, USA), and Nacalai Tesque (Kyoto, Japan), respectively. The experimental treatments were devised using a completely randomized design (CRD). During the analysis stage, three replications were randomly selected for each treatment, and each replication consisted of a group of 100 individuals.

5.2.2. Germination Process

Rice germination was performed according to the procedure outlined by Hasan et al. (2023). Initially, brown rice (BR) grains underwent sterilization with 0.1% NaOCl for 30 minutes, followed by a thorough wash with clean tap water. Subsequently, 150 g of grains in each pot underwent germination at 30 °C with a 75 mM NaCl salt concentration for a duration of 4 days in darkness. The process was conducted under a relative humidity of 65%.

5.2.3. Cooking Process

After the germination process, both germinated brown rice (GBR) and non-GBR underwent a washing process with distilled water and were left to drain for 5 minutes. Subsequently, the rice underwent uniform cooking using an electric rice cooker (Tiger IH Rice Cooker, Osaka, Japan) under consistent conditions for all samples. Ultrapure distilled water, purified through the Direct-Q® UV Water Purification System (Merck KGaA, Darmstadt, Germany), was utilized in the cooking process, ensuring the removal of impurities, ions, and microorganisms. Details of the samples are provided in Table 5.1.

Table 5.1. Description of treatments

Sample code	Cooking process	Extraction condition	Sample code	Cooking process	Extraction condition
KB1	Non-cooked	Sonication, heat, and 80% methanol	KB7	Non-cooked	Sonication, heat, and 80% ethanol
GKB1	Non-cooked	Sonication, heat, and 80% methanol	GKB7	Non-cooked	Sonication, heat, and 80% ethanol
MQ1	Non-cooked	Sonication, heat, and 80% methanol	MQ7	Non-cooked	Sonication, heat, and 80% ethanol
GMQ1	Non-cooked	Sonication, heat, and 80% methanol	GMQ7	Non-cooked	Sonication, heat, and 80% ethanol
KB2	Cooked	Sonication, heat, and 80% methanol	KB8	Cooked	Sonication, heat, and 80% ethanol
GKB2	Cooked	Sonication, heat, and 80% methanol	GKB8	Cooked	Sonication, heat, and 80% ethanol
MQ2	Cooked	Sonication, heat, and 80% methanol	MQ8	Cooked	Sonication, heat, and 80% ethanol
GMQ2	Cooked	Sonication, heat, and 80% methanol	GMQ8	Cooked	Sonication, heat, and 80% ethanol
KB3	Non-cooked	Sonication and 80% methanol	KB9	Non-cooked	Sonication and 80% ethanol
GKB3	Non-cooked	Sonication and 80% methanol	GKB9	Non-cooked	Sonication and 80% ethanol
MQ3	Non-cooked	Sonication and 80% methanol	MQ9	Non-cooked	Sonication and 80% ethanol
GMQ3	Non-cooked	Sonication and 80% methanol	GMQ9	Non-cooked	Sonication and 80% ethanol
KB4	Cooked	Sonication and 80% methanol	KB10	Cooked	Sonication and 80% ethanol
GKB4	Cooked	Sonication and 80% methanol	GKB10	Cooked	Sonication and 80% ethanol
MQ4	Cooked	Sonication and 80% methanol	MQ10	Cooked	Sonication and 80% ethanol
GMQ4	Cooked	Sonication and 80% methanol	GMQ10	Cooked	Sonication and 80% ethanol
KB5	Non-cooked	Heat and 80% methanol	KB11	Non-cooked	Heat and 80% ethanol
GKB5	Non-cooked	Heat and 80% methanol	GKB11	Non-cooked	Heat and 80% ethanol
MQ5	Non-cooked	Heat and 80% methanol	MQ11	Non-cooked	Heat and 80% ethanol
GMQ5	Non-cooked	Heat and 80% methanol	GMQ11	Non-cooked	Heat and 80% ethanol
KB6	Cooked	Heat and 80% methanol	KB12	Cooked	Heat and 80% ethanol
GKB6	Cooked	Heat and 80% methanol	GKB12	Cooked	Heat and 80% ethanol
MQ6	Cooked	Heat and 80% methanol	MQ12	Cooked	Heat and 80% ethanol
GMQ6	Cooked	Heat and 80% methanol	GMQ12	Cooked	Heat and 80% ethanol

KB: Koshihikari brown rice; GKB: Germinated Koshihikari brown rice; MQ: Milky Queen brown rice; GMQ: Germinated Milky Queen brown rice; Extraction with heat was conducted at 80 °C. Extraction with sonication was conducted for 2 h.

5.2.4. Extraction

After the cooking process, both the cooked and non-cooked samples underwent drying in an oven at 40 °C for 7 days. The resulting dried samples were finely ground into powder. Subsequently, 10 g of the powder was subjected to extraction using two distinct solvents—80% methanol and 80% ethanol. The extraction process involved three different sonication techniques: (i) 2 hours of sonication at 80 °C; (ii) 2 hours of sonication at room temperature (RT); and (iii) 2 hours of heating at 80 °C. Following this, the liquid phase obtained went through filtration before being evaporated at 45 °C to yield crude extracts.

5.2.5. Identification and Quantification of Momilactones A (MA) and B (MB) by Ultra-Performance Liquid Chromatography–Electrospray Ionization-Mass Spectrometry (UPLC–ESI-MS)

The ultra-performance liquid chromatography–electrospray ionization-mass spectrometry (UPLC–ESI-MS) system comprised a mass spectrometer (LTQ Orbitrap XL, Thermo Fisher Scientific, Waltham, MA, USA) with an electrospray ionization (ESI) source. Methanolic sample (3.0 µL) was injected by an autosampler (Vanquish, Thermo Fisher Scientific, Waltham, MA, USA) into a column (1.7 µm, 50 × 2.1 mm i.d.) (Acquity UPLC® BEH C18, Waters Cooperation, Milford, MA, USA) maintained at 25 °C. A gradient mobile phase consisted of solvents A and B, which were trifluoroacetic acid in water (0.1:99.9, v/v) and trifluoroacetic acid in acetonitrile (0.1:99.9, v/v), respectively. The gradient program and MS analysis followed the procedure described in the report of Anh et al. (2022). Calibration curves for MA and MB were established using various standard concentrations (0.5, 1, 5, and 10 µg/mL). Quantities of MA and MB in each sample were determined by applying the peak areas detected in each sample to the respective standard curves.

5.2.6. Identification and Quantification of Phenolic and Flavonoid Compounds by High-Performance Liquid Chromatography (HPLC)

High-performance liquid chromatography (HPLC) was employed for the identification and quantification of tricin, caffeic acid, *ρ*-hydroxybenzoic acid, *ρ*-coumaric acid, ferulic acid, salicylic acid, and cinnamic acid. The HPLC system included a pump (PU-4180 RHPLC, Jasco, Tokyo, Japan), controller (LC-Net II/ADC, Jasco, Japan), and detector (UV-4075 UV/VIS, Jasco, Tokyo, Japan). A column (130 Å, 5 µm, 2.1 × 100 mm) (XBridge BEH Shield RP18, Waters Cooperation, Milford, MA, USA) was used as the stationary phase. The gradient mobile phases, solvent A (0.1% formic acid in water) and solvent B (acetonitrile), were conducted

according to the procedure presented by Anh et al. (2022). The analysis of the compounds, lasting 35 min at room temperature, was operated with peak scanning at 350 nm for tricin and at 280 nm for caffeic acid, *ρ*-hydroxybenzoic acid, *ρ*-coumaric acid, ferulic acid, salicylic acid, and cinnamic acid. Quantification of these compounds was based on the corresponding peak areas.

5.2.7. Antioxidant Activities

The radical scavenging abilities of the samples were determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) assays, following the procedures presented by Anh et al. (2021). In the DPPH assay, 80 µL of methanolic sample, 40 µL of DPPH working solution (0.5 mM), and 80 µL of acetate buffer (0.1 mM, pH 5.5) were mixed and underwent a 20 min incubation at 25 °C in darkness. For the ABTS assay, 20 µL of the methanolic sample and 180 µL of ABTS working solution underwent a 30 min incubation at 25 °C in darkness. The radical scavenging activities (%) were determined by assessing the reduced absorbance at 517 and 734 for the DPPH and ABTS assays, respectively, in comparison with the control (methanol).

$$\text{Radical scavenging activity (\%)} = (Ac - (As - Ab)/Ac) \times 100 \quad (5.1)$$

where Ac is the absorbance of the control, As is the absorbance of the sample, and Ab is the absorbance of blank (without radical) solution.

5.2.8. α-Amylase Inhibition Assay

α-Amylase inhibitory activity was evaluated based on the starch–iodine method demonstrated by Quan et al. (2019c) with slight modifications. The extracts were liquefied in 0.2 M phosphate-buffered saline (pH 6.9). α-Amylase solution (5U) was generated by dissolving α-amylase from porcine pancreas (type VI-B, Sigma-Aldrich, St. Louis, MO, USA) in the buffer. Starch (0.5%) and iodine (0.25 mM) solutions were prepared in deionized distilled water. At first, 20 µL of α-amylase solution was diluted and incubated with 20 µL of sample at 37 °C for 9 min. Thereafter, 30 µL of starch (0.5%) solution was added to the mixture followed by incubation for 7 min at 37 °C. Subsequently, 20 µL of HCl (1 M) and 100 µL of iodine solution were pipetted. The mixture was evaluated at 565 nm using a microplate reader (MR, MultiskanTM Microplate Spectrophotometer, Thermo Fisher Scientific, Osaka, Japan). The inhibition percentage of the sample against α-amylase was calculated by the following formula:

$$\text{Inhibition (\%)} = (A - C) / (B - C) \times 100 \quad (5.2)$$

where A is the absorbance of the reaction with the presence of the sample, B is the absorbance of the reaction without enzyme, and C is the absorbance of the reaction with absence of the sample. Acarbose was used as a standard inhibitor.

5.2.9. α-Glucosidase Inhibition Assay

α-Glucosidase inhibitory activity of the extract sample was evaluated using the method described by Quan et al. (2019c) with few modifications. At the beginning, 20 µL of sample in 40 µL of 0.1 M potassium phosphate buffer (pH 7) was premixed with 20 µL of 0.5 U α-glucosidase enzyme (from Saccharomyces cerevisiae, Sigma Aldrich, St Louis, MO, USA). A 20 µL aliquot of 5 mM p-nitrophenyl-α-D-glucopyranoside (pNPG) substrate (in the buffer) was added after 5 min of incubation at 25 °C. Subsequently, the prepared mixture was incubated for 10 min at 25 °C. Finally, the reaction was suspended by adding 100 µL of 0.1 M Na_2CO_3. The resulting mixture was measured at 405 nm by a MR. The inhibition percentage of the sample against α-glucosidase was calculated by the following formula:

$$\text{Inhibition (\%)} = (1 - As/Ac) \times 100 \quad (5.3)$$

where As is the absorbance of the reaction with sample or standard inhibitor (acarbose) and Ac is the absorbance of the reaction with DMSO as a negative control.

5.2.10. Statistical Analysis

All experiments were replicated three times. Statistical analyses, involving one-way ANOVA, were carried out using Minitab software (Minitab 16.2.3, Minitab Inc., State College, PA, USA). The results are presented as mean ± standard deviation (SD). Additionally, the software was utilized to compute Pearson's correlation coefficients among the parameters under examination.

An ordinary least squares (OLS) regression analysis was conducted using the following equation to assess the influence of various factors on the extracted momilactone and phenolic contents from both BR and GBR:

$$Y = a_0 + \beta i x i + ui \quad (5.4)$$

where Y = outcome, a_0 = constant, βi = impact (treatment effect), xi = treatment, and ui = error term.

5.3. Results and Discussion

5.3.1. Quantities of Momilactones A (MA) and B (MB) in Rice Samples

Momilactones A (MA) and B (MB) were soon introduced as allelochemicals derived from rice husk (Kato et al., 1973). Correspondingly, preceding studies have mostly aimed at the identification and quantification of MA and MB in strong allelopathic rice plants (Kong et al., 2004; Kong et al., 2006). Recently, they have been reported to have multiple human health beneficial properties, including antioxidant, anti-diabetes, anti-obesity, anti-skin-aging, and anti-cancer properties (Goufo & Trindade, 2014; Wongsa et al., 2021). Previous screening of 99 rice varieties indicated that rice varieties with awns and later maturing times contained higher levels of MA and MB (Chung et al., 2006), but most of the studied cultivars were not commercial rice. Hence, our study focused on two popular commercial rice varieties, Koshihikari and Milky Queen, to enrich their momilactone contents and optimize the extraction of these compounds. Figure 5.1. presents results regarding the contents of MA and MB in germinated brown rice (GBR) and non-GBR of the varieties Koshihikari and Milky Queen through the cooking process and different extraction techniques. Our study revealed a notably higher concentration of MA (147.73 μg/g DW) and MB (118.8 μg/g DW) in Koshihikari compared to Milky Queen where MA (22.59 μg/g DW) and MB (40.09 μg/g DW) were observed. This could be attributed to the genetic diversity among rice varieties. Previous studies have also indicated variations in momilactone contents in different rice varieties (Chung et al., 2006; Xuan et al., 2016). It has been observed that the expression of momilactone biosynthetic genes, including syncopalyl diphosphate synthase-like (OsCPS4), synpimara-7,15-diene synthase-like (OsKSL4), 9-beta-pimara-7,15-diene oxidase-like (CYP99A3), and momilactone A synthase-like (OsMAS and OsMAS2), may vary among rice cultivars, origins, and subtypes Indica and Japonica (Xuan et al., 2016). The activation of these genes may determine different the MA and MB quantities in studied rice cultivars with different characteristics (Xuan et al., 2016).

The current investigation, for the first time, reveals a significant increase in the quantities of MA and MB in GBR compared to non-GBR, as depicted in Figure 5.1.

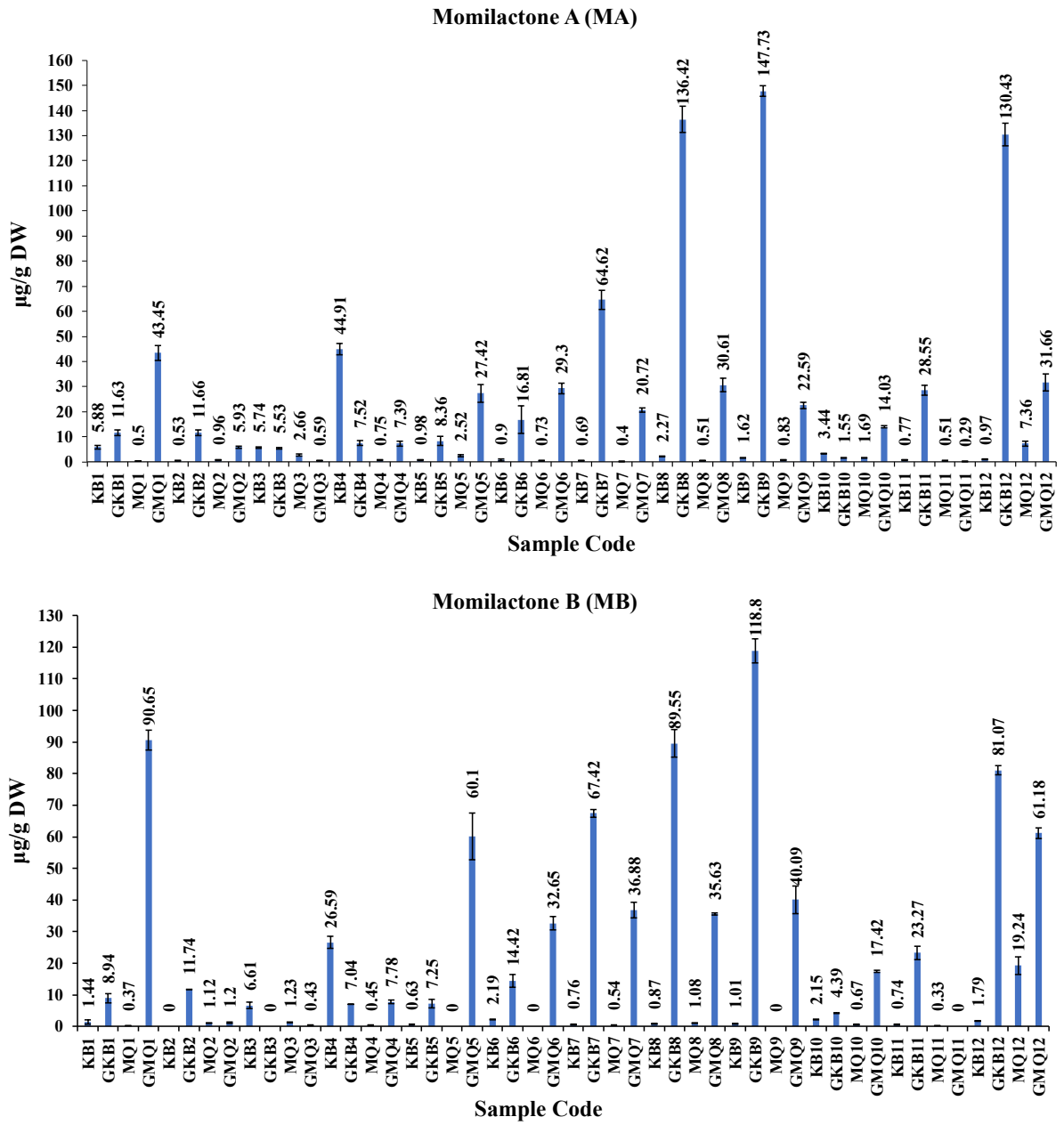

Figure 5.1. Quantities of momilactones A (MA) and B (MB) (µg/g DW) in rice samples. Whiskers enclosed in a column express the standard deviation (SD). DW: dry weight; ND: not detected. The description of sample codes is presented in Table 5.1.

This aspect underscores the importance of considering germination as a factor contributing to the enrichment of MA and MB in BR. This phenomenon could be attributed to the elevated expression of genes associated with momilactone biosynthesis, such as OsCPS4, OsKSL4, CYP99A3, OsMAS, and OsMAS2 (Anh et al., 2022). Germination may trigger the activation of these genes, leading to a higher production of momilactones in GBR, which needs further elaboration. Upon examining the outcomes presented in Figure 5.1., it became evident

that the cooking process resulted in a slight reduction in MA and MB contents (130.43 and 81.07 μg/g DW, respectively) compared to the non-cooking procedure (147.73 and 118.8 μg/g DW, respectively).

In the research of Minh et al. (2018), rice husks subjected to 100 °C, along with a combination of EtOAc and MeOH (v/v), had higher concentrations of both MA and MB compared to untreated rice husks, with MB showing a more significant increase than MA; the most substantial yields of MA (58.76 μg/g DW) and MB (104.43 μg/g DW) were achieved with the EtOAc extract obtained from samples dried at 100 °C for 1 h and subsequently immersed in MeOH at 100% for 1 week (Minh et al., 2018). Our research revealed that utilizing 80% ethanol for an 80 °C heat extraction significantly increased the concentrations of MA (130.43 μg/g DW) and MB (81.07 μg/g DW) in cooked Koshihikari GBR compared to other samples. The use of 80% ethanol, coupled with heat at 80 °C, proved to be an effective approach for extracting MA and MB, emphasizing the importance of solvent and temperature conditions in optimizing the extraction process from BR. On the other hand, our research indicated that 80% ethanolic extraction and 2 h of sonication at room temperature are more efficient for quantifying MA (147.73 μg/g DW) and MB (118.80 μg/g DW) in brown rice.

As previously mentioned, the potential roles of MA and MB in preventing chronic diseases and cancer have been being explored. Several potential approaches, including induced gene expression, metabolic engineering techniques, and genetic modifications, have been proposed to enhance the biosynthesis of MA and MB within rice sources to increase their exploitable value (Bourgaud et al., 2001). For instance, a study highlighted that N-methyl-N-nitrosourea mutations could lead to increased accumulation of MA and MB in mutated rice lines (Kakar et al., 2019). In this study, we propose, for the first time, several methods to enrich and extract MA and MB from BR. Among the 48 samples, non-cooked Koshihikari GBR extracted using 80% ethanol and 2 h sonication at room temperature (RT) demonstrated the highest concentrations of MA and MB (147.73 and 118.8 μg/g DW, respectively), and non-cooked Milky Queen BR extracted using 80% ethanol and 2 h sonication at 80 °C demonstrated the lowest concentrations of MA and MB (0.4 and 0.54 μg/g DW, respectively), as detailed in Figure 5.1.

5.3.2. Contents of Tricin, Caffeic acid, ρ-Hydroxybenzoic Acid, ρ-Coumaric Acid, Ferulic Acid, Salicylic Acid, and Cinnamic Acid in Rice Samples

The results in Figure 5.2. show that cooked Koshihikari GBR extracted using 80% MeOH and sonication at RT (GKB4) exhibited the highest concentrations of tricin, caffeic acid, *ρ*-hydroxybenzoic acid, *ρ*-coumaric acid, ferulic acid, salicylic acid, and cinnamic acid (1714.39, 1011.24, 616.58, 452.88, 942.47, 2495.77, and 369.27 µg/g DW, respectively). Conversely, non-cooked Milky Queen BR extracted using 80% EtOH and sonication at 80 °C showed the lowest contents of these phenolic compounds (23.64, 33.33, 0.93, 15.56, 16.97, 23.39, and 1.99 µg/g DW, respectively) (Figure 5.2). According to Tian et al. (2004), the swift rise in free phenolic acid during seed germination is primarily attributed to the activation of endogenous esterase. The elevation of bound phenolic acids in GBR is likely a result of polymerization originating from free phenolics, as suggested by Ti et al. (2014). The increased concentration of free phenolic acid in GBR may be attributed to the protective role of rice, preventing the loss and oxidation of free phenolic acid.

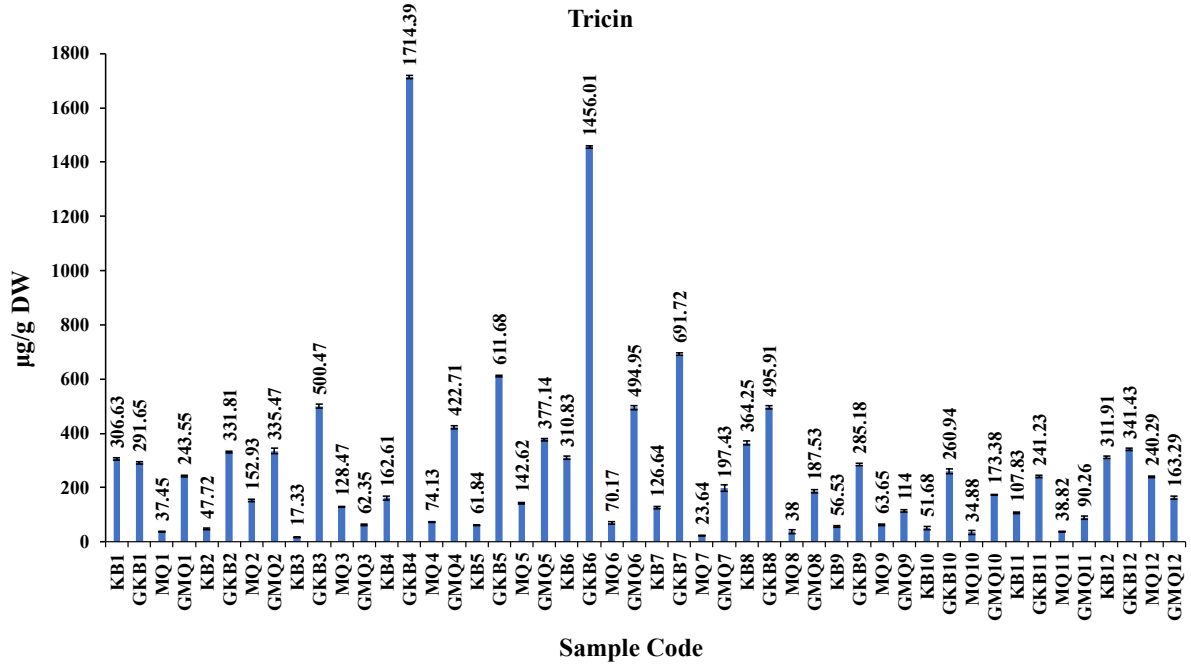

Figure 5.2a. Quantities of tricin, (µg/g DW) in rice samples.

Whiskers enclosed in a column express the standard deviation (SD). DW: dry weight; ND: not detected. The description of sample codes is presented in Table 5.1.

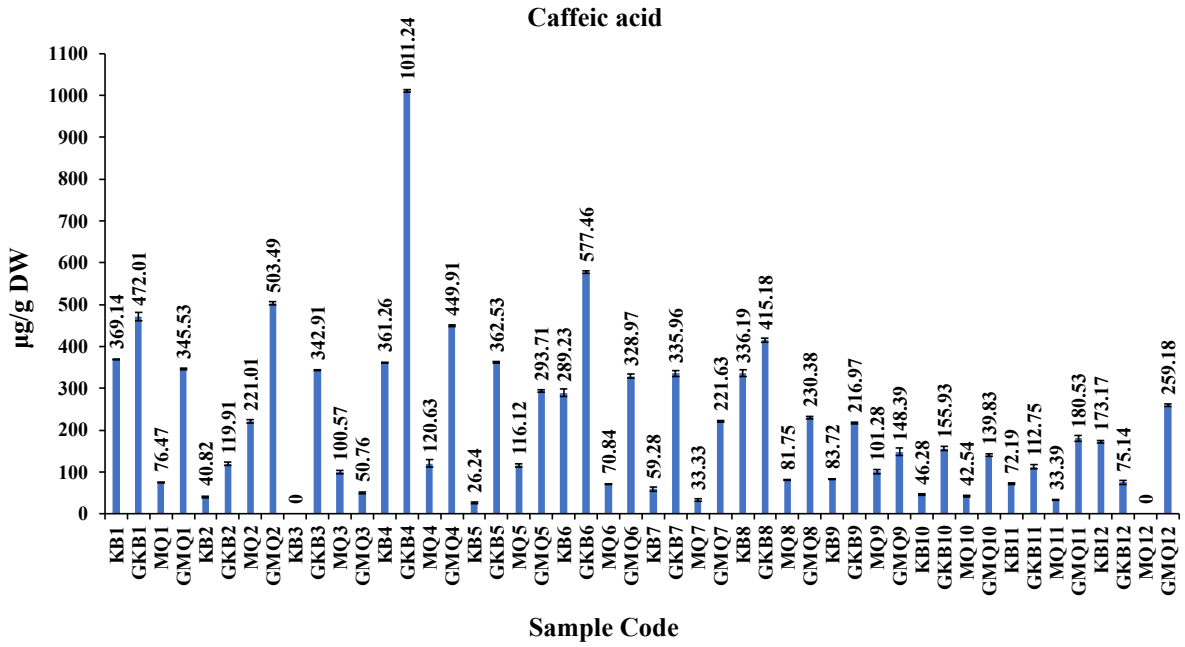

Figure 5.2b. Quantities of caffeic acid (µg/g DW) in rice samples.

Whiskers enclosed in a column express the standard deviation (SD). DW: dry weight; ND: not detected. The description of sample codes is presented in Table 5.1.

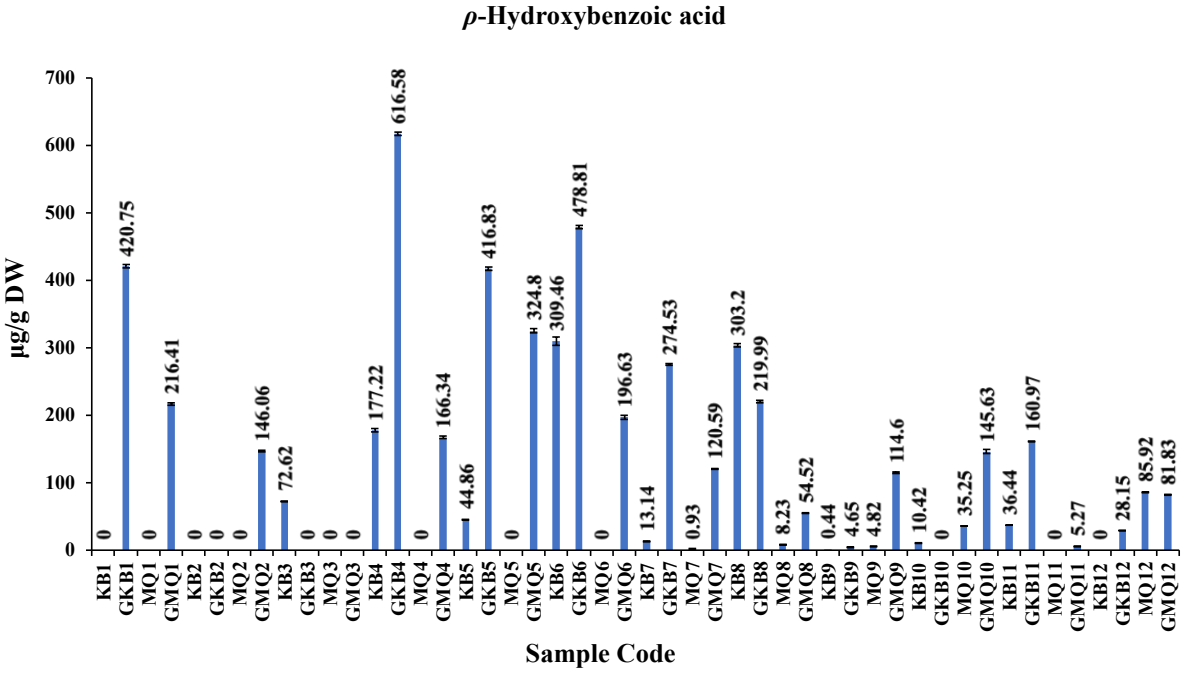

Figure 5.2c. Quantities of *ρ*-hydroxybenzoic acid (µg/g DW) in rice samples.

Whiskers enclosed in a column express the standard deviation (SD). DW: dry weight; ND: not detected. The description of sample codes is presented in Table 5.1.

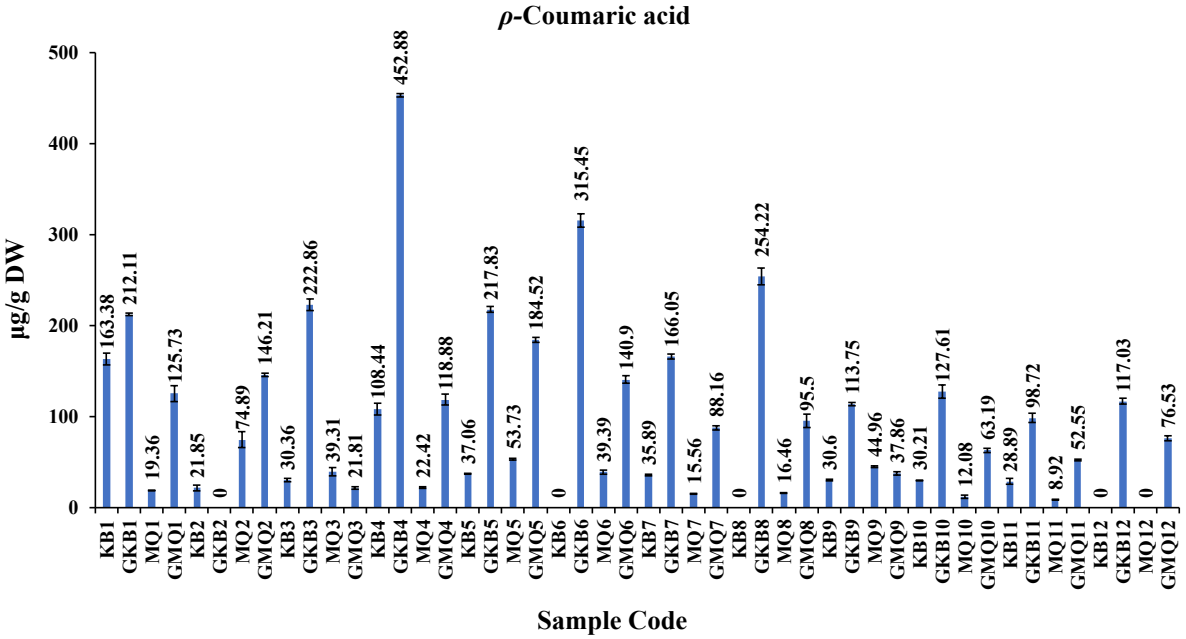

Figure 5.2d. Quantities of *ρ*-coumaric acid (µg/g DW) in rice samples.

Whiskers enclosed in a column express the standard deviation (SD). DW: dry weight; ND: not detected. The description of sample codes is presented in Table 5.1.

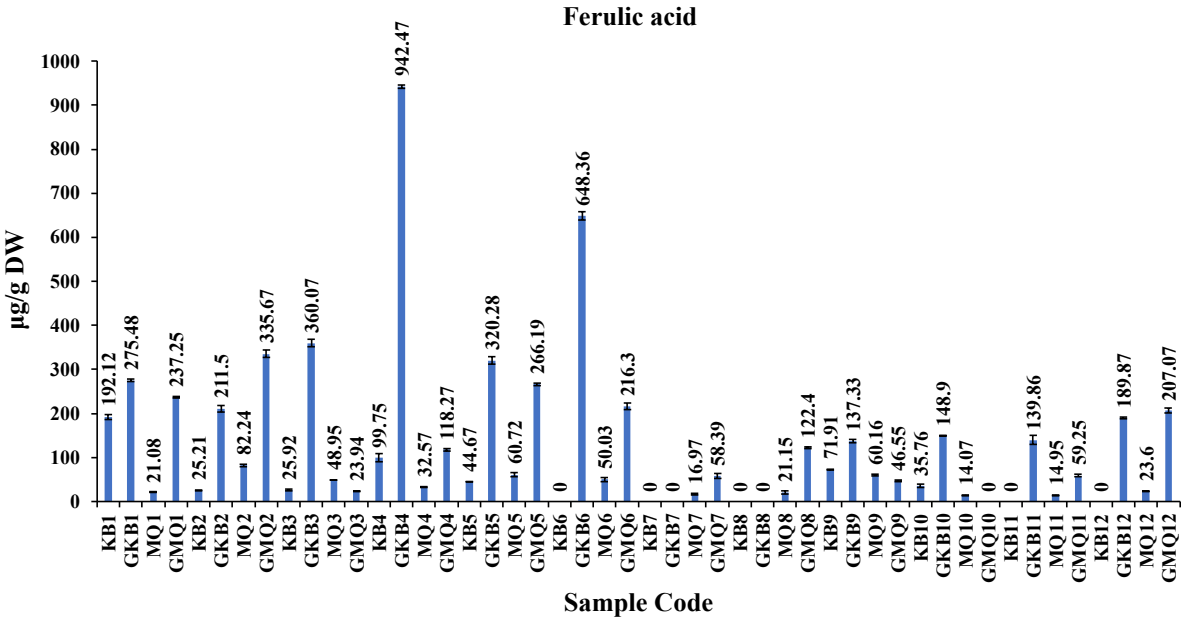

Figure 5.2e. Quantities of ferulic acid (µg/g DW) in rice samples.

Whiskers enclosed in a column express the standard deviation (SD). DW: dry weight; ND: not detected. The description of sample codes is presented in Table 5.1.

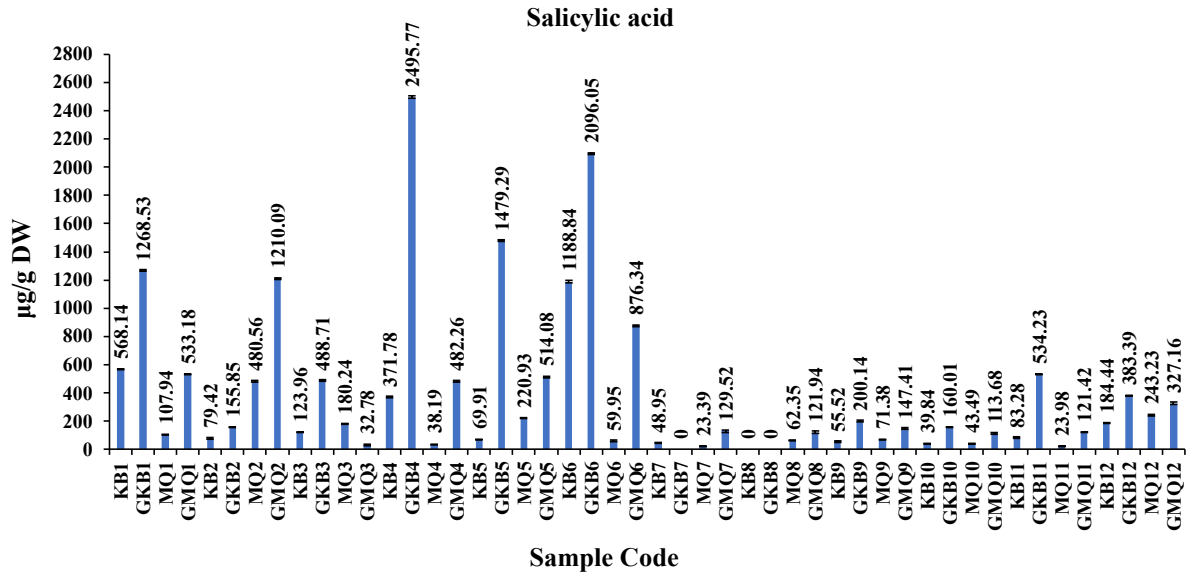

Figure 5.2f. Quantities of salicylic acid (µg/g DW) in rice samples.

Whiskers enclosed in a column express the standard deviation (SD). DW: dry weight; ND: not detected. The description of sample codes is presented in Table 5.1.

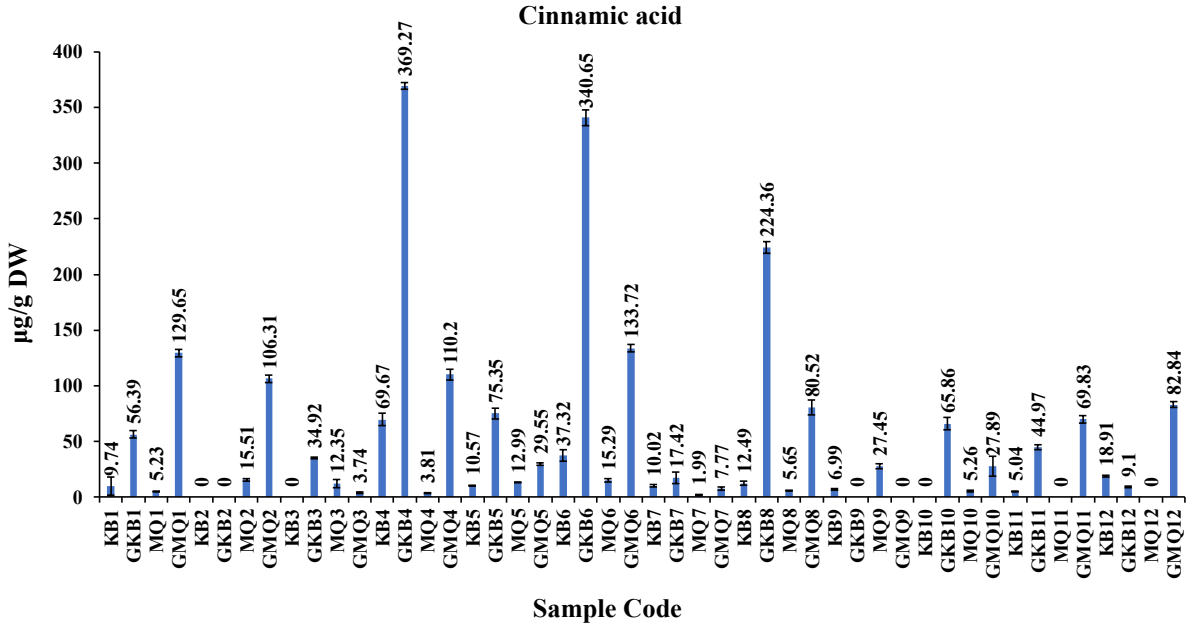

Figure 5.2g. Quantities of cinnamic acid (µg/g DW) in rice samples.

Whiskers enclosed in a column express the standard deviation (SD). DW: dry weight; ND: not detected. The description of sample codes is presented in Table 5.1.

In the previous study, tricin, *ρ*-coumaric acid, ferulic acid, salicylic acid, and cinnamic acid (107.63, 93.77, 139.03, 46.05, and 596.26 µg/g DW, respectively) exhibited ascending concentrations in GBR (Hasan et al., 2023). In the current study, all these compounds

significantly in-creased after cooking, with the highest amounts found in sample GKB4 (cooked Koshihikari GBR, extracted using 80% methanol with 2 h of sonication at RT). For extraction, water, methanol, 80% acetone, and 80% ethanol were identified as more effective solvents for obtaining phenolic and flavonoid compounds and antioxidant capacities (Ly & Lee, 2014; Thi Phuong Lien, 2015). However, the current study indicated that 80% methanolic extract was relatively more effective in acquiring phenolics and flavonoids. It is possible that the inclusion of other controls in the study, such as germination, the cooking process, and sonication, has contributed to the higher levels of phenolics and flavonoids in BR.

5.3.3. Average Values of Momilactones A (MA) and B (MB), Phenolic Acids, and Flavonoids in Rice Varieties under Germination Conditions, Cooking Processes, and Extraction Methods

Figure (5.3–5.6), presents the average values of MA, MB, tricin, caffeic acid, *ρ*-hydroxybenzoic acid, *ρ*-coumaric acid, ferulic acid, salicylic acid, and cinnamic acid in Koshihikari and Milky Queen under different germination conditions, cooking processes, and extraction procedures. The outcomes showed that Koshihikari had higher contents of MA, MB, tricin, caffeic acid, *ρ*-hydroxybenzoic acid, *ρ*-coumaric acid, ferulic acid, salicylic acid, and cinnamic acid than Milky Queen (Figure 5.3).

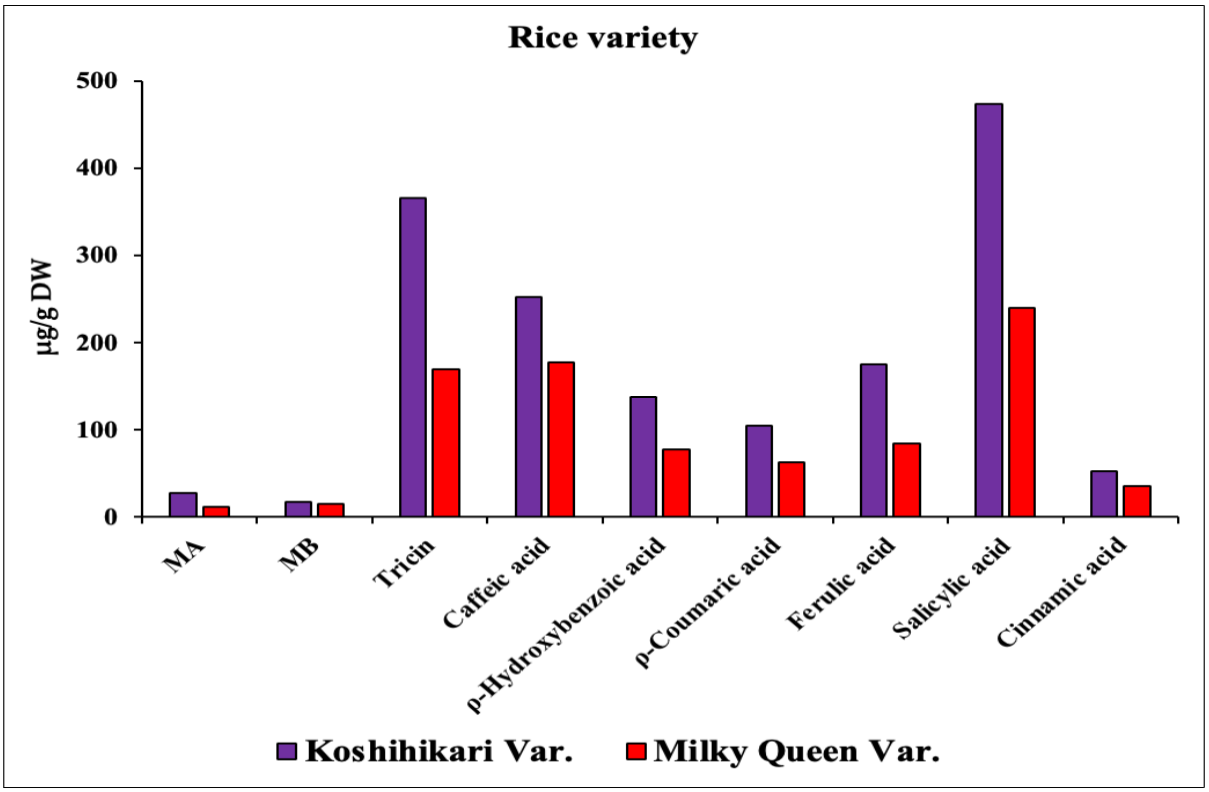

Figure 5.3. Average contents of momilactones A (MA) and B (MB), phenolic acids, and flavonoids among rice varieties.

In Figure 5.4, it is evident that GBR contained higher amounts of all compounds compared to non-GBR. Our findings could contribute to enhancing the value of GBR, which can be directly consumed as a component in dishes, harnessing its health-beneficial properties. Although less common than white rice, GBR might have been consumed since ancient times (Patil & Khan, 2011). For example, the total consumption of soaked GBR in Japan is approximately 9 tons annually (Patil & Khan, 2011). GBR is also served in restaurants and frequently featured in health and fashion magazines. Both older and younger generations, particularly those interested in health, highly appreciate GBR (Patil & Khan, 2011). It is utilized in various products, such as rice balls, soups, bread, pastries, rice burgers, etc., often combined with other ingredients. GBR has been incorporated into numerous cuisines worldwide, such as Italian risotto, Spanish paella, Brazilian feijoada, etc. (Patil & Khan, 2011).

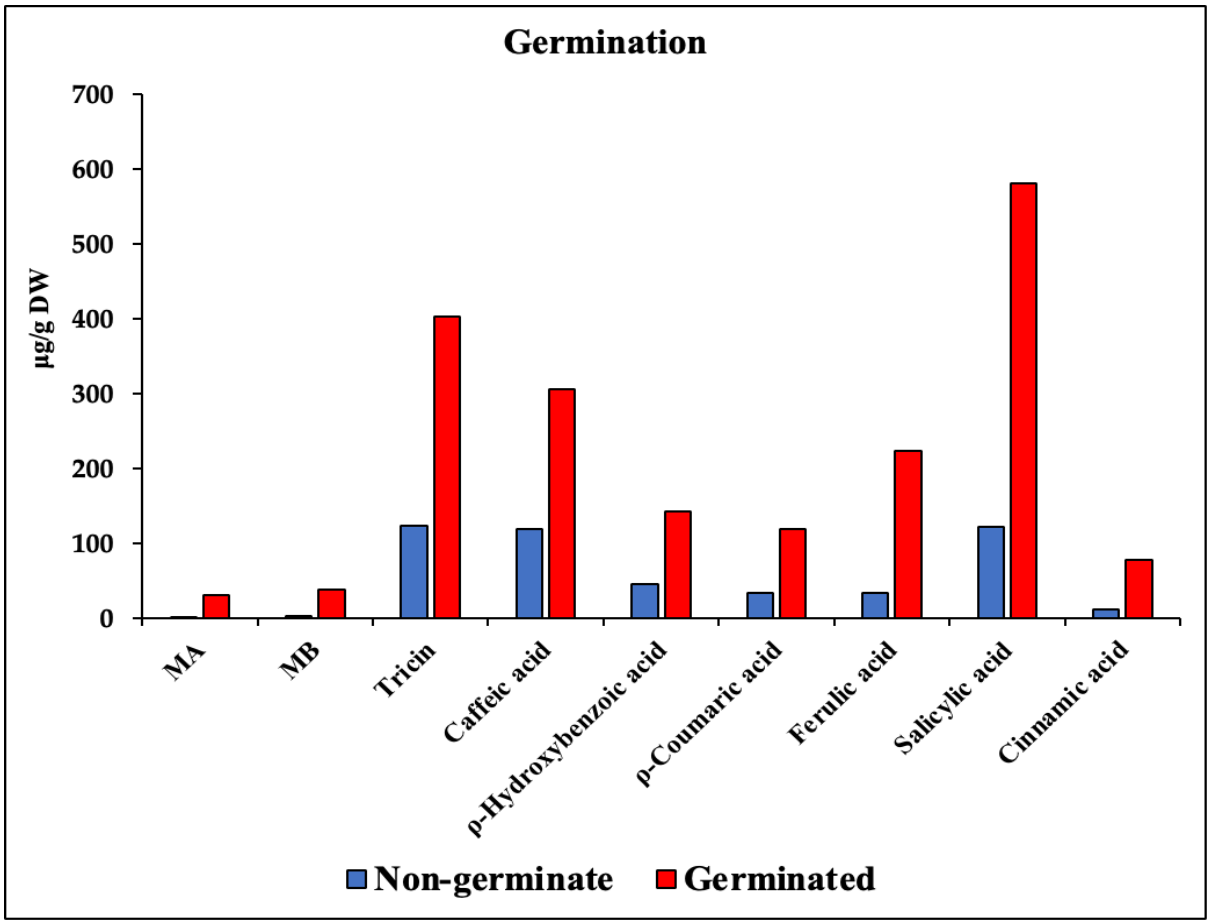

Figure 5.4. Average contents of momilactones A (MA) and B (MB), phenolic acids, and flavonoids among germination conditions.

During the use of rice as a food source, the cooking process induces changes in its physical, chemical, and biological composition, consequently altering its structural characteristics and nutritional contents, as reported by Ma et al. (2023). Beneficial effects of

cooking include enhanced digestibility and food detoxification, contributing to safer consumption for humans (Palermo et al., 2014). Conversely, cooking can modify the levels of bioactive compounds, thereby altering the biological value and nutritional profile of the food (da Silva Lindemann et al., 2021). Therefore, the impact of the cooking process on momilactones in rice is a topic of our current interest, which has not been investigated in other studies. After cooking, the availability of MA, tricin, caffeic acid, *ρ*-hydroxybenzoic acid, ferulic acid, salicylic acid, and cinnamic acid in rice samples significantly increased, while MB and *ρ*-coumaric acid decreased (Figure 5.5).

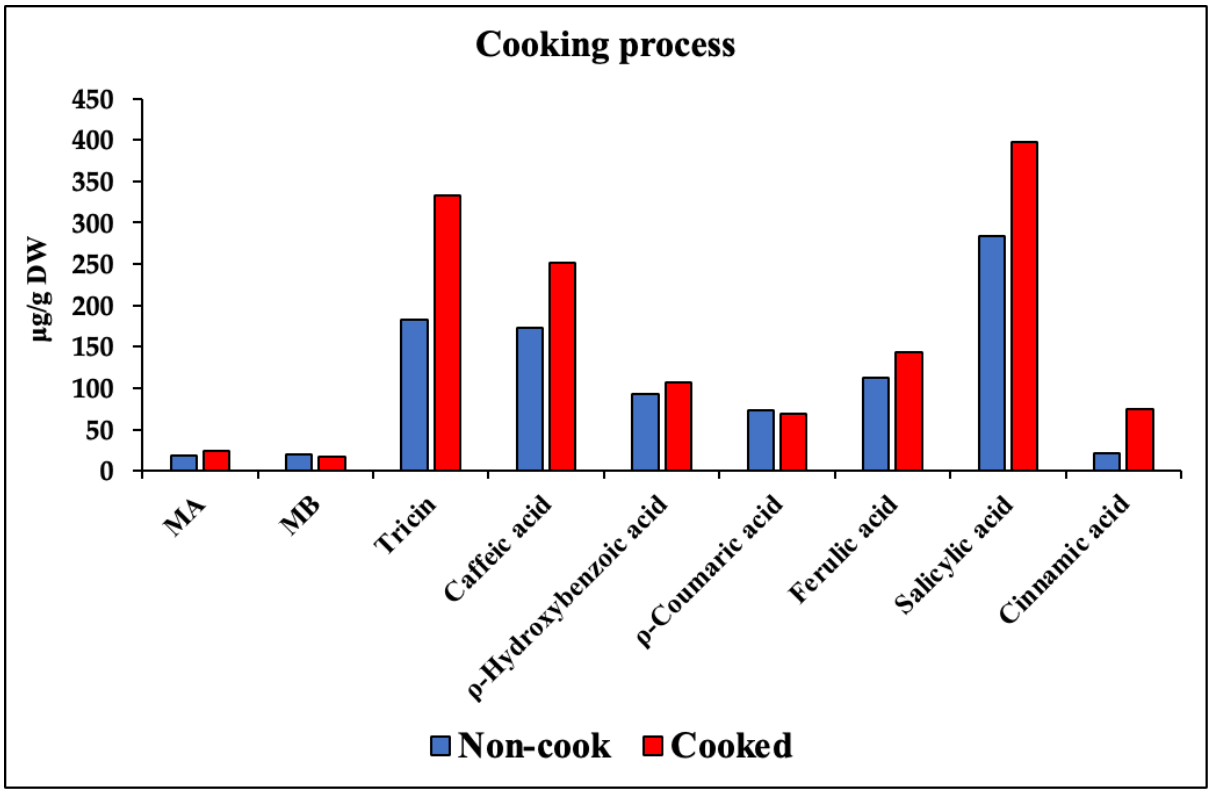

Figure 5.5. Average contents of momilactones A (MA) and B (MB), phenolic acids, and flavonoids among cooking processes.

In the extraction process of bioactive phytocompounds, various solvents (e.g., water, methanol, ethanol, hexane, ethyl acetate, etc.) have been used (Aires-de-Sousa, 2017). Among them, aqueous organic solvents have been demonstrated to be more effective than individual organic solvents. Particularly, plant extraction using aqueous methanol yields the highest phenolic content compared to other solvents (Iloki-Assanga et al., 2015; Metrouh-Amir et al., 2015). On the other hand, the most effective extraction of MA and MB was achieved through 100% methanolic extraction of rice husks treated at 100 °C (Minh et al., 2018). In another study, higher enrichment of MA and MB was observed with Soxhlet extraction employing a

combination of MeOH and MeOH:water, surpassing other methods (Ahmad et al., 2019). While aqueous methanol proves to be an effective solvent in extracting phenolics and momilactones from rice materials, its toxicity renders it less preferable in applications intended for human consumption of the final product (Chan & Chan, 2018). Compared to methanol, ethanol exhibits almost similar polarity and also is extensively used to extract a wide range of natural substances (Alzeer & Abou Hadeed, 2016). Despite also posing potential toxicity risks to humans, ethanol is considered less toxic than methanol and might be preferred in the food and pharmaceutical industries (Alzeer & Abou Hadeed, 2016). Notably, we observed that extraction with 80% ethanol yielded the greatest quantities of momilactones, while extraction with 80% methanol revealed the highest amount of phenolic and flavonoid compounds, as depicted in Figure 5.5.

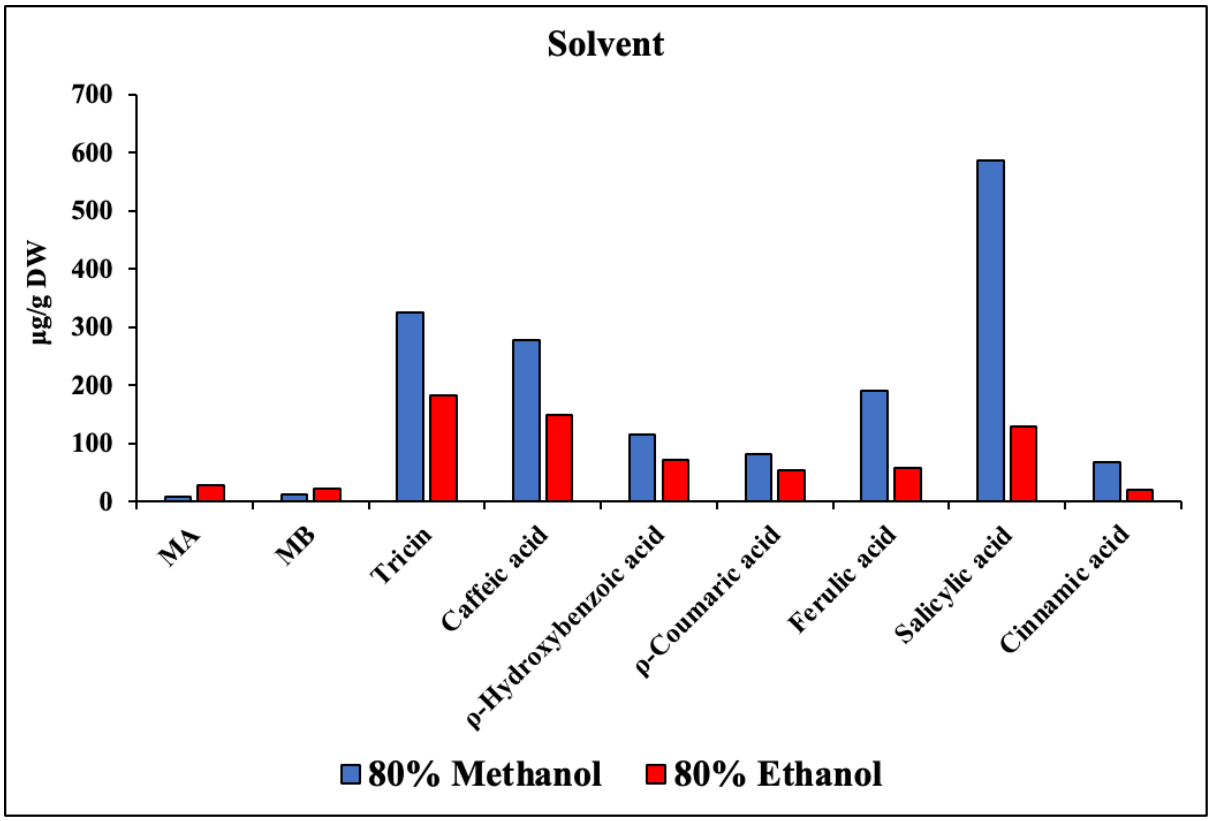

Figure 5.6. Average contents of momilactones A (MA) and B (MB), phenolic acids, and flavonoids among various solvents.

Our results may contribute to standardizing an extraction method for screening rice samples with dominant quantities of phenolics and momilactones. Additionally, our findings might contribute to developing extraction processes to obtain high levels of target compounds from rice on an industrial scale, aimed at producing functional products with health-beneficial properties. However, further extensive investigations are needed to confirm their possibility

and efficacy in industrial implications. On the other hand, strict adherence to safety regulations is paramount when handling ethanol in industrial settings and applications that directly involve consumers.

Sonication plays a crucial role in extracting bioactive compounds from plant samples, enhancing the efficiency of the extraction process, and improving the yield of these compounds. The application of sonication is instrumental in breaking down cell structures and facilitating the release of compounds from the rice matrix (Ahmad et al., 2019). In Figure 5.6, 2 h of sonication at 80 °C resulted in the highest amounts of MB and caffeic acid, while 2 h of sonication at RT led to the highest amount of MA. Additionally, 2 h of heat at 80 °C determined a significantly higher quantity of tricin, *ρ*-hydroxybenzoic acid, *ρ*-coumaric acid, ferulic acid, salicylic acid, and cinnamic acid compared to 2 h of sonication at RT and 80 °C.

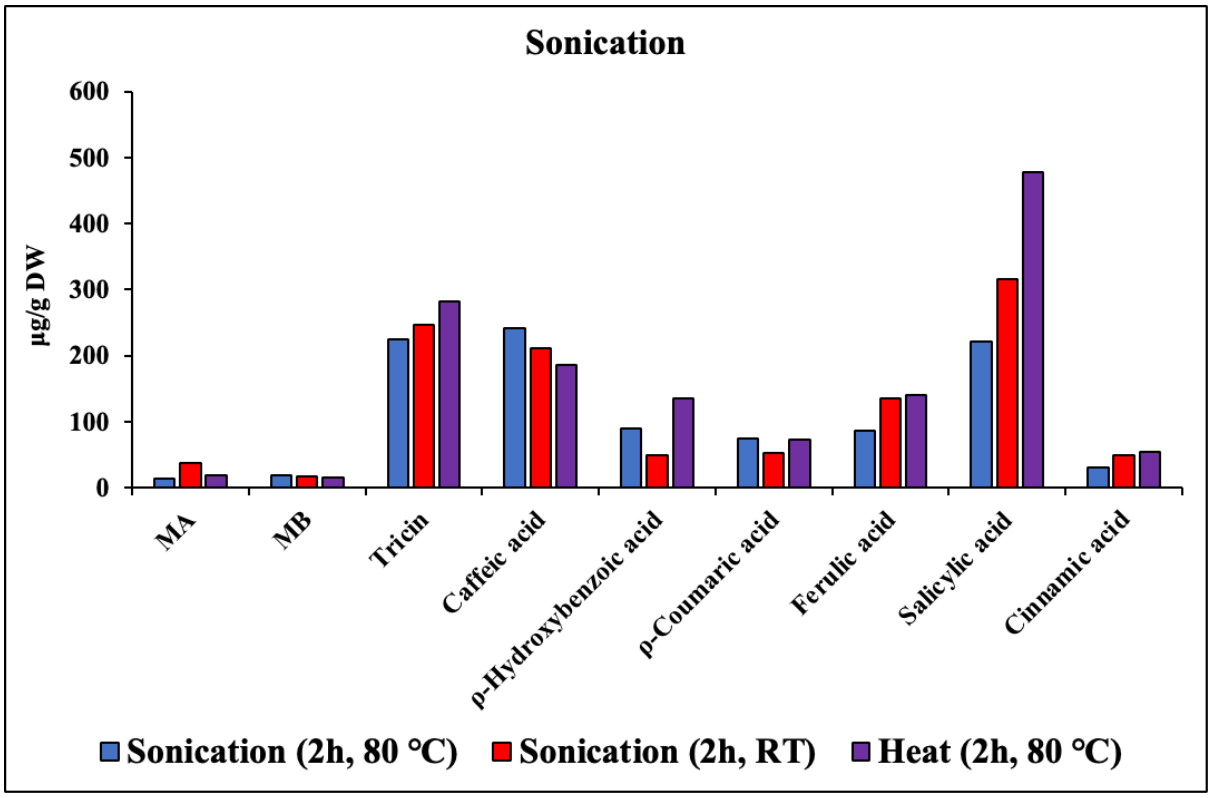

Figure 5.7. Average contents of momilactones A (MA) and B (MB), phenolic acids, and flavonoids among various extraction methods.

Another key consideration is the impact of the drying process on phenolic and momilactone contents in rice samples. Studies have highlighted variations in the levels of bioactive compounds during the drying process, indicating increases or losses depending on the compound types (ElGamal et al., 2023). Under the drying process, bioactive compounds react with oxygen at high temperatures, leading to their degradation owing to high reducibility.

The diverse molecular structures of various bioactive components cause varying susceptibilities to temperature (ElGamal et al., 2023). Specifically, phenolic compounds, being reducing agents, are susceptible to oxidation under the drying process. Moreover, higher drying temperatures escalate the reaction rates in phenolics. Therefore, lower drying temperatures are favored to achieve a higher preservation rate for these compounds (ElGamal et al., 2023). However, concerning momilactones, no studies have explored the impact of the drying process on these compounds; thus, investigating this aspect is highly recommended in subsequent studies. In general, the decomposition of bioactive compounds during convective drying primarily occurs due to prolonged exposure of the sample to high temperatures (ElGamal et al., 2023). In this study, we employed the convective drying method for rice samples at 40 °C for 7 days. However, the actual impact of these conditions on variations in the levels of phenolics and momilactones in the samples has not been evaluated. Thus, the optimization of drying methods for these targeted compounds necessitates thorough exploration in future investigations. Other drying methods, such as microwave freeze drying, infrared, and vacuum drying may also hold potential advantages over convective drying in terms of product quality that should be also considered (ElGamal et al., 2023). Overall, research and development to achieve optimal drying methods with high product quality, low cost, energy efficiency, and environmental friendliness are necessary to advance commercial products based on momilactones and phenolic-enriched rice grains.

5.3.4. Ordinary Least Square Regression (OLS) Estimation for the Effect of Different Treatments on Brown Rice

Table 5.2 presents the average treatment effects of rice variety on momilactones, phenolics, and flavonoids in BR, revealing a significant increase in Koshihikari at a 5% significance level according to the OLS estimate. In contrast, tricin, *ρ*-hydroxybenzoic acid, and *ρ*-coumaric acid decreased by -218.4, -3815, -78.38, and -53.59, respectively, compared to Milky Queen. Table 5.3 indicates that germination had positive and statistically significant effects on momilactones, phenolics, and flavonoids, with significance observed at a 1% level for all compounds. Germination enhanced the availability of MA, MB, tricin, caffeic acid, *ρ*-hydroxybenzoic acid, *ρ*-coumaric acid, ferulic acid, salicylic acid, and cinnamic acid in BR. Table 5.4 reveals that cooking had a positive and statistically significant impact only on cinnamic acid at a 5% significance level, with an increase of 48.24. Cooking showed no statistically significant impact on the other nine components. In Table 5.5, the average treatment effects of 80% methanol and 80% ethanol on nine compounds indicate that 80%

ethanol significantly elevated MA and MB at a 1% significance level, while 80% methanol had positive and statistically significant impacts on tricin, caffeic acid, *ρ*-hydroxybenzoic acid, *ρ*-coumaric acid, ferulic acid, salicylic acid, and cinnamic acid at a 5% significance level. Table 5.6 demonstrates that 2 h of sonication at 80 °C and room temperature had no statistically significant impacts on any tested compounds, but 2 h of heat at 80 °C had positive and statistically significant impacts on tricin, caffeic acid, *ρ*-hydroxybenzoic acid, *ρ*-coumaric acid, ferulic acid, salicylic acid, and cinnamic acid.

Table 5.2. OLS estimation for different rice varieties

Variables	MA	MB	Tricin	Caffeic acid	ρ-Hydroxybenzoic acid	ρ–Coumaric acid	Ferulic acid	Salicylic acid	Cinnamic acid
Milky queen Var.	-16.09	-2.901	-218.4**	-79.37	-78.38*	-53.59**	-72.15	-246.3	-22.35
	-9.732	-8.534	-88.5	-54.18	-42.3	-25.95	-50.19	-153.9	-22.92
Koshihikari Var.	26.65***	19.94***	381.2***	252.3***	149.5***	116.0***	161.2***	503.2***	59.13***
	-6.882	-6.035	-62.58	-38.31	-29.91	-18.35	-35.49	-108.8	-16.21
Observations	48	48	48	48	48	48	48	48	48
R-squared	0.056	0.003	0.117	0.045	0.069	0.085	0.043	0.053	0.02

*, **, and *** indicate significances at $p < 0.05$, 0.01, and 0.001, respectively; MA: momilactone A; MB: momilactone B.

Table 5.3. OLS estimation for non-germinate and germinated samples

Variables	MA	MB	Tricin	Caffeic acid	ρ-Hydroxybenzoic acid	ρ–Coumaric acid	Ferulic acid	Salicylic acid	Cinnamic acid
Germinated	29.86***	31.17***	296.4***	187.3***	128.8***	109.0***	171.8***	395.9***	72.46***
	-8.997	-7.204	-83.42	-48.06	-39.53	-21.85	-44.62	-146.9	-20.55
Non-germinated	3.672	2.909	123.8**	119.0***	45.96	34.74**	39.24	182.1*	11.72
	-6.362	-5.094	-58.99	-33.98	-27.95	-15.45	-31.55	-103.9	-14.53
Observations	48	48	48	48	48	48	48	48	48
R-squared	0.193	0.289	0.215	0.248	0.188	0.351	0.244	0.136	0.213

*, **, and *** indicate significances at $p < 0.05$, 0.01, and 0.001, respectively; MA: momilactone A; MB: momilactone B.

Table 5.4. OLS estimation for non-cook and cooked samples

Variables	MA	MB	Tricin	Caffeic acid	ρ-Hydroxybenzoic acid	ρ–Coumaric acid	Ferulic acid	Salicylic acid	Cinnamic acid
Cooked	3.49	-1.97	142.5	78.96	34.65	7.674	43.46	174.5	48.24**
	-10	-8.54	-91.8	-54.19	-43.55	-27.11	-50.91	-156	-22.04
Non-cooked	16.86**	19.48***	200.8***	173.1***	93.03***	85.42***	103.4***	292.8**	23.83
	-7.074	-6.039	-64.92	-38.32	-30.8	-19.17	-36	-110.3	-15.58
Observations	48	48	48	48	48	48	48	48	48
R-squared	0.003	0.001	0.05	0.044	0.014	0.002	0.016	0.026	0.094

*, **, and *** indicate significances at $p < 0.05$, 0.01, and 0.001, respectively; MA: momilactone A; MB: momilactone B.

Table 5.5. OLS estimation for 80% ethanol and 80% methanolic samples

Variables	MA	MB	Tricin	Caffeic acid	ρ-Hydroxybenzoic acid	ρ–Coumaric acid	Ferulic acid	Salicylic acid	Cinnamic acid
80% ethanol	16.98***	13.42***	-152.3	-129.0**	-78.24*	-52.28*	-136.3***	-500.2***	-35.95
	-9.699	-8.313	-91.46	-52.06	-42.31	-26.01	-47.21	-139.9	-22.54
80% methanol	10.11	11.78	348.1***	277.1***	149.5***	115.4***	193.3***	630.1***	65.93***
	-6.858	-5.878	-64.67	-36.81	-29.92	-18.39	-33.38	-98.9	-15.94
Observations	48	48	48	48	48	48	48	48	48
R-squared	0.062	0.054	0.057	0.118	0.069	0.081	0.153	0.218	0.052

*, **, and *** indicate significances at $p < 0.05$, 0.01, and 0.001, respectively; MA: momilactone A; MB: momilactone B.

Table 5.6. OLS estimation for sonicated samples

Variables	MA	MB	Tricin	Caffeic acid	ρ-Hydroxybenzoic acid	ρ–Coumaric acid	Ferulic acid	Salicylic acid	Cinnamic acid
Sonication (RT)	-4.263	-7.096	15.65	-30.6	-26.86	2.616	35.45	15.96	3.71
	-12.84	-11.09	-113	-73.77	-51.94	-34.39	-64.56	-182.2	-27.92
Heat (80°C)	21.05**	21.76***	242.0***	241.4***	111.1***	89.71***	99.97**	299.4**	42.38**
	-9.076	-7.844	-79.88	-52.16	-36.73	-24.32	-45.65	-128.8	-19.74
Observations	32	32	32	32	32	32	32	32	32
R-squared	0.004	0.013	0.001	0.006	0.009	0	0.01	0	0.001
Sonication (80°C)	-3.076	-2.708	74.25	-55.67	24.48	-3.991	40.11	226	13
	-12.07	-10.49	-97.56	-55.79	-52.98	-30.24	-51.28	-181.2	-26.45
Heat (80°C)	21.05**	21.76***	242.0***	241.4***	111.1***	89.71***	99.97***	299.4**	42.38**
	-8.538	-7.42	-68.99	-39.45	-37.47	-21.39	-36.26	-128.1	-18.7
Observations	32	32	32	32	32	32	32	32	32
R-squared	0.002	0.002	0.019	0.032	0.007	0.001	0.02	0.049	0.008
Sonication (80°C)	1.187	4.388	58.6	-25.06	51.34	-6.606	4.658	210.1	9.295
	-12.24	-9.966	-134.6	-73.28	-56.25	-35.85	-72	-211.5	-31.24
Sonication (RT)	16.79*	14.67**	257.7**	210.8***	84.29**	92.33***	135.4**	315.3**	46.09**
	-8.652	-7.047	-95.16	-51.81	-39.77	-25.35	-50.91	-149.5	-22.09
Observations	32	32	32	32	32	32	32	32	32
R-squared	0	0.006	0.006	0.004	0.027	0.001	0	0.032	0.003

*, **, and *** indicate significances at $p < 0.05$, 0.01, and 0.001, respectively; MA: momilactone A; MB: momilactone B.

5.3.5. Antioxidant Activity by the DPPH and ABTS Radical Scavenging Assay

After assessing the momilactone and phenolic levels, three samples were chosen: GKB4 (Koshihikari GBR cooked and extracted with 80% methanol using 2 hours of sonication at room temperature), GKB9 (non-cooked Koshihikari GBR extracted with 80% ethanol and 2 hours of sonication at room temperature), and MQ7 (non-cooked Milky Queen non-GBR extracted with 80% ethanol and 2 hours of sonication at 80 °C). Among them, GKB4 stood out for its high phenolic and flavonoid contents, GKB9 had the highest levels of MA and MB, while MQ7 had the lowest amounts of momilactones and phenolics. The DPPH radical scavenging activity results are summarized in Table 5.7, revealing that GKB4 demonstrated the most potent inhibition against DPPH cations (IC_{50} = 1.47 mg/mL), followed by GKB9 and MQ7 (IC_{50} = 1.56 and 4.98 mg/mL, respectively). In the ABTS assay, MQ7 exhibited the weakest activity with an IC_{50} value of 3.49 mg/mL, while GKB4 and GKB9 displayed robust activity with IC_{50} values of 1.70 and 1.80 mg/mL, respectively.

Table 5.7. Antioxidant, α-amylase, and α-glucosidase inhibitory activity of selected rice samples

	IC_{50} value (mg/mL)			
Sample	**DPPH**	**ABTS**	**α-Amylase**	**α-Glucosidase**
GKB4	1.47±0.07^{b}	1.7±0.10^{b}	0.55±0.01^{b}	0.69±0.03^{b}
GKB9	1.56±0.06^{b}	1.80±0.04^{b}	0.48±0.02^{a}	0.15±0.01^{a}
MQ7	4.98±0.12^{a}	3.49±0.07^{a}	4.89±0.51^{c}	NA
BHT	0.02±0.67	0.06±0.44	ND	ND
Acarbose	ND	ND	0.26±0.08	2.48±0.13

Data express means ± SD (standard deviation). Different superscript letters (^{a,b,c}) in a column indicate significant differences at $p < 0.05$; DPPH: 2,2-diphenyl-1-picrylhydrazyl; ABTS: 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid); BHT: butylated hydroxytoluene; IC_{50}: required concentration for 50% inhibition; NA: no activity; ND: not determined. The description of sample codes is presented in Table 5.1.

GKB4 not only showed the strongest antioxidant activity but also had enriched phenolics and flavonoid contents. In a prior investigation, it was highlighted that the antioxidant effectiveness of whole rice grain is strongly linked to the levels of both free and bound phenolics (Min et al., 2012). The diminished antioxidant effects observed in MQ7 are associated with its lower quantities of phenolics and flavonoids. These results enhance our comprehension of the connection between particular compounds and the antioxidant capabilities in rice, underscoring the significance of phenolics and flavonoids in bestowing antioxidant properties to entire rice grains.

5.3.6. α-Amylase and α-Glucosidase Inhibition Assay

The inhibitory effects on α-amylase and α-glucosidase were evaluated for three chosen samples: GKB4 (Koshihikari GBR cooked extract using 80% methanol with 2 hours of sonication at room temperature), GKB9 (non-cooked Koshihikari GBR extract using 80% ethanol with 2 hours of sonication at room temperature), and MQ7 (non-cooked Milky Queen non-GBR extract using 80% ethanol with 2 hours of sonication at 80 °C), as outlined in Table 5.7. GKB9 demonstrated the highest inhibitory activity against α-amylase (IC_{50} = 0.48 mg/mL) and α-glucosidase (IC_{50} = 0.15 mg/mL). In contrast, MQ7 exhibited the lowest inhibitory activity against α-amylase (IC_{50} = 4.89 mg/mL) and minimal effects on α-glucosidase. This study found that GKB9 displayed the most potent inhibition against α-amylase and α-glucosidase, aligning with its elevated MA and MB contents. Remarkably, Quan et al. (2019a) reported that MA and MB, identified and isolated from rice, were effective inhibitors of α-amylase and α-glucosidase, suggesting their potential as promising candidates for novel antidiabetic therapy.

5.3.7. Comparation of Extraction Techniques for MA and MB from Different Rice

Table 5.8 presents a comparison between previous and current studies, encompassing various parts, varieties, and extraction methods for rice. For example, MA and MB were found in straw with concentrations of 3.8 and 2.01 μg/g DW, respectively (Lee et al., 1999). The levels of these compounds were determined in the aerial parts of 30 rice varieties (ranging from 69.9 to 99.3 μg/g DW for MA and 64.4 to 114.1 μg/g DW for MB) (Xuan et al., 2016). MA and MB were also quantified in various plant parts (e.g., husks and grains) (Minh et al., 2018; Quan et al., 2019c) (Table 5.8). In our present study, GBR exhibited elevated levels of MA and MB (147.73 and 118 μg/g DW, respectively).

Table 5.8. Comparation of extraction techniques for MA and MB from different rice samples

Plant Part	Extraction	Methods	MA (µg/g DW)	MB (µg/g DW)	Reference
Straw (*O. sativa*)	Extraction: 80% aqueous MeOH	HPLC-MS-MS (positive-ion mode)	3.8	2.01	(Lee et al., 1999)
Aerial parts of 30 rice (O. sativa) varieties	Extraction: MeOH	GC-MS	69.9 - 99.3	64.4 - 114.1	(Xuan et al., 2016)
Husks (*O. sativa*)	Extraction: EtOAc, MeOH, and distilled water	RP-HPLC	11.8 - 58.8	3.0 - 104.4	(Minh et al., 2018)
	Heat (100 °C) and pressure (120 kPa)				
Different plant parts (*O. sativa*)	MeOH	HPLC	2.07 - 16.44	1.06 - 12.73	(Quan et al., 2019c)
Husks (*O. sativa*)	MeOH	HPLC-MS-MS	51.96	42.33	(Quan, et al., 2019d)
Grains (*O. sativa*)	MeOH	UPLC-ESI-MS (positive-ion mode)	0.05 - 1.56	0.05 - 1.61	(Quan et al., 2019c)
Germinated brown rice	MeOH	UPLC-ESI-MS (positive-ion mode)	1.70 - 18.94	7.20 - 41.00	(Hasan et al., 2023)
Germinated brown rice and non-germinated brown rice	80% MeOH	UPLC-ESI-MS (positive-ion mode)	0.29 - 147.73	0.33 - 118.8	Current study
	80% EtOH				
	Cooked and non-cooked				
	2 hours sonication at 80 °C				
	2 hours sonication at RT				
	2 hours heat at 80 °C				

MA: momilactone A; and MB: momilactone B.

Furthermore, the levels of MA and MB in rice grain (1.56 and 1.61 µg/g DW, respectively) (Quan et al., 2019a) and GBR (18.94 and 41.00 µg/g DW, respectively) (Hasan et al., 2023) in previous findings were significantly lower than those observed in the present study. Our current study demonstrated that 80% ethanol was more effective for the extraction of MA and MB than 80% methanol. In contrast, this study indicates that utilizing 80% ethanolic

extraction with sonication at room temperature can result in higher yields of MA and MB in GBR.

5.4. Conclusions

This research, for the first time, established an optimized method to enhance and extract momilactones A (MA) and B (MB) along with phenolic compounds from both germinated brown rice (GBR) and non-GBR of the Koshihikari and Milky Queen varieties through the cooking process. Specifically, extracts from cooked Koshihikari GBR using 80% methanol and 2 hours of sonication (GKB4) displayed the highest levels of phenolic compounds, showing a strong correlation with their potent antioxidant activity. In contrast, the non-cooked Koshihikari GBR extract using 80% ethanol and 2 hours of sonication (GKB9) demonstrated the highest concentrations of MA and MB, consistent with its ability to inhibit α-amylase and α-glucosidase. Notably, the effectiveness of GKB9 was comparable to the diabetes drug acarbose. Given the undervaluation of brown rice (BR), leading to its improper utilization or wastage, our research findings hold promise in enhancing the nutritional value of BR and promoting the development of rice-derived products that contribute to improving human health. Thus, this study aims to encourage the consumption of BR by highlighting its intrinsic value and potential benefits. Moreover, these findings are anticipated to contribute to the achievement of the Sustainable Development Goals (SDGs) by enhancing the collective well-being of individuals, eliminating hunger and poverty, and ensuring global food security, especially in rice-dependent nations.

CHAPTER VI.
GENERAL DISCUSSION

6.1. Discussion

The in-depth exploration of rice, a key element in global diets, reveals a wealth of bioactive compounds that go beyond basic nutritional value (Fukagawa & Ziska, 2019). Playing a crucial role in providing about 25% of the world's energy intake, rice stands as a vital cereal crop, notably driven by the efforts of Asian farmers (Phillips et al., 2024). This study's research findings highlight rice's importance as a rich source of bioactive compounds, specifically focusing on phenolics, flavonoids, and momilactones. These compounds showcase a diverse range of positive effects, such as antioxidant properties (Quan et al., 2019a), anti-diabetic benefits (Quan et al., 2019a, 2019c), anti-obesity effects (Quan et al., 2019c), anti-skin aging contributions (Quan et al., 2019a), antimicrobial qualities (Fukuta et al., 2007), anti-inflammatory actions (Cho et al., 2015), and even anti-cancer potential (Wongsa et al., 2021).

Moreover, the discovery and measurement of these bioactive elements in white rice, brown rice, and germinated brown rice offer valuable insights into the chemical makeup of diverse rice varieties. In white rice (WR), Momilactone A (MA) was detected at a minimal concentration of 0.01 μg/g DW, while Momilactone B (MB) exhibited slightly higher levels at 0.09 μg/g DW. Tricin and ρ-coumaric acid were present at 0.89 μg/g DW and 1.73 μg/g DW, respectively. Conversely, brown rice (BR) displayed elevated concentrations of momilactones, with MA and MB values at 0.98 μg/g DW and 0.63 μg/g DW, respectively. Remarkably, brown rice exhibited significantly higher quantities of tricin and ρ-coumaric acid, measuring 34.84 μg/g DW and 37.06 μg/g DW, respectively. Germinated brown rice (GBR) surpassed all others in chemical content, with notable increases in Momilactones A and B at 7.33 μg/g DW and 18.68 μg/g DW, respectively. Additionally, tricin and ρ-coumaric acid demonstrated impressive concentrations of 44.43 μg/g DW and 46.43 μg/g DW, respectively. These findings underscore the potential of rice, particularly germinated brown rice, as a functional food rich in bioactive components, holding implications for both nutritional and medicinal applications.

The study's novel approach, which involves optimizing extraction conditions and investigating the impact of salinity and germination periods on the accumulation of bioactive compounds in germinated brown rice, introduces new possibilities for elevating the

pharmaceutical and medicinal properties of rice grains. Significant variations in total phenolic contents (TPCs) and total flavonoid contents (TFCs) were observed across different treatments, with the B2 treatment showing the highest TPCs and the lowest recorded in treatment A2. The study underscores that a moderate salinity level of 75 mM, combined with a 4-day germination period, represents optimal conditions for enhancing TPC and TFC in germinated brown rice. While the commonly used Folin–Ciocalteu method for TPC analysis has limitations, HPLC analysis was employed for a more precise determination of specific phenolic compounds in germinated brown rice. Phenolic compounds like tricin, *ρ*-coumaric acid, ferulic acid, cinnamic acid, and salicylic acid, known for their diverse health benefits, exhibited increased quantities in treatment B2 (75 mM salinity and 4-day germination). However, under extreme salinity levels (150 mM), there was a notable decrease in these phenolic compounds in germinated brown rice. The study highlights that optimal conditions for enhancing bioactive phenolics in germinated brown rice involve a salinity of 75 mM maintained for 4 days during germination. Momilactones A (MA) and B (MB), recognized for their diverse health benefits, are valuable bioactive compounds derived from rice. This study, for the first time, explores the impact of salinity and germination periods on the accumulation of MA and MB in germinated brown rice (GBR). The results indicate that treatment B2, with moderate salinity (75 mM) and a 4-day germination period, significantly enhances the contents of MA and MB, potentially contributing to the pharmaceutical and medicinal values of GBR. Oxidative stress, intricately linked with inflammation in human physiology, plays a pivotal role in chronic diseases. The study assesses the antioxidant properties of germinated brown rice (GBR) under varying salinity levels and germination periods, with treatment B2 exhibiting the highest antiradical activities. The findings suggest that a moderate salinity level of 75 mM is conducive to enhancing the antioxidant activity of GBR, highlighting the delicate balance required in determining salt concentration for optimal health benefits. The study establishes strong correlations between antioxidant activities and the accumulation of bioactive compounds, including MA, MB, tricin, *ρ*-coumaric acid, ferulic acid, cinnamic acid, and salicylic acid in GBR. While the antioxidant abilities of tricin, *ρ*-coumaric acid, ferulic acid, cinnamic acid, and salicylic acid are well-known, the underlying mechanisms of MA and MB remain unclear. The simultaneous increase in these bioactive compounds and antioxidant activity in GBR suggests potential synergistic effects with positive implications for human health. The study suggests avenues for enhancing rice consumption values and developing pharmaceuticals, functional foods, and supplements, such as utilizing GBR in the production of fermented functional

beverages like kombucha. However, future investigations should delve into the bioaccessibility and bioavailability of these compounds throughout the digestive process.

Moreover, the study's examination of inhibiting crucial enzymes such as α-amylase to address type 2 diabetes and tyrosinase to prevent skin hyperpigmentation highlights the potential of customized germination treatments in augmenting the nutritional and bioactive profile of brown rice. GBR5 particularly stands out in inhibiting key enzymes associated with carbohydrate digestion and melanin formation, displaying low IC_{50} values in α-amylase, α-glucosidase, and tyrosinase assays. This suggests the potential therapeutic use of GBR5 in conditions related to skin pigmentation and carbohydrate metabolism, positioning it as a natural therapeutic alternative. The overall inhibitory properties of GBR extracts, especially GBR5, underscore their potential as functional foods with medicinal implications, prompting further investigation into their bioactive components for a comprehensive understanding of their health benefits. The measurable enhancements in palmitic acid content and the diverse effects of external treatments on the antioxidant, anti-diabetic, and anti-skin aging potentials of brown rice emphasize the importance of this research in revealing innovative strategies for harnessing the health-promoting properties of rice grains.

The results concerning the extraction of momilactones A and B and phenolic compounds from both germinated and non-germinated brown rice varieties through cooking offer promising insights into the development of rice-derived products with health-enhancing properties. The study underscores significant variations in momilactone concentrations (MA and MB) among rice varieties, attributing these differences to genetic diversity. Germinated brown rice (GBR), particularly GBR5, exhibits a notable increase in momilactone content compared to non-GBR, emphasizing the significance of germination in enriching these compounds. Extraction conditions, such as employing 80% ethanol and 2 hours of sonication, influence the efficiency of obtaining higher momilactone concentrations in brown rice. The study delves into the potential health benefits of momilactones in preventing chronic diseases and cancer, highlighting the importance of extraction methods in optimizing their presence in rice. Cooked Koshihikari GBR (GKB4: cooked Koshihikari GBR with 80% methanol extraction), extracted with 80% MeOH and sonication at room temperature, demonstrates the highest concentrations of various phenolic compounds, including tricin, caffeic acid, and cinnamic acid. Conversely, non-cooked Milky Queen BR displays the lowest levels of these compounds. The research suggests that the cooking process, along with specific extraction conditions, influences the phenolic content in brown rice, with 80% methanol identified as a

relatively more effective solvent for acquiring phenolics and flavonoids. Among the three selected samples−GKB4 (cooked Koshihikari GBR with 80% methanol extraction), GKB9 (non-cooked Koshihikari GBR with 80% ethanol extraction), and MQ7 (non-cooked Milky Queen non-GBR with 80% ethanol extraction)−GKB4 stands out for its high phenolic and flavonoid contents. GKB9 exhibits the highest levels of momilactones (MA and MB), while MQ7 has the lowest amounts of both momilactones and phenolics. GKB4 demonstrates the strongest antioxidant activity, aligning with its enriched phenolic and flavonoid contents. GKB9 exhibits the most potent inhibition against α-amylase and α-glucosidase, correlating with its elevated MA and MB contents, suggesting potential antidiabetic properties. Therefore, these findings could contribute to enhancing the value of GBR, which can be directly consumed as a component in dishes, harnessing its health-beneficial properties. Although less common than white rice, GBR might have been consumed since ancient times (Ly et al., 2014). For example, the total consumption of soaked GBR in Japan is approximately 9 tons annually (Patil et al., 2011). GBR is also served in restaurants and frequently featured in health and fashion magazines. Both older and younger generations, particularly those interested in health, highly appreciate GBR (Patil et al., 2011). It is utilized in various products such as rice balls, soups, bread, pastries, rice burgers, etc., often combined with other ingredients. GBR has been incorporated into numerous cuisines worldwide such as Italian risotto, Spanish paella, Brazilian feijoada, etc. (Patil et al., 2011). The distinctive profiles and identified strategies for enhancing the nutritional value of brown rice open up new possibilities for utilizing rice by-products in creating value-added products with enhanced health benefits. On the other hand, these results indicated that the extraction with 80% ethanol yielded the greatest quantities of momilactones, while the extraction with 80% methanol revealed the highest amount of phenolic and flavonoid compounds. Our results may contribute to standardizing an extraction method for screening rice samples with dominant quantities of phenolics and momilactones. Additionally, the findings might contribute to developing extraction processes to obtain high levels of target compounds from rice on an industrial scale, aimed at producing functional products with health-beneficial properties. However, further extensive investigations are needed to confirm their possibility and efficacy in industrial implications.

In summary, this research makes a noteworthy contribution to comprehending the medicinal capabilities of rice grains enriched with bioactive compounds. Through clarifying the diverse advantages of particular treatments during the germination stage and pinpointing optimal conditions to augment the bioactive profile of rice grains, this study lays the foundation

for future progress in creating rice-based products with heightened medicinal properties. The anticipated impact extends to the pursuit of Sustainable Development Goals (SDGs), encompassing objectives like poverty eradication, promotion of healthy lives, attainment of food security, enhancement of nutrition, and advancement of sustainable agriculture on a global scale.

6.2. Conclusions

This extensive study reveals rice's richness in bioactive compounds, surpassing basic nutritional value, playing a vital role in global diets and supplying 25% of global energy. Focusing on phenolics, flavonoids, and momilactones, it highlights diverse health benefits, from antioxidants to anti-cancer potential. Different rice varieties show varied chemical compositions, with germinated brown rice standing out. The research optimizes extraction conditions, linking salinity and germination to bioactive compound accumulation, suggesting new avenues for medicinal rice applications. Inhibitory effects on key enzymes for diabetes and skin pigmentation are explored, emphasizing GBR5's potential. Cooking influences momilactone and phenolic extraction, impacting health-enhancing properties. The study's insights into rice's diverse benefits contribute to developing pharmaceuticals and functional foods, aligning with Sustainable Development Goals (SDGs) for global well-being.

6.3. New Findings

- Momilactone A and B, tricin, and *ρ*-coumaric acid were identified and quantified in white rice, brown rice, and germinated brown rice. After comparing the amounts of Momilactone A and B, tricin, and *ρ*-coumaric acid among white rice, brown rice, and germinated brown rice, it was reported for the first time that the highest amount of momilactones A and B was calculated in germinated brown rice.
- For the first time, this research has pinpointed an optimized treatment (75 mM NaCl and 4-day germination) that markedly enhances the accumulation of valuable bioactive compounds, such as phenolics and momilactones A and B, in germinated brown rice (GBR, var. Koshihikari).
- This study delved into the impact of specific stress conditions on germinated brown rice for the first time, revealing significant antioxidant properties and potent inhibitory effects against crucial enzymes (α-amylase, α-glucosidase, and tyrosinase), indicating potential benefits for diabetes and anti-skin aging.

- In this study, momilactone A and B were quantified using 80% methanol and 80% ethanol, revealing significantly higher amounts of momilactone A and B.
- The first time we conducted research on momilactone A and B in cooked germinated brown rice, we observed a significant amount of both momilactone A and B.
- For the first time, we observed interesting results regarding the antioxidant and anti-diabetic potential compounds in both cooked and non-cooked germinated brown rice using various extraction methods.

REFERENCES

Ahmad, A., Xuan, T.D., Minh, T.N., Siddiqui, N.A. and Van Quan, N. Comparative extraction and simple isolation improvement techniques of active constituents' momilactone A and B from rice husks of Oryza sativa by HPLC analysis and column chromatography. *Saudi pharmaceutical journal*, **2019**, *27*, 17–24.

Aires-de-Sousa, M. Methicillin-resistant Staphylococcus aureus among animals: current overview. *Clinical Microbiology and Infection*, **2017**, *23*, 373–380.

Ali, A.S.; Elozeiri, A.A. Metabolic processes during seed germination. *Advances in seed biology*, **2017**, *2017*,141–166.

Alves, G.H.; Ferreira, C.D.; Vivian, P.G.; Monks, J.L.F.; Elias, M.C.; Vanier, N.L.; de Oliveira, M. The revisited levels of free and bound phenolics in rice: Effects of the extraction procedure. *Food Chemistry*, **2016**, *208*, 116–123.

Alzeer, J.; Abou Hadeed, K. Ethanol and its Halal status in food industries. *Trends in Food Science & Technology*, **2016**, *58*, 14–20.

Ambade, A.; Mandrekar, P. Oxidative stress and inflammation: essential partners in alcoholic liver disease. *International journal of hepatology*, **2012**, *2012*.

Andriana, Y.; Xuan, T.D.; Quy, T.N.; Minh, T.N.; Van, T.M.; Viet, T.D. Antihyperuricemia, antioxidant, and antibacterial activities of Tridax procumbens L. *Foods*, **2019**, *8*, 21.

Anh, L.H.; Khanh, T.D.; Xuan, T.D. Biological roles of momilactones: Achievements, challenges, and promising approaches to exploit their beneficial properties. *Front. Nat. Prod*, **2023**, *2*, 1245869.

Anh, L.H.; Lam, V.Q.; Takami, A.; Khanh, T.D.; Quan, N.V.; Xuan, T.D. Cytotoxic mechanism of momilactones A and B against acute promyelocytic leukemia and multiple myeloma cell Lines. *Cancers*, **2022**, *14*, 4848.

Anh, L.H.; Quan, N.V.; Lam, V.Q.; Iuchi, Y.; Takami, A.; Teschke, R.; Xuan, T.D. Antioxidant, anti-tyrosinase, anti-α-amylase, and cytotoxic potentials of the invasive weed Andropogon virginicus. *Plants*, **2020**, *10*, 69.

Anh, N.T.H.; Anh, L.H.; Mai, N.P.; Quan, N.V.; Xuan, T.D. Identification and isolation techniques for plant growth inhibitors in Rice. *Separations*, **2023**, *10*, 105.

Anh, L.H.; Quan, N.V.; Lam, V.Q.; Takami, A.; Khanh, T.D.; Xuan, T.D. Rice momilactones and phenolics: Expression of relevant biosynthetic genes in response to UV and chilling stresses. *Agronomy*, **2022**, *12*, 1731.

Anh, L.H.; Quan, N.V.; Nghia, L.T.; Xuan, T.D. Phenolic allelochemicals: Achievements, limitations, and prospective approaches in weed management. *Weed Biology and Management*, **2021**, *21*, 37–67.

Barber, T.M.; Kabisch, S.; Pfeiffer, A.F.; Weickert, M.O. The health benefits of dietary fibre. *Nutrients*, **2020**, *12*, 3209.

Barclay, A.W.; Petocz, P.; McMillan-Price, J.; Flood, V.M.; Prvan, T.; Mitchell, P.; Brand-Miller, J.C. Glycemic index, glycemic load, and chronic disease risk—a meta-analysis of observational studies. *The American journal of clinical nutrition*, **2008**, *87*, 627–637.

Biswas, S.K. Does the interdependence between oxidative stress and inflammation explain the antioxidant paradox?. *Oxidative medicine and cellular longevity*, **2016**, *2016*.

Bonto, A.P.; Tiozon Jr, R.N.; Sreenivasulu, N.; Camacho, D.H. Impact of ultrasonic treatment on rice starch and grain functional properties: A review. *Ultrasonics Sonochemistry*, **2021**, *71*, 105383.

Bourgaud, F.; Gravot, A.; Milesi, S.; Gontier, E. Production of plant secondary metabolites: a historical perspective. *Plant science*, **2001**, *161*, 839–851.

Bueno-Costa, F.M.; Zambiazi, R.C.; Bohmer, B.W.; Chaves, F.C.; da Silva, W.P.; Zanusso, J.T.; Dutra, I. Antibacterial and antioxidant activity of honeys from the state of Rio Grande do Sul, Brazil. *LWT-Food Science and Technology*, **2016**, *65*, 333–340.

Cáceres, P.J.; Peñas, E.; Martinez-Villaluenga, C.; Amigo, L.; Frias, J. Enhancement of biologically active compounds in germinated brown rice and the effect of sun-drying. *Journal of Cereal Science*, **2017**, *73*, 1–9.

Cai, H.; Hudson, E.A.; Mann, P.; Verschoyle, R.D.; Greaves, P.; Manson, M.M.; Steward, W.P.; Gescher, A.J. Growth-inhibitory and cell cycle-arresting properties of the rice bran constituent tricin in human-derived breast cancer cells in vitro and in nude mice in vivo. *British Journal of Cancer*, **2004**, *91*, 1364–1371.

Capanzana, M.V.; Buckle, K.A. Optimisation of germination conditions by response surface methodology of a high amylose rice (*Oryza sativa*) cultivar. *LWT-Food Science and Technology*, **1997**, *30*, 155–163.

Chaiyasut, C.; Sivamaruthi, B.S.; Pengkumsri, N.; Saelee, M.; Kesika, P.; Sirilun, S.; Fukngoen, P.; Jampatip, K.; Khongtan, S.; Peerajan, S. Optimization of conditions to achieve high content of gamma amino butyric acid in germinated black rice, and changes in bioactivities. *Food Science and Technology*, **2017**, *37*, 83–93.

Chan, A.P.; Chan, T.Y. Methanol as an unlisted ingredient in supposedly alcohol-based hand rub can pose serious health risk. *International journal of environmental research and public health*, **2018**, *15*, 1440.

Chinma, C.E.; Adedeji, O.E.; Jolayemi, O.S.; Ezeocha, V.C.; Ilowefah, M.A.; Rosell, C.M.; Adebo, J.A.; Wilkin, J.D.; Adebo, O.A. Impact of germination on the techno-functional properties, nutritional composition, and health-promoting compounds of brown rice and its products: A review. *Journal of food science*, **2023**.

Chiu, C.J.; Taylor, A. Dietary hyperglycemia, glycemic index and metabolic retinal diseases. *Progress in retinal and eye research*, **2011**, *30*, 18–53.

Cho, D.H.; Lim, S.T. Germinated brown rice and its bio-functional compounds. *Food Chemistry*, **2016**, *196*, 259–271.

Cho, J.G.; Cha, B.J.; Min Lee, S.; Shrestha, S.; Jeong, R.H.; Sung Lee, D.; Kim, Y.C.; Lee, D.G.; Kang, H.C.; Kim, J.; Baek, N.I. Diterpenes from the roots of *Oryza sativa* L. and their inhibition activity on NO production in LPS-stimulated RAW264. 7 macrophages. *Chemistry & Biodiversity*, **2015**, *12*, 1356–1364.

Choe, H.; Sung, J.; Lee, J.; Kim, Y. Effects of calcium chloride treatment on bioactive compound accumulation and antioxidant capacity in germinated brown rice. *Journal of Cereal Science*, **2021**, *101*, 103294.

Choi, I.D.; Kim, D.S.; Son, J.R.; Yang, C.I.; Chun, J.Y.; Kim, K.J. Physico-chemical properties of giant embryo brown rice (Keunnunbyeo). *Applied Biological Chemistry*, **2006**, *49*, 95–100.

Chung, I.M.; Hahn, S.J.; Ahmad, A. Confirmation of potential herbicidal agents in hulls of rice, *Oryza sativa*. *Journal of chemical ecology*, **2005**, *31*, 1339–1352.

Chung, I.M.; Kim, J.T.; Kim, S.H. Evaluation of allelopathic potential and quantification of momilactone A, B from rice hull extracts and assessment of inhibitory bioactivity on paddy field weeds. *Journal of Agricultural and Food Chemistry*, **2006**, *54*, 2527–2536.

Chung, S.I.; Lo, L.M.P.; Kang, M.Y. Effect of germination on the antioxidant capacity of pigmented rice (*Oryza sativa* L. cv. Superjami and Superhongmi). *Food Science and Technology Research*, **2016**, *22*, 387–394.

Cui, L.; Pan, Z.; Yue, T.; Atungulu, G.G.; Berrios, J. Effect of ultrasonic treatment of brown rice at different temperatures on cooking properties and quality. *Cereal chemistry*, **2010**, *87*, 403–408.

da Silva Lindemann, I.; Dittgen, C.L.; de Souza Batista, C.; Dos Santos, J.P.; Bruni, G.P.; Elias, M.C.; Vanier, N.L. Rice and common bean blends: Effect of cooking on in vitro starch digestibility and phenolics profile. *Food Chemistry*, **2021**, *340*, 127908.

De La Peña, R.; Sattely, E.S. Rerouting plant terpene biosynthesis enables momilactone pathway elucidation. *Nature Chemical Biology*, **2021**, *17*, 205–212.

Delbaere, K.; Close, J.C.; Brodaty, H.; Sachdev, P.; Lord, S.R. Determinants of disparities between perceived and physiological risk of falling among elderly people: cohort study. *Bmj*, **2010**, *341*.

Demeekul, K.; Suthammarak, W.; Petchdee, S. Bioactive compounds from germinated brown rice protect cardiomyocytes against simulated ischemic/reperfusion injury by ameliorating mitochondrial dysfunction. *Drug Design, Development and Therapy*, **2021**, 1055–1066.

Demirseren, D.D.; Emre, S.; Akoglu, G.; Arpacı, D.; Arman, A.; Metin, A.; Cakır, B. Relationship between skin diseases and extracutaneous complications of diabetes mellitus: clinical analysis of 750 patients. *American journal of clinical dermatology*, **2014**, *15*, 65–70.

Ding, J.; Yang, T.; Feng, H.; Dong, M.; Slavin, M.; Xiong, S.; Zhao, S. Enhancing contents of γ-aminobutyric acid (GABA) and other micronutrients in dehulled rice during germination under normoxic and hypoxic conditions. *Journal of Agricultural and Food Chemistry*, **2016**, *64*, 1094–1102.

do Nascimento, L.Á.; Abhilasha, A.; Singh, J.; Elias, M.C.; Colussi, R. Rice germination and its impact on technological and nutritional properties: A review. *Rice Science*, **2022**, *29*, 201–215.

Dorairaj, D.; Govender, N.T. Rice and paddy industry in Malaysia: governance and policies, research trends, technology adoption and resilience. *Frontiers in Sustainable Food Systems*, **2023**, *7*, 1093605.

ElGamal, R.; Song, C.; Rayan, A.M.; Liu, C.; Al-Rejaie, S.; ElMasry, G. Thermal Degradation of Bioactive Compounds during Drying Process of Horticultural and Agronomic Products: A Comprehensive Overview. *Agronomy*, **2023**, *13*, 1580.

El-Maarouf-Bouteau, H.; Bailly, C. Oxidative signaling in seed germination and dormancy. *Plant signaling & behavior*, **2008**, *3*, 175–182.

Falcinelli, B.; Sileoni, V.; Marconi, O.; Perretti, G.; Quinet, M.; Lutts, S.; Benincasa, P. Germination under moderate salinity increases phenolic content and antioxidant activity in rapeseed (Brassica napus var oleifera Del.) sprouts. *Molecules*, **2017**, *22*, 1377.

Floegel, A.; Kim, D.O.; Chung, S.J.; Koo, S.I.; Chun, O.K. Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. *Journal of food composition and analysis*, **2011**, *24*, 1043–1048.

Fukagawa, N.K.; Ziska, L.H. Rice: Importance for global nutrition. *Journal of nutritional science and vitaminology*, **2019**, *65*, S2–S3.

Fukuta, M.; Dang Xuan, T.; Deba, F.; Tawata, S.; Dang Khanh, T.; Min Chung, I. Comparative efficacies in vitro of antibacterial, fungicidal, antioxidant, and herbicidal activities of momilatones A and B. *Journal of Plant Interactions*, **2007**, *2*, 245–251.

Ganie, S.A.; Bhat, J.A.; Devoto, A. The influence of endophytes on rice fitness under environmental stresses. *Plant Molecular Biology*, **2022**, *109*, 447–467.

Germain, J.; Deslongchamps, P. Total synthesis of (±)-momilactone A. *The Journal of organic chemistry*, **2002**, *67*, 5269–5278.

Getoff, N.; 2007. Anti-aging and aging factors in life. The role of free radicals. *Radiation Physics and Chemistry*, **2007**, *76*, 1577–1586.

Ghasemzadeh, A.; Karbalaii, M.T.; Jaafar, H.Z.; Rahmat, A. Phytochemical constituents, antioxidant activity, and antiproliferative properties of black, red, and brown rice bran. *Chemistry Central Journal*, **2018**, *12*, 1–13.

Ghose, B.; Kpoghomou, M.A.; Shamsitdinov, H.; Mondal, A.K.; Sarker, S. Nutraceutical potential of rice and wheat antioxidants and their impacts on health. *Oxid. Antioxid. Med. Sci*, **2013**, *2*, 245–249.

Gong, E.S.; Luo, S.; Li, T.; Liu, C.; Zhang, G.; Chen, J.; Zeng, Z.; Liu, R.H. Phytochemical profiles and antioxidant activity of processed brown rice products. *Food Chemistry*, **2017**, *232*, 67–78.

Goufo, P.; Trindade, H. Rice antioxidants: phenolic acids, flavonoids, anthocyanins, proanthocyanidins, tocopherols, tocotrienols, γ-oryzanol, and phytic acid. *Food science & nutrition*, **2014**, *2*, 75–104.

Gujral, H.S.; Sharma, P.; Bajaj, R.; Solah, V. Effects of incorporating germinated brown rice on the antioxidant properties of wheat flour chapatti. *Food science and technology international*, **2012**, *18*, 47–54.

Guzmán-Ortiz, F.A.; Castro-Rosas, J.; Gómez-Aldapa, C.A.; Mora-Escobedo, R.; Rojas-León, A.; Rodríguez-Marín, M.L.; Falfán-Cortés, R.N.; Román-Gutiérrez, A.D. Enzyme activity during germination of different cereals: A review. *Food Reviews International*, **2019**, *35*, 177–200.

Haj Sghaier, A.; Tarnawa, Á.; Khaeim, H.; Kovács, G.P.; Gyuricza, C.; Kende, Z. The Effects of Temperature and Water on the Seed Germination and Seedling Development of Rapeseed (*Brassica napus* L.). *Plants*, **2022**, *11*, 2819.

Hasan, M.; Quan, N.V.; Anh, L.H.; Khanh, T.D.; Xuan, T.D. Salinity treatments promote the accumulations of momilactones and phenolic compounds in germinated brown rice. *Foods*, **2023**, *12*, 2501.

Huong, C.T.; Anh, T.T.T.; Dat, T.D.; Khanh, T.D.; Xuan, T.D. Uniparental inheritance of salinity tolerance and beneficial phytochemicals in rice. *Agronomy*, **2020**, *10*, 1032.

Iloki-Assanga, S.B.; Lewis-Luján, L.M.; Lara-Espinoza, C.L.; Gil-Salido, A.A.; Fernandez-Angulo, D.; Rubio-Pino, J.L.; Haines, D.D. Solvent effects on phytochemical constituent profiles and antioxidant activities, using four different extraction formulations for analysis of *Bucida buceras* L. and Phoradendron californicum. *BMC research notes*, **2015**, *8*, 1–14.

Imam, M.U.; Azmi, N.H.; Bhanger, M.I.; Ismail, N.; Ismail, M. Antidiabetic properties of germinated brown rice: a systematic review. *Evidence-based complementary and alternative medicine*, **2012**, *2012*.

Islam, M.Z.; Shim, M.J.; Jeong, S.Y.; Lee, Y.T. Effects of soaking and sprouting on bioactive compounds of black and red pigmented rice cultivars. *International Journal of Food Science & Technology*, **2022**, *57*, 201–209.

Izadi, V.; Azadbakht, L. Is there any association between rice consumption and some of the cardiovascular diseases risk factors? A systematic review. *ARYA atherosclerosis*, **2015**, *11*, 109.

Johnson, M.H.; Lucius, A.; Meyer, T.; Gonzalez de Mejia, E. Cultivar evaluation and effect of fermentation on antioxidant capacity and in vitro inhibition of α-amylase and α-glucosidase by highbush blueberry (Vaccinium corombosum). *Journal of Agricultural and Food Chemistry*, **2011**, *59*, 8923–8930.

Joung, Y.H.; Lim, E.J.; Kim, M.S.; Lim, S.D.; Yoon, S.Y.; Lim, Y.C.; Yoo, Y.B.; Ye, S.K.; Park, T.; Chung, I.M.; Bae, K.Y. Enhancement of hypoxia-induced apoptosis of human breast cancer cells via STAT5b by momilactone B. *International journal of oncology*, **2008**, *33*, 477–484.

Kakar, K.; Xuan, T.D.; Quan, N.V.; Wafa, I.K.; Tran, H.D.; Khanh, T.D.; Dat, T.D. Efficacy of N-methyl-N-nitrosourea mutation on physicochemical properties, phytochemicals, and momilactones A and B in rice. *Sustainability*, **2019**, *11*, 6862.

Kato, T.; Kabuto, C.; Sasaki, N.; Tsunagawa, M.; Aizawa, H.; Fujita, K.; Kato, Y.; Kitahara, Y.; Takahashi, N. Momilactones, growth inhibitors from rice, *Oryza sativa* L. *Tetrahedron Lett.* **1973**, *14*, 3861–3864.

Khaeim, H.; Kende, Z.; Jolánkai, M.; Kovács, G.P.; Gyuricza, C.; Tarnawa, Á. Impact of temperature and water on seed germination and seedling growth of maize (*Zea mays* L.). *Agronomy*, **2022**, *12*, 397.

Kim, S.J.; Park, H.R.; Park, E.; Lee, S.C. Cytotoxic and antitumor activity of momilactone B from rice hulls. *Journal of Agricultural and Food Chemistry*, **2007**, *55*, 1702–1706.

Kim, T.H.; Kim, E.K.; Lee, M.S.; Lee, H.K.; Hwang, W.S.; Choe, S.J.; Kim, T.Y.; Han, S.J.; Kim, H.J.; Kim, D.J.; Lee, K.W. Intake of brown rice lees reduces waist circumference

and improves metabolic parameters in type 2 diabetes. *Nutrition research*, **2011**, *31*, 131–138.

Kobayashi, A.; Hori, K.; Yamamoto, T.; Yano, M. Koshihikari: a premium short-grain rice cultivar–its expansion and breeding in Japan. *Rice*, **2018**, *11*, 1–12.

Kong, C.H.; Li, H.B.; Hu, F.; Xu, X.H.; Wang, P. Allelochemicals released by rice roots and residues in soil. *Plant and soil*, **2006**, *288*, 47–56.

Kong, Liang, Xu, X.; Hu, F.; Wang, P.; Jiang, Y. Release and activity of allelochemicals from allelopathic rice seedlings. *Journal of agricultural and food chemistry*, **2004**, *52*, 2861–2865.

Kruk, J.; Aboul-Enein, B.H.; Duchnik, E.; Marchlewicz, M. Antioxidative properties of phenolic compounds and their effect on oxidative stress induced by severe physical exercise. *The Journal of Physiological Sciences*, **2022**, *72*, 1–24.

Kuwabara, H.; Mouri, K.; Otsuka, H.; Kasai, R.; Yamasaki, K. Tricin from a malagasy connaraceous plant with potent antihistaminic activity. *Journal of Natural Products*, **2003**, *66*, 1273–1275.

Lee, C.W.; Yoneyama, K.; Takeuchi, Y.; Konnai, M.; Tamogami, S.; Kodama, O. Momilactones A and B in rice straw harvested at different growth stages. *Bioscience, biotechnology, and biochemistry*, **1999**, *63*, 1318–1320.

Lee, S.C.; Chung, I.M.; Jin, Y.J.; Song, Y.S.; Seo, S.Y.; Park, B.S.; Cho, K.H.; Yoo, K.S.; Kim, T.H.; Yee, S.B.; Bae, Y.S. Momilactone B, an allelochemical of rice hulls, induces apoptosis on human lymphoma cells (Jurkat) in a micromolar concentration. *Nutrition and cancer*, **2008,** *60*, 542–551.

Lin, P.Y.; Li, S.C.; Lin, H.P.; Shih, C.K. Germinated brown rice combined with Lactobacillus acidophilus and Bifidobacterium animalis subsp. lactis inhibits colorectal carcinogenesis in rats. *Food science & nutrition*, **2019**, *7*, 216–224.

Lin, Y.T.; Pao, C.C.; Wu, S.T.; Chang, C.Y. Effect of different germination conditions on antioxidative properties and bioactive compounds of germinated brown rice. *BioMed Research International*, **2015**, *2015*.

Ly, D.; Lee, S.G. Comparisons of phenolic compounds, flavonoids contents and antioxidant activity in eco-friendly cultivated and conventional cultivated rice. *Free Radic. Antioxid.* **2014**, *141*, 378–385.

Ma, Y.; Zhang, S.; Feng, D.; Duan, N.; Rong, L.; Wu, Z.; Shen, Y. Effect of different doses of nitrogen fertilization on bioactive compounds and antioxidant activity of brown rice. *Frontiers in Nutrition*, **2023**, *10*, 1071874.

Ma, Z.; Zhai, X.; Zhang, N.; Tan, B. Effects of germination, fermentation and extrusion on the nutritional, cooking and sensory properties of brown rice products: A comparative study. *Foods*, **2023**, *12*, 1542.

Mattioli, R.; Francioso, A.; Mosca, L.; Silva, P. Anthocyanins: A comprehensive review of their chemical properties and health effects on cardiovascular and neurodegenerative diseases. *Molecules*, **2020**, *25*, 3809.

Metrouh-Amir, H.; Duarte, C.M.; Maiza, F. Solvent effect on total phenolic contents, antioxidant, and antibacterial activities of Matricaria pubescens. *Industrial crops and products*, **2015**, *67*, 249–256.

Min, B.; Gu, L.; McClung, A.M.; Bergman, C.J.; Chen, M.H. Free and bound total phenolic concentrations, antioxidant capacities, and profiles of proanthocyanidins and anthocyanins in whole grain rice (*Oryza sativa* L.) of different bran colours. *Food Chemistry*, **2012**, *133*, 715–722.

Minh, L.T.; Khang, D.T.; Ha, P.T.; Tuyen, P.T.; Minh, T.N.; Quan, N.V.; Xuan, T.D. Effects of salinity stress on growth and phenolics of rice (*Oryza sativa* L.). *Int. Lett. Nat. Sci.* **2016**, 57.

Ngoc Minh, T.; Xuan, T.D.; Ahmad, A.; Abdelghany Elzaawely, A.; Teschke, R.; Van, T.M. Momilactones A and B: Optimization of yields from isolation and purification. *Separations*, **2018**, *5*, 28.

Mittal, P.; Dhankhar, S.; Chauhan, S.; Garg, N.; Bhattacharya, T.; Ali, M.; Chaudhary, A.A.; Rudayni, H.A.; Al-Zharani, M.; Ahmad, W.; Khan, S.U.D. A review on natural antioxidants for their role in the treatment of parkinson's disease. *Pharmaceuticals*, **2023**, *16*, 908.

Mohidem, N.A.; Hashim, N.; Shamsudin, R.; Che Man, H. Rice for food security: Revisiting its production, diversity, rice milling process and nutrient content. *Agriculture*, **2022**, *12*, 741.

Mukherjee, A.; Gaurav, A.K.; Singh, S.; Yadav, S.; Bhowmick, S.; Abeysinghe, S.; Verma, J.P. The bioactive potential of phytohormones: a review. *Biotechnol. Rep*. **2022**, *35*, e00748.

Munarko, H.; Sitanggang, A.B.; Kusnandar, F.; Budijanto, S. Effect of different soaking and germination methods on bioactive compounds of germinated brown rice. *Int. J. Food Sci. Technol.* **2021**, *56*, 4540–4548.

Nascimento, L.Á.D.; Avila, B.P.; Colussi, R.; Elias, M.C. Effect of abiotic stress on bioactive compound production in germinated brown rice. *Cereal Chem.* **2020**, *97*, 868–876.

Nemzer, B.; Al-Taher, F. Analysis of Fatty Acid Composition in Sprouted Grains. *Foods*, **2023**, *12*, 1853.

Ng, L.T.; Huang, S.H.; Chen, Y.T.; Su, C.H. Changes of tocopherols, tocotrienols, γ-oryzanol, and γ-aminobutyric acid levels in the germinated brown rice of pigmented and nonpigmented cultivars. *J. Agric. Food Chem.* **2013**, *61*, 12604–12611.

Joye, I.J. Dietary fibre from whole grains and their benefits on metabolic health. *Nutrients*, **2020**, *12*, 3045.

Nkhata, S.G.; Ayua, E.; Kamau, E.H.; Shingiro, J.B. Fermentation and germination improve nutritional value of cereals and legumes through activation of endogenous enzymes. *Food science & nutrition*, **2018**, *6*, 2446–2458.

Nonogaki, H. Seed germination—the biochemical and molecular mechanisms. *Breed. Sci.* **2006**, *56*, 93–105.

Olszowy, M.; Dawidowicz, A.L. Is it possible to use the DPPH and ABTS methods for reliable estimation of antioxidant power of colored compounds?. *Chem. Zvesti.* **2018**, *72*, 393–400.

Owolabi, I.O.; Chakree, K.; Takahashi Yupanqui, C. Bioactive components, antioxidative and anti-inflammatory properties (on RAW 264.7 macrophage cells) of soaked and germinated purple rice extracts. *Int. J. Food Sci. Technol.* **2019**, *54*, 2374–2386.

Palermo, M.; Pellegrini, N.; Fogliano, V. The effect of cooking on the phytochemical content of vegetables. *J. Sci. Food Agric.* **2014**, *94*, 1057–1070.

Pandey, K.B.; Rizvi, S.I. Plant polyphenols as dietary antioxidants in human health and disease. *Oxid. Med. Cell. Longev.* **2009**, *2*, 270–278.

Park, C.; Jeong, N.Y.; Kim, G.Y.; Han, M.H.; Chung, I.M.; Kim, W.J.; Yoo, Y.H.; Choi, Y.H. Momilactone B induces apoptosis and G1 arrest of the cell cycle in human monocytic leukemia U937 cells through downregulation of pRB phosphorylation and induction of the cyclin-dependent kinase inhibitor p21Waf1/Cip1. *Oncol. Rep.* **2014**, *31*, 1653–1660.

Patil, S.B.; Khan, M.K. Germinated brown rice as a value-added rice product: A review. *J. Food Sci. Technol.* **2011**, *48*, 661–667.

Phillips, J.; Durand-Morat, A.; Nalley, L.L.; Graterol, E.; Bonatti, M.; de la Pava, K.L.; Urioste, S.; Yang, W. Understanding demand for broken rice and its potential food security implications in Colombia. *J. Agric. Food Res*. **2024**, *15*, 100884.

Prior, R.L.; Wu, X.; Schaich, K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J. Agric. Food Chem.* **2005**, *53*, 4290–4302.

Quan, N.V.; Thien, D.D.; Khanh, T.D.; Tran, H.D.; Xuan, T.D. Momilactones A, B, and tricin in rice grain and by-products are potential skin aging inhibitors. *Foods*, **2019a**, *8*, 602.

Quan, N.V.; Tran, H.D.; Xuan, T.D.; Ahmad, A.; Dat, T.D.; Khanh, T.D.; Teschke, R. Momilactones A and B are α-amylase and α-glucosidase inhibitors. *Molecules*, **2019b**, *24*, 482.

Quan, N.V.; Xuan, T.D.; Tran, H.D.; Ahmad, A.; Khanh, T.D.; Dat, T.D. Contribution of momilactones A and B to diabetes inhibitory potential of rice bran: Evidence from in vitro assays. *Saudi Pharm. J.* **2019c**, *27*, 643–649.

Quan, N.V.; Xuan, T.D.; Tran, H.D.; Thuy, N.T.D.; Trang, L.T.; Huong, C.T.; Andriana, Y.; Tuyen, P.T. Antioxidant, α-amylase and α-glucosidase inhibitory activities and potential constituents of Canarium tramdenum bark. *Molecules***, 2019d,** *24*, 605.

Quan, N.V.; Xuan, T.D.; Anh, L.H.; Tran, H.D. Bio-guided isolation of prospective bioactive constituents from roots of Clausena indica (Dalzell) Oliv. *Molecules*, **2019e**, *24*, 4442.

Quan, N.V.; Xuan, T.D.; Tran, H.D.; Thuy, N.T.D. Inhibitory activities of momilactones A, B, E, and 7-ketostigmasterol isolated from rice husk on paddy and invasive weeds. *Plants*, **2019f**, *8*, 159.

Quan, N.V.; Iuchi, Y.; Anh, L.H.; Hasan, M.; Xuan, T.D. Simple isolation of cordycepin from cordyceps militaris by dual-normal phase column chromatography and its potential for making kombucha functional products. *Separations*, **2022**, *9*.

Rahim, M.A.; Umar, M.; Habib, A.; Imran, M.; Khalid, W.; Lima, C.M.G.; Shoukat, A.; Itrat, N.; Nazir, A.; Ejaz, A.; Zafar, A. Photochemistry, functional properties, food applications, and health prospective of black rice. *J. Chem*. **2022**, *2022*.

Rahman, M.M.; Rahaman, M.S.; Islam, M.R.; Rahman, F.; Mithi, F.M.; Alqahtani, T.; Almikhlafi, M.A.; Alghamdi, S.Q.; Alruwaili, A.S.; Hossain, M.S.; Ahmed, M. Role of phenolic compounds in human disease: current knowledge and future prospects. *Molecules*, **2021**, *27*, 233.

Rajendran, P.; Nandakumar, N.; Rengarajan, T.; Palaniswami, R.; Gnanadhas, E.N.; Lakshminarasaiah, U.; Gopas, J.; Nishigaki, I. Antioxidants and human diseases. *Clin. Chim. Acta.* **2014**, 436, 332–347.

Ramazani, E.; Akaberi, M.; Emami, S.A.; Tayarani-Najaran, Z. Biological and pharmacological effects of gamma-oryzanol: An updated review of the molecular mechanisms. *Curr. Pharm. Des.* **2021**, *27*, 2299–2316.

Ravichanthiran, K.; Ma, Z.F.; Zhang, H.; Cao, Y.; Wang, C.W.; Muhammad, S.; Aglago, E.K.; Zhang, Y.; Jin, Y.; Pan, B. Phytochemical profile of brown rice and its nutrigenomic implications. *Antioxidants*, **2018**, *7*, 71.

Razak, A.M.; Tan, J.K.; Mohd Said, M.; Makpol, S. Modulating effects of zingiberaceae phenolic compounds on neurotrophic factors and their potential as neuroprotectants in brain disorders and age-associated neurodegenerative disorders: a review. *Nutrients*, **2023**, *15*, 2564.

Trachoo, N.; Boudreaux, C.; Moongngarm, A.; Samappito, S.; Gaensakoo, R. Effect of germinated rough rice media on growth of selected probiotic bacteria. *Pak. J. Biol. Sci*. **2006**, *9*, 2657–2661.

Seechamnanturakit, V.; Karrila, T.T.; Sontimuang, C.; Sukhoom, A. The natural pigments in pigmented rice bran and their relation to human health: a literature review. *Applied Science and Engineering Progress*, **2018**, *11*, 3–13.

Sen, S.; Chakraborty, R.; Kalita, P. Rice-not just a staple food: A comprehensive review on its phytochemicals and therapeutic potential. *Trends Food Sci. Technol.* **2020**, *97*, 265–285.

Senguttuvel, P., Padmavathi, G., Jasmine, C., Sanjeeva Rao, D., Neeraja, C. N., Jaldhani, V., Beulah, P., Gobinath, R., Aravind Kumar, J., Sai Prasad, S. V., Subba Rao, L. V., Hariprasad, A. S., Sruthi, K., Shivani, D., Sundaram, R. M., & Govindaraj, M. Rice

biofortification: breeding and genomic approaches for genetic enhancement of grain zinc and iron contents. *Front. Plant Sci.* **2023**, *14*, 1138408.

Shahabi Mohammadabadi, S.; Goli, M.; Naji Tabasi, S. Optimization of bioactive compound extraction from eggplant peel by response surface methodology: ultrasound-assisted solvent qualitative and quantitative effect. *Foods*, **2022**, *11*, 3263.

Shahidi, F.; Ambigaipalan, P. Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects–A review. *J. Funct. Foods.* **2015**, *18*, 820–897.

Shalini, V.; Bhaskar, S.; Kumar, K.S.; Mohanlal, S.; Jayalekshmy, A.; Helen, A. Molecular mechanisms of anti-inflammatory action of the flavonoid, tricin from Njavara rice (*Oryza sativa* L.) in human peripheral blood mononuclear cells: possible role in the inflammatory signaling. *Int. Immunopharmacol.* **2012**, *14*, 32–38.

Shalini, V.; Pushpan, C.K.; Sindhu, G.; Jayalekshmy, A.; Helen, A. Tricin, flavonoid from Njavara reduces inflammatory responses in hPBMCs by modulating the p38MAPK and PI3K/Akt pathways and prevents inflammation associated endothelial dysfunction in HUVECs. *Immunobiology*, **2016**, *221*, 137–144.

Sharif, M.K.; Butt, M.S.; Anjum, F.M.; Khan, S.H. Rice bran: A novel functional ingredient. *Crit. Rev. Food Sci. Nutr.* **2014**, *54*, 807–816.

Sharifi-Rad, M;, Anil Kumar, N.V.; Zucca, P.; Varoni, E.M.; Dini, L.; Panzarini, E.; Rajkovic, J.; Tsouh Fokou, P.V.; Azzini, E.; Peluso, I.; Prakash Mishra, A. Lifestyle, oxidative stress, and antioxidants: Back and forth in the pathophysiology of chronic diseases. *Front. Physiol.* **2020**, *11*, 694.

Sharma, A.; Thakur, S.; Kumar, V.; Kanwar, M.K.; Kesavan, A.K.; Thukral, A.K.; Bhardwaj, R.; Alam, P.; Ahmad, P. Pre-sowing seed treatment with 24-epibrassinolide ameliorates pesticide stress in Brassica juncea L. through the modulation of stress markers. *Front. Plant Sci.* **2016**, *7*, 1569.

Soong, Y.Y.; Barlow, P.J. Antioxidant activity and phenolic content of selected fruit seeds. *Food chem.* **2004**, *88*, 411–417.

Sukegawa, H.; Kokawa, M.; Kitamura, Y. Establishment of a cultivation method for sprouted brown rice and elucidation of its functional food characteristics. *Food Sci. Technol. Res.* **2021**, *27*, 341–349.

Tan, B.L.; Norhaizan, M.E.; Chan, L.C. Rice Bran: From waste to nutritious food ingredients. *Nutrients*, **2023**, *15*, 2503.

Tehami, W.; Nani, A.; Khan, N.A.; Hichami, A. New Insights Into the Anticancer Effects of p-Coumaric Acid: Focus on Colorectal Cancer. *Dose-Response*, **2023**, *21*, 15593258221150704.

Lien, D.T.P.; Tram, P.T.B.; Toan, H.T. Effects of extraction process on phenolic content and antioxidant activity of soybean. *J. Food Nutr. Sci*, **2015**, *3*, 33–38.

Ti, H.; Zhang, R.; Li, Q.; Wei, Z.; Zhang, M. Effects of cooking and in vitro digestion of rice on phenolic profiles and antioxidant activity. *Food Res. Int.* **2015**, *76*, 813–820.

Ti, H.; Zhang, R.; Zhang, M.; Li, Q.; Wei, Z.; Zhang, Y.; Tang, X.; Deng, Y.; Liu, L.; Ma, Y. Dynamic changes in the free and bound phenolic compounds and antioxidant activity of brown rice at different germination stages. *Food chem.* **2014**, *161*, 337–344.

Tian, S.; Nakamura, K.; Kayahara, H. Analysis of phenolic compounds in white rice, brown rice, and germinated brown rice. *J. Agric. Food Chem.* **2004**, *52*, 4808–4813.

Tucker, P.S.; Scanlan, A.T.; Dalbo, V.J. Chronic kidney disease influences multiple systems: describing the relationship between oxidative stress, inflammation, kidney damage, and concomitant disease. *Oxid. Med. Cell. Longev*. **2015**, *2015*.

Tungmunnithum, D.; Thongboonyou, A.; Pholboon, A.; Yangsabai, A. Flavonoids and other phenolic compounds from medicinal plants for pharmaceutical and medical aspects: An overview. *Medicines*, **2015**, *5*, 93.

Tuyen, P.T.; Xuan, T.D.; Khang, D.T.; Ahmad, A.; Quan, N.V.; Tu Anh, T.T.; Anh, L.H.; Minh, T.N. Phenolic compositions and antioxidant properties in bark, flower, inner skin, kernel and leaf extracts of Castanea crenata Sieb. et Zucc. *Antioxidants*, **2017**, *6*, 31.

Tyagi, A.; Shabbir, U.; Chen, X.; Chelliah, R.; Elahi, F.; Ham, H.J.; Oh, D.H. Phytochemical profiling and cellular antioxidant efficacy of different rice varieties in colorectal adenocarcinoma cells exposed to oxidative stress. *Plos one*. **2022**, *17*, e0269403.

Tyagi, A.; Yeon, S.J.; Daliri, E.B.M.; Chen, X.; Chelliah, R.; Oh, D.H. Untargeted metabolomics of Korean fermented brown rice using UHPLC Q-TOF MS/MS reveal an abundance of potential dietary antioxidative and stress-reducing compounds. *Antioxidants*, **2021**, *10*, 626.

Un, S.; Quan, N.V.; Anh, L.H.; Lam, V.Q.; Takami, A.; Khanh, T.D.; Xuan, T.D. Effects of in vitro digestion on anti-α-amylase and cytotoxic potentials of Sargassum spp. *Molecules*, **2022**, *27*, 2307.

Valavanidis, A.; Vlachogianni, T.; Fiotakis, K.; Loridas, S. Pulmonary oxidative stress, inflammation and cancer: respirable particulate matter, fibrous dusts and ozone as major causes of lung carcinogenesis through reactive oxygen species mechanisms. *Int. J. Environ. Res. Public Health.* **2013**, *10*, 3886–3907.

Valko, M.; Rhodes, C.J.B.; Moncol, J.; Izakovic, M.M.; Mazur, M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem. Biol. Interact*. **2006**, *160*, 1–40.

Verardo, V.; Gómez-Caravaca, A.M.; Marconi, E.; Segura-Carretero, A.; Garrido-Frenich, A.; Fernández-Gutiérrez, A. Determination of lipophilic and hydrophilic bioactive compounds in raw and parboiled rice bran. *RSC Adv*. **2016**, *6*, 50786–50796.

Wada, K.; Oba, S.; Nagata, C. Rice-based diet and cardiovascular disease mortality in Japan: From the Takayama Study. *J. Nutr.* **2022**, *14*, 2291.

Waliat, S.; Arshad, M.S.; Hanif, H.; Ejaz, A.; Khalid, W.; Kauser, S.; Al-Farga, A. A review on bioactive compounds in sprouts: extraction techniques, food application and health functionality. *Int. J. Food Prop.* **2023**, *26*, 647–665.

Wang, W.; Li, Y.; Dang, P.; Zhao, S.; Lai, D.; Zhou, L. Rice secondary metabolites: structures, roles, biosynthesis, and metabolic regulation. *Molecules*, **2018**, *23*, 3098.

Wang, Y.; Shen, J.Z.; Chan, Y.W.; Ho, W.S. Identification and growth inhibitory activity of the chemical constituents from Imperata Cylindrica aerial part ethyl acetate extract. *Molecules*, **2018**, *23*, 1807.

Wisetkomolmat, J.; Arjin, C.; Hongsibsong, S.; Ruksiriwanich, W.; Niwat, C.; Tiyayon, P.; Jamjod, S.; Yamuangmorn, S.; Prom-U-Thai, C.; Sringarm, K. Antioxidant Activities and Characterization of Polyphenols from Selected Northern Thai Rice Husks: Relation with Seed Attributes. *Rice Sci*. **2023**, *30*, 148–159.

Wojtunik-Kulesza, K.A.; Oniszczuk, A.; Oniszczuk, T.; Waksmundzka-Hajnos, M. The influence of common free radicals and antioxidants on development of Alzheimer's Disease. *Biomed. Pharmacother.* **2016**, *78*, 39–49.

Wongsa, P. Phenolic compounds and potential health benefits of pigmented rice. *Recent Advances in Rice Research*, **2020**, *4*, 19–21.

Wu, X.; Guo, T.; Luo, F.; Lin, Q. Brown rice: a missing nutrient-rich health food. *Food Sci. Hum. Wellness.* **2023**, *12*, 1458–1470.

Xia, Q.; Green, B.D.; Zhu, Z.; Li, Y.; Gharibzahedi, S.M.T.; Roohinejad, S.; Barba, F.J. Innovative processing techniques for altering the physicochemical properties of wholegrain brown rice (*Oryza sativa* L.)–opportunities for enhancing food quality and health attributes. *Crit. Rev. Food Sci. Nutr.* **2019**, *59*, 3349–3370.

Xuan, T.D.; Huong, C.T.; Quan, N.V.; Anh, L.H.; Khanh, T.D.; Rayee, R. Improvement of salinity tolerance in rice seedlings by exogenous magnesium sulfate application. *Soil Systems*, **2022**, *6*, 69.

Xuan, T.D.; Khang, D.T. Effects of exogenous application of protocatechuic acid and vanillic acid to chlorophylls, phenolics and antioxidant enzymes of rice (*Oryza sativa* L.) in submergence. *Molecules*, **2018**, *23*, 620.

Xuan, T.D.; Minh, T.N.; Anh, L.H.; Khanh, T.D. Allelopathic momilactones A and B are implied in rice drought and salinity tolerance, not weed resistance. *Agron Sustain Dev.* **2016**, *36*, 1–8.

Zahra, N.; Jabeen, S. Brown rice as useful nutritional source. *Pakistan Journal of Agricultural Research*, **2020**, *33*, 445.

Zhang, H.; Tsao, R. Dietary polyphenols, oxidative stress and antioxidant and anti-inflammatory effects. *Curr. Opin*. *Food Sci.* **2016**, *8*, 33–42.

Zhao, M.; Cheng, J.; Guo, B.; Duan, J.; Che, C.T. Momilactone and related diterpenoids as potential agricultural chemicals. *J. Agric. Food Chem.* **2018**, *66*, 7859–7872.

Zubair, M.; Anwar, F.; Nadeem Zafar, M.; Faizan Nazar, M.; Ijaz Hussain, A.; Mughal, E.U. Effect of Ultrasonic Extraction Regimes on Phenolics and Antioxidant Attributes of Rice (*Oryza Sativa* L.) Cultivars. *Iran. J. Chem. Chem. Eng.* **2018**, *37*, 109–119.