



Research article

Green valorization of defatted rice bran: unlocking bioactive and prebiotic potential using deep eutectic solvent extraction

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Abstract

Importance of the work: Defatted rice bran (DFRB), a nutrient-rich byproduct of rice oil extraction, is often discarded as waste. Utilizing a sustainable and eco-friendly extraction method can help to unlock its potential for applications in functional foods, nutraceuticals and agriculture. This study explored the use of deep eutectic solvents (DES) as a green extraction technology to recover bioactive and prebiotic compounds from DFRB.

Objectives: To extract bioactive compounds from DFRB using a sodium propionate/glycerol (Na:Gly) DES and to evaluate their phenolic composition, antioxidant activity and prebiotic properties.

Materials and Methods: DFRB was extracted using Na:Gly DES (1:8, weight per volume) at 70°C for 5 hr. The extract was analyzed for its phenolic content, phenolic composition, protein, reducing sugars and phytic acid. Its antioxidant activity was assessed using 2,2-diphenyl-1-picrylhydrazyl and 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid assays, while its prebiotic potential was tested based on monitoring *L. plantarum* growth. In addition, the extract's ability to inhibit harmful bacteria was evaluated.

Results: The extraction method recovered a substantial proportion of glycosylated (47.09%), esterified (45.26%) and free phenolic (7.65%) compounds, with ferulic acid, p-coumaric acid and sinapic acid as the dominant phenolic acids. In addition, the extract contained 7.08 mg/g of protein, 4.26 mg/g of reducing sugars and 1.14 mg/g of phytic acid. The carbon and nitrogen sources in the DFRB extract were suitable for the growth of *L. plantarum*, while the phenolic acids inhibited the growth of *S. aureus* and *P. aeruginosa*.

Main finding: Applying Na:Gly DES was an effective, sustainable and eco-friendly method for extracting valuable bioactive compounds from DFRB. These findings support its potential use in functional foods, gut health applications and sustainable agriculture.

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Introduction

Defatted rice bran (DFRB) is produced as a byproduct during the rice bran oil refining process. As the demand for rice bran oil continues to rise, the production of DFRB has also increased (Alexandri et al., 2020). Unfortunately, a large portion of DFRB is underused, with much of it being discarded as waste or utilized as low-value animal feed (Gul et al., 2015; Alexandri et al., 2020). However, DFRB is rich in nutrients and functional compounds and utilizing its potential through sustainable extraction techniques could lead to new applications in functional foods, prebiotics and nutraceuticals.

DFRB is naturally rich in bioactive compounds, particularly phenolic acids, known for their antioxidant and anti-inflammatory properties (Zhao et al., 2018; Yin et al., 2019). These compounds have been associated with anti-cancer and anti-obesity effects (Dokkaew et al., 2019; Zheng et al., 2024) and are reported to support gut health through their prebiotic properties (Alsaleem et al., 2024).

DFRB is not only abundant in phenolic acids but also contains high levels of phytic acid (IP6), with concentrations in the range 5.90–6.48% on a dry weight basis. Studies have shown that IP6 has several health benefits, including antimicrobial properties, antioxidative activity and potential protective effects against colon cancer (Kaur et al., 2020; Canan et al., 2021; Nassar et al., 2021). Furthermore, DFRB is recognized for its protein content, which contributes to its functional properties such as emulsification, foaming ability and gel formation (Zhang et al., 2012). In addition, it is considered hypoallergenic, making it a valuable ingredient for specialized food products (Al-Doury et al., 2018). Beyond its role in food applications, DFRB can be fermented to produce reducing sugars, which have promising applications in food processing and bioenergy production (Alexandri et al., 2020). The utilization of DFRB in value-added products contributes to economic growth and expanded market opportunities.

Bound phenolic compounds have been recognized widely for their health benefits, including prebiotic effects (Arruda et al., 2018; Wang et al., 2022; Zhong et al., 2022). For example, phenolic compounds extracted from oats have been reported to exhibit both antioxidant and prebiotic properties (Zhang et al., 2023). Similarly, soluble-bound polyphenols in wheat bran exhibit substantial prebiotic activity (Zhang et al., 2023), while bound polyphenols in carrot dietary fiber contribute to both antioxidant and prebiotic properties (Liu et al., 2019).

In DFRB, bioactive compounds are often bound within cell walls, either as insoluble macromolecules or integrated with structural components such as cellulose and proteins (Zhao et al., 2018). Solvent-based methods are commonly used to efficiently extract these phenolic compounds (Shahidi et al., 2023). However, conventional extraction methods using strong acids or alkaline solutions pose environmental risks and conflict with sustainable and eco-friendly practices.

These limitations have been addressed through the emergence of deep eutectic solvents (DESs) as an innovative, eco-friendly alternative for bioactive compound extraction (Abbott et al., 2003). DESs consist of halide salts or hydrogen-bond donors and acceptors, offering cost-effective and simple preparation while producing high-purity compounds with low energy requirements, while also being biodegradable and compatible with biological systems (Zhang et al., 2012; Hayyan et al., 2013; Yang et al., 2019). Their low volatility, non-toxicity, non-flammability and high selectivity and solubility make them ideal for extraction and separation processes (Hizaddin et al., 2022).

Sodium propionate/glycerol (Na:Gly) as a DES has gained attention as a sustainable method for extracting phenolic compounds from agricultural residues (Slim et al., 2018; Stefou et al., 2019; Alibade et al., 2021). While other research has focused primarily on the ability of this form of DES to extract bioactive compounds, the present study highlighted the effectiveness of various DES types in extracting a range of phenolic acids and value-added biomolecules, such as phytic acid, reducing sugars and proteins (Sombutsuwan et al., 2024).

Despite these advancements, much remains to be understood about the effectiveness of Na:Gly DES in recovering these compounds, particularly in terms of their prebiotic potential. Exploring the prebiotic properties of these extracts could lead to novel health and wellness applications that are currently underexplored.

Therefore, the present study aimed to bridge these gaps by comprehensively analyzing the bioactive composition of DFRB extracts obtained using Na:Gly DES and evaluating their prebiotic potential. The results should provide valuable insights into how agricultural byproducts can be optimized for sustainable use, contributing to the development of health-promoting ingredients.

Materials and Methods

Plant material

The DFRB was obtained from Surin Rice Bran Oil Co., Ltd. (Surin province, Thailand). The material was dried using a vacuum hot-air oven at 60°C to reduce the moisture content to approximately 5% (weight per weight, w/w) and subsequently stored at -20°C in the dark to prevent degradation. The proximate composition was determined according to official methods (Association of Official Analytical Chemists, 2005) and the Van Soest sequential analytical method (Van Soest et al., 1991).

Deep eutectic solvent preparation and its properties

The DES was prepared based on the method described by Sombutsuwan et al. (2024), as part of earlier research by the current authors. In this method, sodium propionate, acting as the hydrogen bond acceptor and glycerol, the hydrogen bond donor, were combined at a molar ratio of 1:8. This ratio was selected based on preliminary screening to achieve a stable, homogeneous liquid DES. A similar formulation using glycerol and sodium propionate has been reported as highly effective for polyphenol extraction from plant materials (Slim et al., 2018). The temperature was set at 70°C to form DES until a transparent and uniform liquid was achieved. The properties of the prepared DES, including pH and polarity, were analyzed following the protocols of Laokuldilok et al. (2011), Mulia et al. (2019) and Sombutsuwan et al. (2024). The pH of the DES was measured using a pH meter (Model S220; Mettler Toledo, Switzerland). The polarity was evaluated using Nile Red dye and the absorbance spectra were recorded in the range of 400–700 nm using microplate spectrophotometer (Multiskan GO; Thermo Fisher Scientific, Waltham, USA). The polarity of the DES was quantified using the Nile Red polarity index (E_{NR} ; measured in kilocalories per mole), calculated using the formula in Equation 1, as described by Laokuldilok et al. (2011) and Mulia et al. (2019):

$$E_{NR} \text{ (kcal/mol)} = 28,591 / \lambda_{\text{abs.max}} \text{ (nm)}$$

where $\lambda_{\text{abs.max}}$ is the wavelength of maximum absorption (measured in nanometers)

All analyses were performed in triplicate.

Extraction procedure

The DFRB was prepared as described by Sombutsuwan et al. (2024). Briefly, 2.5 g of DFRB was mixed with 25 g of Na:Gly DES in an Erlenmeyer flask and 20% (w/w) distilled water was added to reduce viscosity. The mixture was agitated in a water bath shaker (Model SBS40; Stuart, UK) at 150 revolutions per minute (rpm) and 70°C for 5 hr, then centrifuged at 3,500 rpm for 15 min. The supernatant was collected and stored at -20°C for further analysis, without separating the Na:Gly DES.

Phenolic compounds categorization from defatted rice bran extracted using deep eutectic solvent of sodium propionate and glycerol

Phenolic compounds (PCs) in the DFRB extract were categorized into three groups: free phenolic compounds (FPCs), soluble esterified bound phenolic compounds (SEBPCs) and soluble glycosylated bound phenolic compounds (SGBPCs) based on classification following the method described by Lou et al. (2020), with minor modifications to suit the conditions of the current study.

Extraction of FPCs

The FPCs were adjusted to pH 2 using 6 M HCl and then extracted with ethyl acetate at a 1:1 volume ratio three times. The extracted FPCs in the ethyl acetate phase were dried using Na_2SO_4 , filtered and evaporated under nitrogen gas. Then, the dried sample was redissolved in 5 mL of ethanol for further analysis (Lou et al., 2020; Sombutsuwan et al., 2024). Next the FPCs were extracted three times using ethyl acetate with a 1:1 volume ratio. Anhydrous Na_2SO_4 was added to dry the ethyl acetate phase; then, the mixture was filtered and evaporated using nitrogen gas. The residue was redissolved in 5 mL of ethanol for further analysis, following the procedures outlined by Lou et al. (2020) and Sombutsuwan et al. (2024).

Extraction of SEBPCs

After extracting the FPCs, the aqueous phase was treated with 4 M NaOH, 10 mM ethylenediaminetetraacetic acid and 1% ascorbic acid, then incubated at room temperature for 4 hr to induce hydrolysis (Sombutsuwan et al., 2024). The solution was acidified to pH 2 with 6 M HCl and the SEBPCs were extracted using the same method as for the FPCs (Lou et al., 2020; Sombutsuwan et al., 2024).

Extraction of SGBPCs

The aqueous phase after SEBPC extraction was treated with 6 M HCl to facilitate hydrolysis, with the reaction occurring at 75°C for 60 min. Following this, the SGBPCs were extracted using the same procedure as for the FPCs (Lou et al., 2020; Sombutsuwan et al., 2024).

Quantification of total phenolic content using Folin-Ciocalteu method

The total phenolic content in each fraction was determined using the Folin-Ciocalteu method, according to Sombutsuwan et al. (2024). A mixture was prepared by adding 20 µL of sample to 80 µL of Folin-Ciocalteu reagent, followed by 200 µL of 7.5% Na₂CO₃ solution and 700 µL of distilled water. After incubation in the dark for 2 hr, absorbance was recorded at 765 nm using a microplate spectrophotometer. Results were expressed as mg gallic acid equivalents per g DFRB (mg GAE/g DFRB). Each sample was analyzed in triplicate.

Evaluation of antioxidant activity through 2,2-diphenyl-1-picrylhydrazyl and 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid assays

The antioxidant activity of the FPCs, SEBPCs and SGBPCs was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) radical scavenging assays, following the protocols described by Laokuldilok et al. (2011) and Sombutsuwan et al. (2021).

2,2-Diphenyl-1-picrylhydrazyl assay

The DPPH radical scavenging activity was measured using a method adapted from Laokuldilok et al. (2011). Briefly, 20 µL of the sample was mixed with 20 µL of ethanol and then added to a 96-well plate containing 560 µL of 0.1 mM DPPH solution in ethanol. The mixture was incubated in the dark at room temperature for 30 min. The absorbance at 517 nm was measured using the microplate spectrophotometer and antioxidant capacity was reported as millimoles of gallic acid equivalents per gram of DFRB (mmol GAE/g DFRB). Each sample was analyzed in triplicate.

2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid assay

The antioxidant activity was further evaluated using the ABTS assay, following Sombutsuwan et al. (2021).

To prepare the mixture, 20 µL of the sample was added to a 96-well plate, followed by 180 µL of ABTS solution, with ethanol used to adjust the absorbance to 0.7. After standing for 6 min, absorbance was measured at 734 nm using the microplate spectrophotometer. The ABTS scavenging activity was calculated as described by Sombutsuwan et al. (2021) and antioxidant capacity was expressed as in the DPPH assay. Each sample was analyzed in triplicate.

Analysis and measurement of phenolic acids using high-performance liquid chromatography

High-performance liquid chromatography (HPLC) was used to determine the phenolic acid profile of the samples, as described by Sombutsuwan et al. (2021, 2024). The analysis was performed using a Waters 515 pump (Waters Associates, Milford, USA) a 10 µL loop Rheodyne 7125 injector and a Shimadzu photodiode array detector (PDA; Shimadzu, Kyoto, Japan). A Mightysil Si60 column (250 mm × 4.6 mm, 5 µm diameter; Kanto Chemical Co. Inc, Japan) was used for component separation, with a guard column of the same type (10 mm × 4.6 mm, 5 µm) to protect the main column. The mobile phase, composed of hexane, ethyl acetate and acetic acid in a 70:30:0.2 (volume per volume per volume) ratio, was passed through the column at a flow rate of 1 mL/min. UV absorbance measured using the microplate reader and recorded across the 257–320 nm range; subsequently, the data were processed using the LC Solution software (version 1.24; Japan). Quantitative analysis of 4-hydroxybenzoic acid, vanillic acid, p-coumaric acid, ferulic acid, syringic acid, sinapic acid and caffeic acid was conducted using calibration curves obtained from external standards. The compound identification was confirmed by comparing retention times and UV spectra with those of the standards.

Analysis of value-added biomolecules

The concentrations of key value-added biomolecules in the DFRB extract were determined using standard analytical procedures. The phytic acid content was quantified following the method described by Gao et al. (2007), with slight modification. Briefly, 5 mL of extracted supernatant was passed through a Dowex anion exchange column (Thermo Fisher Scientific, USA). Subsequently, the column was eluted with 7.5 mL of 0.1 M NaCl followed by 7.5 mL of 0.7 M NaCl to obtain the phytate containing fraction. Then, a 3 mL eluted sample was mixed with 1 mL of Wade reagent and incubated

at room temperature for 10 min. Absorbance was measured at 500 nm using the microplate spectrophotometer. DES was used as a blank. The phytic acid concentration was calculated using a calibration curve constructed from phytic acid standard.

The protein content was measured using the Bradford assay, which was performed according to the manufacturer's instructions using the Protein Assay Kit (Bio-Rad Laboratories Ltd, Bangkok, Thailand), as originally described by Bradford (1976). Briefly, 10 μ L of extracted solution was combined with 200 μ L of Bradford reagent for 5 min. The absorbance was recorded at 595 nm using a microplate spectrophotometer. DES was used as a blank reference. The protein content was calculated based on a bovine serum albumin standard curve.

The reducing sugar concentration was determined using the DNS assay (Miller, 1959; Sombutsuwan et al., 2024). A 100 μ L aliquot of the extracted supernatant was mixed with 100 μ L of DNS reagent and heated at 80°C for 30 min in a water bath. After cooling to room temperature, the absorbance was recorded at 575 nm using the microplate spectrophotometer. DES was used as a blank. The reduced sugar concentration was determined based on a glucose standard curve.

Bacterial preparation

The probiotic strain *Lactobacillus plantarum* ATCC 14917 was cultivated anaerobically at 37°C for 24 hr in DeMan, Rogosa and Sharpe medium. Pathogenic strains, consisting of *Escherichia coli* ATCC 25922, *Salmonella* Typhi and *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923 (all obtained from the Division of Microbiology, Faculty of Science, Prince of Songkla University, Thailand) were grown aerobically at 37°C for 24 hr in trypticase soy medium. Prior to experimentation, the optical density of both the probiotic and pathogenic strains was adjusted to 0.5 McFarland, equivalent to approximately 1×10^8 CFU/mL.

Prebiotic properties

The prebiotic properties of the DFRB extract were evaluated by comparing the growth of *L. plantarum* and *E. coli* in minimal medium supplemented with 1% (volume per volume, v/v) DFRB extract, without glucose. In the control group, both bacteria were cultured in minimal medium with 1% (weight per volume) glucose. Growth was assessed by counting bacterial colonies after incubating *L. plantarum* anaerobically and *E. coli* aerobically at 37°C for 0 and 24 hr, using the pour

plate method. The prebiotic activity score was calculated using Equation 2 (Huebner et al., 2007):

$$\text{Prebiotic activity score} = X - Y \quad (2)$$

$$\text{where } X = \frac{(\text{Log of probiotic activity on prebiotic at 24 hr} - \text{Log of probiotic activity on prebiotic at 0 hr})}{(\text{Log of probiotic activity on glucose at 24 hr} - \text{Log of probiotic activity on glucose at 0 hr})}$$

$$\text{and } Y = \frac{(\text{Log of enteric activity on prebiotic at 24 hr} - \text{Log of enteric activity on prebiotic at 0 hr})}{(\text{Log of enteric activity on glucose at 24 hr} - \text{Log of enteric activity on glucose at 0 hr})}$$

and the logarithm (log) of probiotic and enteric activity is measured in colony forming units per milliliter (CFU/mL).

Prebiotic property of defatted rice bran extract on growth of probiotic

L. plantarum was cultured in a minimal medium, as described by Olano-Martin et al. (2000), which contained 10 g/L peptone, 5 g/L yeast extract, 10 g/L beef extract, 1 mL/L Tween 80, 2 g/L ammonium citrate, 5 g/L sodium acetate, 0.1 g/L magnesium sulfate hydrate, 0.05 g/L manganese sulfate hydrate and 2 g/L dipotassium phosphate. The bacteria were inoculated at 1% (v/v) into the medium, supplemented with either 1% (v/v) glucose or 1% (v/v) DFRB extract, with a control group inoculated in medium without glucose. Then, the cultures were incubated at 37°C for 0, 12, 24, 36 and 48 hr under anaerobic conditions. After incubation, the cultures were monitored using the microplate reader to measure the optical cell density at 660 nm (OD_{660}), with each experiment repeated three times.

Pathogenic bacteria inhibition

The agar well diffusion test was used to assess the inhibitory effect of DFRB extracted in DES against pathogenic bacteria. Mueller-Hinton agar plates were drilled with a 5 mm diameter cork borer and then swabbed with *E. coli*, *S. Typhi*, *P. aeruginosa* and *S. aureus*. Subsequently, 50 μ L of DFRB extract was added into the wells on the agar plates, followed by incubation at 37°C for 24 hr. After incubation, the diameters of the inhibition zones were measured. DES without DFRB extract served as the negative control, while 15 μ g/mL of erythromycin and 30 μ g/mL of tetracycline were used as positive controls for Gram-positive and Gram-negative bacteria, respectively.

Statistical analysis

Values were expressed as mean $\pm$ SD from at least three independent replicates. Statistical analysis was conducted using two-way analysis of variance (ANOVA) followed by Tukey's multiple-comparisons test in GraphPad Prism (version 9.3.0; GraphPad Software, USA). Differences were considered statistically significant at $p < 0.05$.

Results and Discussion

Defatted rice bran compositions and deep eutectic solvent properties

The proximate composition analysis of the DFRB identified hemicellulose (23.22%) and protein (18.00%) as the major constituents, along with lower levels of ash (11.74%), cellulose (8.20%), lignin (4.27%) and moisture (5.11%). The Na:Gly DES, characterized by a neutral pH (7.39 ± 0.03) and high polarity ($E_{NR} = 48.96$ kcal/mol), enhanced the extraction efficiency of bioactive compounds, particularly PC and other valuable biomolecules known for their antioxidant properties.

Efficiency of Na:Gly deep eutectic solvent for phenolic compound extraction

Fig. 1A illustrates the phenolic profiles in the DFRB extract, categorized into FPCs, SEBPCs and SGBPCs.

The Na:Gly DES system predominantly extracted glycosylated (47.09% w/w) and esterified phenolics (45.26% w/w), with a minor portion of FPCs (7.65% w/w). The total phenolic content recovered from the crude extract was 3.49 mg/g DFRB, based on the sum of quantified phenolic acids across all fractions. These results highlighted the efficiency of Na:Gly DES in targeting bound phenolics, which are typically more difficult to extract than free phenolics. In contrast to previous report, the extraction process greatly favored the recovery of FPCs under extream DES pH condition (Sombutsuwan et al., 2024). The neutral pH of Na:Gly DES facilitated the efficient recovery of both SEBPCs and SGBPCs, while preserving the stability of the phenolic compounds. In addition, the use of Na:Gly DES, which exhibited a near neutral pH (7.39), might have helped to preserve the prebiotic potential of the bioactive compounds extracted from DFRB. This aligned with another study that reported a slightly acidic-to-neutral pH range (6.0–6.5) was optimal for maximizing prebiotic activity, while a very low or very high pH could reduce effectiveness (Palframan et al., 2002).

Composition of phenolic compounds

The identification and quantification of phenolic acids extracted from the DFRB samples were validated using HPLC and comparing the retention times and UV spectra of sample peaks with those of authentic standards. Supplementary Fig. S1 presents chromatograms of individual phenolic acid standards (4-hydroxybenzoic acid, vanillic acid, caffeic acid,

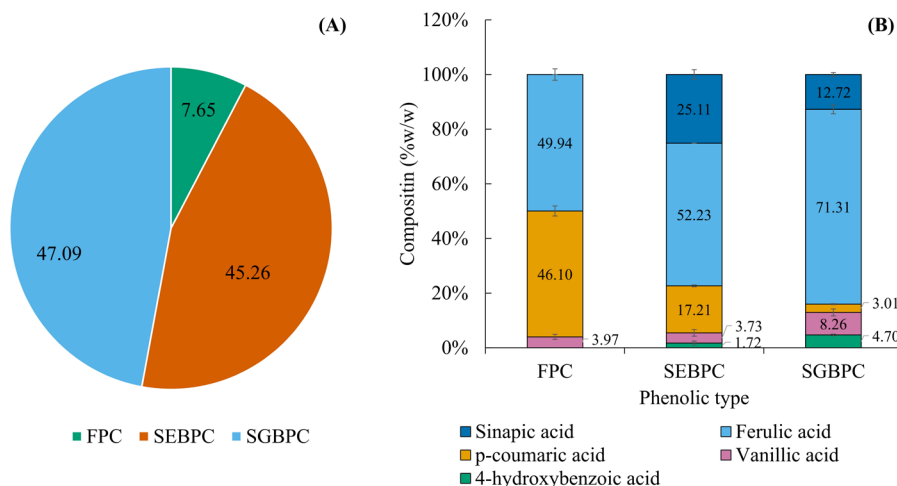


Fig. 1 Distribution of phenolic compound fractions: (A) extracted using Na:Gly deep eutectic solvent; (B) their phenolic acid composition. FPC = free phenolic compounds; SEBPC = soluble esterified bound phenolic compound; SGBPC = soluble glycosylated bound phenolic compound; w/w = weight per weight. Error bars represent mean $\pm$ SD ($n = 3$).

p-coumaric acid, ferulic acid, syringic acid and sinapic acid), each analyzed independently under identical conditions, with detection at their respective $\lambda_{\max}$ values. Supplementary Fig. S2 shows the chromatogram of the standard mixture acquired at 275 nm, confirming the separation and retention order of the compounds. A representative chromatogram of the extracted DFRB sample is shown in Supplementary Fig. S3, demonstrating the successful identification of phenolic acids based on matched retention times and UV absorbance characteristics.

The phenolic composition varied markedly among the different fractions (Fig. 1B). In the FPC fraction, p-coumaric acid (46.10%) and ferulic acid (49.94%) were predominant, with minor contributions from vanillic acid (3.97%), while 4-hydroxybenzoic acid and sinapic acid were not detected. In the SEBPCs, ferulic acid (52.23%) and sinapic acid (25.11%) were the major constituents, followed by p-coumaric acid (17.21%), vanillic acid (3.73%) and 4-hydroxybenzoic acid (1.72%). The SGBPC fraction contained the highest proportion of ferulic acid (71.31%), followed by sinapic acid (12.72%), vanillic acid (8.26%) and 4-hydroxybenzoic acid (4.70%).

The dominance of ferulic acid, p-coumaric acid and sinapic acid in the DFRB phenolic profile suggested their crucial role in the chemical composition of this material. These findings aligned with other studies, which identified ferulic acid as a major phenolic compound in cereal byproducts, such as wheat bran and rice bran, occurring in both free and bound forms (Kim et al., 2006; Laokuldilok et al., 2011; Avinash et al., 2024). In addition, p-coumaric acid and sinapic acid have been reported in free and bound forms in rice bran (Pang et al., 2018).

Antioxidant activities

The antioxidant activities of the phenolic fractions were assessed using the DPPH and ABTS assays (Fig. 2), demonstrating strong radical scavenging capability, particularly in the bound phenolic fractions. The Na:Gly DES system has been reported to enhance the extraction of phenolic compounds, including polyphenols from agricultural byproducts (Slim et al., 2018; Stefou et al., 2019; Alibade et al., 2021).

For the DPPH assay, the antioxidant activity of the SEBPC and SGBPC fractions were approximately 4.79 and 4.85 mmol GAE/g DFRB, respectively, while the FPCs had the lowest activity at approximately 0.62 mmol GAE/g DFRB.

Similarly, the ABTS assay revealed antioxidant activities of approximately 5.48 and 5.41 mmol GAE/g DFRB for the SEBPCs and SGBPCs, respectively, compared with less than 2.0 mmol GAE/g DFRB for the FPCs. These results highlighted the superior performance of the bound phenolic fractions, likely attributable to their higher concentrations of TPCs (Fig. 1A).

These findings were consistent with other reports, demonstrating a strong correlation between bound phenolic content and antioxidant capacity in whole grains and bran fractions (Begum et al., 2015). Furthermore, the radical scavenging activity of phenolics extracted using Na:Gly DES had higher antioxidant potency than those extracted with weak acidic and basic DES (Sombutsuwan et al., 2024).

The ABTS assay showed greater sensitivity than the DPPH assay, with slightly higher antioxidant values observed across all fractions. This difference stemmed from the ABTS assay's broader mechanism coverage, capturing both hydrogen atom transfer and single electron transfer interactions, providing a more comprehensive evaluation of antioxidant capacity (Apak, 2019).

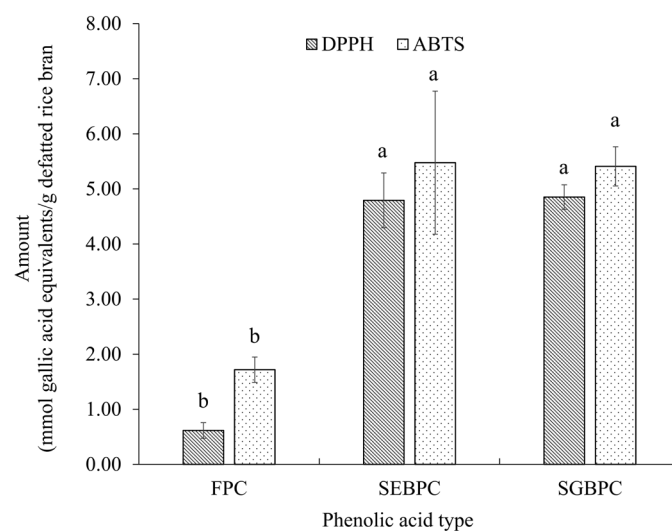


Fig. 2 Antioxidant activities of Na:Gly deep eutectic solvent-extracted fractions for different types of phenolic acid measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assays. FPC = free phenolic compounds; SEBPC = soluble esterified bound phenolic compound; and SGBPC = soluble glycosylated bound phenolic compound. Columns labeled with different letters (a, b) within the same assay (DPPH or ABTS) indicate significant differences ($p < 0.05$) among phenolic acid types. Error bars represent mean $\pm$ SD ($n = 3$).

Value-added biomolecules in defatted rice bran extract

Further evaluation of the DFRB extract revealed specific concentrations of key biomolecules: proteins (7.08 ± 0.46 mg/g), reducing sugars (4.36 ± 0.32 mg/g) and phytic acid (1.14 ± 0.13 mg/g DFRB). The neutral pH of the Na:Gly DES potentially influenced the efficiency of extracting these valuable biomolecules. In the present study, the protein content was similar to that obtained under mildly acidic or alkaline conditions by Sombutsuwan et al. (2024); however, the reducing sugar content was higher than that extracted with weak acidic DES and lower than that with weak basic DES in their study. Additionally, the low phytic acid content was comparable to results obtained using strong basic DES by Sombutsuwan et al. (2024), with the observed variation likely due to the ability of DES to efficiently break down plant cell walls at both low and high pH levels, thereby enhancing the extraction of bioactive compounds into the solvent. For effective extraction of rice bran protein, the optimal pH range generally falls between 7.5 and 11; however, a highly acidic pH range of 0 to 1 is required for the dissociation of phytates from their complexes with iron and proteins (Gifford et al., 1990; Zheng et al., 2019).

Prebiotic properties of defatted rice bran extract

In the present study, the prebiotic property of the DFRB extract had demonstrated ability to support and promote the growth of beneficial organisms (probiotics) while inhibiting the proliferation of pathogenic ones. The growth of *E. coli* and *L. plantarum* significantly increased at 24 hr across all three medium conditions compared to 0 hr, as demonstrated in Table 1. Specifically, the growth of *E. coli* after 24 hr in the presence of glucose (8.42 ± 0.01 log CFU/mL) surpassed that in the DFRB extract (8.23 ± 0.01 log CFU/mL), indicating a restriction of *E. coli* growth by the DFRB extract, as observed in the absence of glucose in the medium (8.22 ± 0.01 log CFU/mL). Notably, the DFRB extract medium effectively promoted

L. plantarum growth (8.91 ± 0.11 log CFU/mL), comparable to the efficacy of glucose medium (8.84 ± 0.05 log CFU/mL). The growth-promoting characteristic of the DFRB extract was specific to probiotic bacteria and restricted the growth of other bacterial species, particularly those that are pathogenic. The DFRB extract had a favorable prebiotic activity score of 0.65 for the growth of *L. Plantarum*, where a score greater than zero signifies the existence of prebiotic properties (Huebner et al., 2007). Valero-Cases et al. (2017) identified various compounds with prebiotic properties, including carbohydrates, polyphenols, polysaccharides and polypeptide polymers, which also had antimicrobial properties in the gastrointestinal tract. Therefore, the DFRB extract containing reducing sugars, protein and polyphenols, exhibited prebiotic characteristics.

Antimicrobial activity of defatted rice bran extract in inhibiting pathogen growth

The antibacterial activity of the DFRB extract against pathogens was determined using the agar well diffusion assay. The DFRB extract showed a significant inhibitory effect on Gram-positive bacteria, particularly *S. aureus*, with an observed inhibition zone of 30.00 ± 0.00 mm at its maximum. However, its inhibitory effects on the Gram-negative bacterium, *P. aeruginosa* were less pronounced, measuring 8.00 ± 0.01 mm (Table 2). According to Kabploy et al. (2022), the crude extract from Sangyod rice had significant inhibitory effects on Gram-positive bacteria (*B. cereus* and *S. aureus*) but did not inhibit the growth of Gram-negative bacteria

Table 2 Antibacterial activity of defatted rice bran (DFRB) extract (inhibition zone, mm) against pathogenic bacteria.

Bacterium	Inhibition zone (mm)
	DFRB extract
<i>Escherichia coli</i>	ND
<i>Salmonella</i> Typhi	ND
<i>Pseudomonas aeruginosa</i>	8.00 ± 0.01
<i>Staphylococcus aureus</i>	30.00 ± 0.00

ND = not detected.

Table 1 Effect of different culture medium conditions on the growth of *Escherichia coli* and *Lactobacillus plantarum*.

Condition	<i>E. coli</i> (log CFU/mL)		<i>L. plantarum</i> (log CFU/mL)	
	0 hr	24 hr	0 hr	24 hr
Without glucose	7.00 ± 0.07^{Aa}	8.22 ± 0.01^{Ba}	5.45 ± 0.06^{Aa}	8.73 ± 0.00^{Ba}
Glucose	7.09 ± 0.01^{Aa}	8.42 ± 0.01^{Bb}	6.04 ± 0.05^{Ab}	8.84 ± 0.05^{Bab}
DFRB extract	7.26 ± 0.01^{Ab}	8.23 ± 0.01^{Ba}	5.05 ± 0.04^{Aa}	8.91 ± 0.11^{Bb}

Values (mean $\pm$ SD; n =3) followed by different lowercase superscripts within a column indicate significant differences ($p < 0.05$), whereas values followed by different capital superscripts within a row for the same bacterial strain indicate significant differences ($p < 0.05$). CFU = colony forming unit; DFRB = defatted rice bran.

(*E. coli* and *P. aeruginosa*). This discrepancy could be attributed to the structural differences in the bacterial cell envelope between Gram-negative and Gram-positive bacteria. Gram-negative bacteria have an outer membrane with a relatively low permeability barrier (Runti et al., 2015). Furthermore, certain hydrophobic substances, such as phenols, esters and tannins, have poor penetration ability through the outer membrane of Gram-negative pathogens, which consists primarily of phospholipids (Duangjai et al., 2016).

Effect of defatted rice bran extract on the growth of *L. plantarum*

The growth of *L. plantarum* cultivated in the DFRB extract medium was compared with that in a glucose medium. Bacterial growth was measured at 0, 12, 24, 36 and 48 hr using spectrophotometry at OD₆₆₀. As illustrated in Table 3, the growth of the probiotic bacterium *L. plantarum* was significantly enhanced at 36 hr and 48 hr of incubation when DFRB extract was used as a carbon source compared to the glucose medium. The decline in growth observed in the glucose medium between 24 and 48 hr may have been associated with nutrient depletion, acid accumulation and self-inhibition from metabolic byproducts. In contrast, rice bran carbohydrates such as xylo-oligosaccharides prolonged *L. plantarum* growth up to 48 hr (Antunes et al., 2023).

Conclusion

This research explored the use of Na:Gly DES for extracting PCs and other beneficial biomolecules from DFRB. The findings indicated successful extraction of soluble bound phenolic acids and other valuable biomolecules, which demonstrated prebiotic activity and the ability to inhibit pathogen growth. This approach should contribute to a more sustainable use of rice bran by valorizing its bioactive components through an eco-friendly extraction method. Ongoing efforts are being directed toward optimizing the composition of the natural extract to maximize its potential as an antioxidant ingredient, thereby expanding its application scope.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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References

- Abbott, A.P., Capper, G., Davies, D.L., Rasheed, R.K., Tambyrajah, V. 2003. Novel solvent properties of choline chloride/urea mixtures. *Chem. Commun.* 1: 70–71. doi.org/10.1039/B210714G
- Al-Doury, M. K. W., Hettiarachchy, N. S., Horax, R. 2018. Rice-endosperm and rice-bran proteins: A review. *J. Am. Oil Chem. Soc.* 95: 943–956. doi.org/10.1002/aocs.12110
- Alexandri, M., López-Gómez, J.P., Olszewska-Widdrat, A., Venus, J. 2020. Valorising agro-industrial wastes within the circular bioeconomy concept: the case of defatted rice bran with emphasis on bioconversion strategies. *Fermentation*. 6: 42. doi.org/10.3390/fermentation6020042
- Alibade, A., Lakka, A., Bozinou, E., Lalas, S.I., Chatzilazarou, A., Makris, D.P. 2021. Development of a green methodology for simultaneous extraction of polyphenols and pigments from red winemaking solid wastes (pomace) using a novel glycerol-sodium benzoate deep eutectic solvent and ultrasonication pretreatment. *Environments*. 8: 90. doi.org/10.3390/environments8090090
- Alsaleem, A.K., Hamouda, A.E.M. 2024. Optimizing probiotic low-fat yogurt: The benefits of incorporating defatted rice bran for enhanced quality and functionality. *Food Sci. Nutr.* 12: 10242–10254. doi.org/10.1002/fsn3.4558
- Antunes, L.L., Back, A.L., Catto Kossar, M.L.B., Spessato, A.G., Colla, E., Drunkler, D.A. 2023. Prebiotic potential of carbohydrates from defatted rice bran – Effect of physical extraction methods. *Food Chem.* 404: 134539. doi.org/10.1016/j.foodchem.2022.134539
- Apak, R. 2019. Current issues in antioxidant measurement. *J. Agric. Food Chem.* 67: 9187–9202. doi/10.1021/acs.jafc.9b03657

Table 3 Growth of *Lactobacillus plantarum*, measured as optical density at 660 nm (OD₆₆₀), in glucose and defatted rice bran (DFRB) media at various incubation times.

Medium	<i>L. plantarum</i> growth (OD ₆₆₀ nm)				
	0 hr	12 hr	24 hr	36 hr	48 hr
Glucose	0.015±0.02 ^{Aa}	0.003±0.00 ^{Ba}	0.236±0.02 ^{Ba}	0.192±0.03 ^{Ba}	0.171±0.05 ^{Ba}
DFRB extract	0.051±0.01 ^{Aa}	0.069±0.03 ^{Aa}	0.321±0.01 ^{Ba}	0.348±0.01 ^{Bb}	0.365±0.01 ^{Bb}

Values (mean ± SD; n = 3) followed by different lowercase superscripts within a column indicate significant differences ($p < 0.05$) between glucose and DFRB medium, whereas values followed by different capital superscripts within a row indicate significant differences ($p < 0.05$) relative to 0 h.

- Arruda, H. S., Pereira, G. A., de Morais, D. R., Eberlin, M. N., Pastore, G. M. 2018. Determination of free, esterified, glycosylated and insoluble-bound phenolics composition in the edible part of araticum fruit (*Annona crassiflora* Mart.) and its by-products by HPLC-ESI-MS/MS. *Food Chem.* 245: 738–749. doi.org/10.1016/j.foodchem.2017.11.120
- Association of Official Analytical Chemists. 2005. Official Methods of Analysis, 18th ed. The Association of Official Analytical Chemists. Gaithersburg, MD, USA.
- Avinash, G., Sharma, N., Prasad, K.R., Kaur, R., Singh, G., Pagidipala, N., Thulasinathan, T. 2024. Unveiling the distribution of free and bound phenolic acids, flavonoids, anthocyanins and proanthocyanidins in pigmented and non-pigmented rice genotypes. *Front. Plant Sci.* 15: 1324825. doi.org/10.3389/fpls.2024.1324825
- Begum, A., Goswami, A., Chowdhury, P. 2015. A comparative study on free and bound phenolic acid content and their antioxidant activity in bran of rice (*Oryza sativa* L.) cultivars of Eastern Himalayan range. *Int. J. Food Sci. Tech.* 50: 2529–2536. doi.org/10.1111/ijfs.12920
- Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72: 248–254. doi.org/10.1016/0003-2697(76)90527-3
- Canan, C., Kalschne, D.L., Ongaratto, G.C., Leite, D.O., Cursino, A.T.C., Flores, E. L. de M., Ida, E. I. 2021. Antioxidant effect of rice bran purified phytic acid on mechanically deboned chicken meat. *J. Food Process. Preserv.* 45: e15716. doi.org/10.1111/jfpp.15716
- Dokkaew, A., Punvittayagul, C., Insuan, O., Limtrakul, P., Wongpoomchai, R. 2019. Protective effects of defatted sticky rice bran extracts on the early stages of hepatocarcinogenesis in rats. *Molecules.* 24: 2142. doi.org/10.3390/molecules24112142
- Duangjai, A., Suphrom, N., Wungthath, J., Ontawong, A., Nuengchamnong, N., Yosboonruang, A. 2016. Comparison of antioxidant, antimicrobial activities and chemical profiles of three coffee (*Coffea arabica* L.) pulp aqueous extracts. *Integr. Med. Res.* 5: 324–331. doi.org/10.1016/j.imr.2016.09.001
- Gao, Y., Shang, C., Maroof, M.A.S., Biyashev, R.M., Grabau, E.A., Kwanyuen, P., Burton, J.W., Buss, G.R. 2007. A modified colorimetric method for phytic acid analysis in soybean. *Crop Sci.* 47: 1797–1803. doi.org/10.2135/cropsci2007.03.0122
- Gifford, S.R., Clydesdale, F.M. 1990. Interactions among calcium, zinc and phytate with three protein sources. *J. Food Sci.* 55: 1720–1724. doi.org/10.1111/j.1365-2621.1990.tb03608.x
- Gul, K., Yousuf, B., Singh, A.K., Singh, P., Wani, A.A. 2015. Rice bran: Nutritional values and its emerging potential for development of functional food—A review. *Bioact. Carbohydr. Diet.* 6: 24–30. doi.org/10.1016/j.bcdf.2015.06.002
- Hayyan, M., Hashim, M.A., Hayyan, A., Al-Saadi, M.A., AlNashef, I.M., Mirghani, M.E.S., Saheed, O.K. 2013. Are deep eutectic solvents benign or toxic?. *Chemosphere.* 90: 2193–2195. doi.org/10.1016/j.chemosphere.2012.11.004
- Hizaddin, H.F., Wazeer, I., Huzaimi, N.A.M., El Blidi, L., Hashim, M.A., L  v  que, J.-M., Hadj-Kali, M.K. 2022. Extraction of phenolic compound from model pyrolysis oil using deep eutectic solvents: Computational screening and experimental validation. *Separations.* 9: 336. doi.org/10.3390/separations9110336
- Huebner, J., Wehling, R.L., Hutkins, R.W. 2007. Functional activity of commercial prebiotics. *Int. Dairy J.* 17: 770–775. doi.org/10.1016/j.idairyj.2006.10.006
- Kabloy, K., Saengsawang, P., Romyasmit, C., et al. 2022. Sangyod rice bran extract enhances *Lactocaseibacillus paracasei* growth during the exponential phase and antibacterial activity of *L. paracasei* supernatant against zoonotic and foodborne pathogens. *Vet. World.* 15: 2466–2474. doi.org/10.14202/vetworld.2022.2466-2474
- Kaur, V., Goyal, A. K., Ghosh, G., Si, S. C., Rath, G. 2020. Development and characterization of pellets for targeted delivery of 5-fluorouracil and phytic acid for treatment of colon cancer in Wistar rat. *Heliyon.* 6: e03125. doi.org/10.1016/j.heliyon.2020.e03125
- Kim, K.H., Tsao, R., Yang, R., Cui, S.W. 2006. Phenolic acid profiles and antioxidant activities of wheat bran extracts and the effect of hydrolysis conditions. *Food Chem.* 95: 466–473. doi.org/10.1016/j.foodchem.2005.01.032
- Laokuldilok, T., Shoemaker, C.F., Jongkaewwattana, S., Tulyathan, V. 2011. Antioxidants and antioxidant activity of several pigmented rice brans. *J. Agric. Food Chem.* 59: 193–199. doi.org/10.1021/jf103649q
- Liu, S., Jia, M., Chen, J., Wan, H., Dong, R., Nie, S., Xie, M., Yu, Q. 2019. Removal of bound polyphenols and its effect on antioxidant and prebiotics properties of carrot dietary fiber. *Food Hydrocoll.* 93: 284–292. doi.org/10.1016/j.foodhyd.2019.02.047
- Lou, X., Xu, H., Hanna, M., Yuan, L. 2020. Identification and quantification of free, esterified, glycosylated and insoluble-bound phenolic compounds in hawthorn berry fruit (*Crataegus pinnatifida*) and antioxidant activity evaluation. *LWT- Food Sci. Technol.* 130: 109643. doi.org/10.1016/j.lwt.2020.109643
- Miller, G.L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.* 31: 426–428. doi.org/10.1021/ac60147a030
- Mulia, K., Fauzia, F., Krisanti, E.A. 2019. Polyalcohols as hydrogen-bonding donors in choline chloride-based deep eutectic solvents for extraction of xanthenes from the pericarp of *Garcinia mangostana* L. *Molecules.* 24: 636. doi.org/10.3390/molecules24030636
- Nassar, M., Nassar, R., Maki, H., Al-Yagoob, A., Hachim, M., Senok, A., Williams, D., Hiraishi, N. 2021. Phytic acid: Properties and potential applications in dentistry. *Front. Mater.* 8: 638909. doi.org/10.3389/fmats.2021.638909
- Olano-Martin, E., Mountzouris, K.C., Gibson, G.R., Rastall, R.A. 2000. In vitro fermentability of dextran, oligodextran and maltodextrin by human gut bacteria. *Br J Nutr.* 83: 247–255. doi.org/10.1017/S0007114500000325
- Palframan, R.J., Gibson, G.R., Rastall, R.A. 2002. Effect of pH and dose on the growth of gut bacteria on prebiotic carbohydrates *in vitro*. *Anaerobe* 8: 287–292. doi.org/10.1006/anae.2002.0434
- Pang, Y., Ahmed, S., Xu, Y., Beta, T., Zhu, Z., Shao, Y., Bao, J. 2018. Bound phenolic compounds and antioxidant properties of whole grain and bran of white, red and black rice. *Food Chem.* 240: 212–221. doi.org/10.1016/j.foodchem.2017.07.095
- Runti, G., Pacor, S., Colomban, S., Gennaro, R., Navarini, L., Scocchi, M. 2015. Arabica coffee extract shows antibacterial activity against *Staphylococcus epidermidis* and *Enterococcus faecalis* and low toxicity towards a human cell line. *LWT- Food Sci. Technol.* 62: 108–114. doi.org/10.1016/j.lwt.2014.12.039

- Shahidi, F., Hossain, A. 2023. Importance of insoluble-bound phenolics to the antioxidant potential is dictated by source material. *Antioxidants*. 12: 203. doi.org/10.3390/antiox12010203
- Slim, Z., Jancheva, M., Grigorakis, S., Makris, D.P. 2018. Polyphenol extraction from *Origanum dictamnus* using low-transition temperature mixtures composed of glycerol and organic salts: Effect of organic anion carbon chain length. *Chem. Eng. Commun.* 205: 1494–1506. doi.org/10.1080/00986445.2018.1458026
- Sombutsuwan, P., Durand, E., Aryusuk, K. 2024. Effect of acidity/alkalinity of deep eutectic solvents on the extraction profiles of phenolics and biomolecules in defatted rice bran extract. *PeerJ anal. chem.* 6: e29. doi.org/10.7717/peerj-achem.29
- Sombutsuwan, P., Jirattisakul, A., Nakornsadet, A., Akepratumchai, S., Chumsantea, S., Pojjanapornpun, S., Lilitchan, S., Krisnangkura, K., Aryusuk, K. 2021. A simple and efficient method for synthesis and extraction of ethyl ferulate from γ -oryzanol. *J Oleo Sci.* 70: 757–767. doi.org/10.5650/jos.ess20180
- Stefou, I., Grigorakis, S., Loupassaki, S., Makris, D. 2019. Development of sodium propionate-based deep eutectic solvents for polyphenol extraction from onion solid wastes. *Clean Technol. Environ. Policy*. 21: 1563–1574. doi.org/10.1007/s10098-019-01727-8
- Valero-Cases, E., Nuncio-Jáuregui, N., Frutos, M.J. 2017. Influence of fermentation with different lactic acid bacteria and in vitro digestion on the biotransformation of phenolic compounds in fermented pomegranate juices. *J. Agric. Food Chem.* 65: 6488–6496. doi.org/10.1021/acs.jafc.6b04854
- Van Soest, P.J., Robertson, J.B., Lewis, B.A. 1991. Methods for dietary fiber, neutral detergent fiber and nonstarch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74: 3584–3597. doi.org/10.3168/jds.S0022-0302(91)78551-2
- Wang, Z., Chen, X., Guo, Z., Feng, X., Huang, P., Du, M., Zalán, Z., Kan, J. 2022. Distribution and natural variation of free, esterified, glycosylated and insoluble-bound phenolic compounds in brocade orange (*Citrus sinensis* L. Osbeck) peel. *Food Res. Int.* 153: 110958. doi.org/10.1016/j.foodres.2022.110958
- Yang, Z. 2019. Toxicity and biodegradability of deep eutectic solvents and natural deep eutectic solvents. In: *Deep Eutectic Solvents: Synthesis, Properties and Applications*. Wiley-VCH. Weinheim, Germany. pp. 43–60.
- Yin, Z.N., Wu, W.J., Sun, C.Z., et al. 2019. Antioxidant and anti-inflammatory capacity of ferulic acid released from wheat bran by solid-state fermentation of *Aspergillus niger*. *Biomed. Environ. Sci.* 32: 11–21. doi.org/10.3967/bes2019.002
- Zhang, L., Wu, T., Zhang, Y., Chen, Y., Ge, X., Sui, W., Zhu, Q., Geng, J., Zhang, M. 2023. Release of bound polyphenols from wheat bran soluble dietary fiber during simulated gastrointestinal digestion and colonic fermentation in vitro. *Food Chem.* 402: 134111. doi.org/10.1016/j.foodchem.2022.134111
- Zhang, Q., De Oliveira Vigier, K., Royer, S., Jérôme, F. 2012. Deep eutectic solvents: syntheses, properties and applications. *Chem. Soc. Rev.* 41: 7108–7146. doi.org/10.1039/C2CS35178A
- Zhao, G., Zhang, R., Dong, L., et al. 2018. A comparison of the chemical composition, in vitro bioaccessibility and antioxidant activity of phenolic compounds from rice bran and its dietary fibres. *Molecules*. 23: 202. doi.org/10.3390/molecules23010202
- Zheng, B., Zhao, X., Ao, T., Chen, Y., Xie, J., Gao, X., Liu, L., Hu, X., Yu, Q. 2024. The role of bound polyphenols in the anti-obesity effects of defatted rice bran insoluble dietary fiber: An insight from multi-omic. *Food Chem.* 459: 140345. doi.org/10.1016/j.foodchem.2024.140345
- Zheng, Y., Gao, N., Wu, J., Yin, B. 2019. Rice bran protein: extraction, nutraceutical properties and potential applications. In: *Rice Bran and Rice Bran Oil*. Academic Press and AOCS Press. Champaign, USA. pp. 271–293.
- Zhong, J., Wang, Y., Li, C., et al. 2022. Natural variation on free, esterified, glycosylated and insoluble-bound phenolics of *Rubus chingii* Hu: correlation between phenolic constituents and antioxidant activities. *Food Res. Int.* 162: 112043. doi.org/10.1016/j.foodres.2022.112043