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ORIGINAL PAPER

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GREEN RECOVERY OF ANTIOXIDANTS FROM "CẨM" PURPLE RICE BRAN BY MICROWAVE-ASSISTED EXTRACTION: OPTIMIZATION AND EVALUATION OF ITS ANTIOXIDANT AND ANTI-DIABETIC PROPERTIES

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ABSTRACT

Background. "Cẩm" rice, a purple rice variety, is one of the specialty cultivars in Vietnam. During the post-harvesting process, dark-purple rice bran, which has high antioxidant content, was retrieved.

Materials and methods. Microwave-assisted extraction, a green recovery technique, was applied to extract antioxidant compounds from the "Cẩm" rice bran. The antioxidant activity and anti-diabetic properties of the extract were determined.

Results. A multiple regression analysis revealed the parameters that give the highest efficiency in the recovery of antioxidants from "Cẩm" rice bran. The observed optimal conditions were extraction at 662 W of microwave power for 6.7 mins. Under these conditions, high antioxidant activity and reduced activity of amylolitic enzymes (α-amylase and α-glycosidase) were observed.

Conclusion. The research showed that "Cẩm" rice bran extract has high antioxidant content as well as antioxidant activity. Furthermore, inhibition of amylotic enzymes was observed, which opens up the possibility of applying local rice as a nutraceutical ingredient in the food industry.

Keywords: pigmented rice, antioxidant activity, inhibition, green technology

INTRODUCTION

Rice is one of the most important staple foods around the world. Globally, 757 million tons of rice and paddy were produced in 2020 (Park et al., 2023). In Vietnam, various rice varieties are cultivated and produced annually. However, rice production generates a large amount of waste, which could be used to create value-added products (Van Tai et al., 2023). One waste product from rice production is rice bran. With a potential global market of 29.3 million tons per year, rice bran is a significant byproduct of the rice milling industry. Because of its rich nutrient content, low cost, easy availability, high antioxidant capacity, and promising effects against numerous metabolic disorders, it is attracting the attention of researchers (Sohail et al., 2017). Recent research has shown that rice bran contains various nutrients as well as antioxidant compounds (Huang and Lai, 2016). The

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presence of significant amounts of bioactive substances, including dietary fiber, tocopherols, tocotrienols, oryzanols, phytic acid, and phenolic compounds, is usually thought to influence the quality of rice (Arab et al., 2011; Thuy et al., 2022a), and these bioactive compounds could potentially reduce its glycemic index and anti-diabetic activity (Ngo et al., 2022). Rice bran also contains many vitamins and nutrients (Thuy et al., 2022a).

"Cẩm" rice, which originated in Tien Giang province (Vietnam), has a purple outer layer and is commonly used in cooking or in the production of various products such as instant rice, germinated rice flour, or gluten-free bread (Loan et al., 2023c). However, after the milling and polishing process, "Cẩm" rice bran is discarded and not properly used (Loan et al., 2023b; 2023c). Recently, the study of Loan et al. (2023a) reported that "Cẩm" rice bran could be utilized as a value-added product due to its high protein content and other nutrients. Other research has shown that these rice varieties have high anthocyanin content, accounting for 46.3 mg/100 g (Loan et al., 2023c). Due to the potentially high antioxidant content of "Cẩm" rice bran, the possible extraction and utilization of this material should be examined.

Recently, the microwave-assisted extraction process has been applied to various food waste materials, such as papaya peel and pulp (Vallejo-Castillo et al., 2020), peach waste (Kurtulbaş et al., 2022), aloe vera skin (Solaberrieta et al., 2022a), and coffee pulp waste (Tran et al., 2022). This method, which makes use of new, green, energy-saving technologies, presents several advantages over conventional technologies: namely, faster extraction kinetics, a shorter extraction time, rapid temperature increase, and a higher efficiency and extraction yield (Alvi et al., 2022). Therefore, the aim of this study was to investigate the effect of different microwave-assisted conditions and find the optimal conditions for recovering polyphenol from Cẩm rice bran by multiple regression analysis. The antioxidant and anti-diabetic properties of the extract retrieved under optimal extraction conditions were also evaluated.

MATERIALS AND METHODS

Materials

"Cẩm" rice was harvested in the spring in Vietnam. After the rice had been dehusked, polished and milled by a local company in Tien Giang province (Vietnam), dark purple "Cẩm" rice bran (CRB) was collected. In order to extract antioxidant compounds from the defatted rice bran following the method of Surin et al. (2020), it was then ground and processed to pass an 80-mesh sieve. Prior to extraction, the powder was kept at 4°C in a dark, sealed bag.

Extraction process

Based on the kinetic study of Loan et al. (2023b), suitable levels of microwave power (X_1: 500, 600, 700 W) were used for this extraction experiment for 2–6 min (X_2, extraction time). The extraction procedure made use of an electric microwave oven (EM-M25D22BM, Electrolux, Korea). The solid-to-liquid ratio used for extractions was 1:10 (w/v) with 60% food-grade ethanol as the extraction solvent, which was used in the preliminary test (Loan et al., 2023b; Van Tai et al., 2021). After extraction, the crude extracts were immediately centrifuged at 10,000 rpm for 1 minute and then filtered through filter paper while being vacuumed (V-700, Büchi, Switzerland). The extracts were then collected in glass vials and kept at 4°C to determine the total phenolic content (TPC). Three replications was conducted for each treatment. The total extraction yield was calculated using vacuum evaporation and further freeze-drying of the crude liquid extract. The percentage of total extractable solids per 100 g of dry matter was used to report the results (%, w/w).

Determination of total phenolic content

Before employing the dried extract to measure the total phenolic content (TPC), the extract was once more dissolved in 80% methanol. To determine the TPC of the extract, the method described by Wanyo et al. (2014) was used. In a nutshell, 2.25 mL of sodium carbonate (60 g/L) solution was added to the combination after 300 mL of extract had been combined with 2.25 mL of Folin-Ciocalteu reagent (10%) and had stood at room temperature for 5 minutes. A spectrophotometer was used to measure the absorbance at 725 nm after 90 min at room temperature. Gallic acid equivalents (mg GAE/g) were used based on the standard curve ($y = 0.0025x + 0.0632$, $R^2 = 0.9957$) to express the results in 1 g of dried sample.

Antioxidant and antiradical properties

Spectrophotometric analysis was used to calculate antioxidant activity. The procedure employs methanolic extract, which has been employed in previous studies (Loan et al., 2023b), and measures the sample's antioxidant activity using the percentage of inhibition of DPPH radicals (% DPPH·). Ferric reducing antioxidant power is referred to as FRAP. The unit of measurement for FRAP is M Trolox/g of dry weight. The results are given in Trolox equivalents (M Trolox/g FW), and a FRAP calculation is carried out by plotting a calibration curve (10–100 M) produced by adding Trolox to the FRAP buffer solution ($y = 1.9527x + 0.2753$, $R^2 = 0.9993$).

Oxygen Radical Absorbance Capacity Assays (ORAC) were used to further test antioxidant activity. Trolox was employed for calibration. Excitation and emission wavelengths for the fluorescence quenching experiment were 485 nm and 528 nm, respectively, and the temperature was 37°C. In order to monitor the reaction's development, observations were made every 2 minutes over a period of 2 hours (Groth et al., 2020).

Antidiabetic properties by inhibition of some digestive enzymes

For this test, methods proposed by Aalim et al. (2019) were used. In 0.1 M phosphate buffer (pH 6.9), 20 mL of CRB or control (arabose), 40 mL of 0.1% starch (w/v), and 20 mL of α-amylase (0.1 mg/mL) were combined for the α-amylase inhibition experiment. The mixture was incubated for 20 minutes at 37°C before adding 80 µL of 0.4 M HCl and 100 µL of 5 mM iodine (in 5 mM KI) to terminate the reaction. The results were expressed as inhibition present (%) after absorbance at 630 nm had been measured in comparison to a blank (without extract).

50 mL of the CRB or control, 50 mL of the α-glucosidase enzyme solution (0.3 U $*mL^{-1}$), and 50 mL of the 5 mM pNPG solution were all mixed together in a 96-well plate with 0.1 M phosphate buffer (pH 6.9) for the α-glucosidase inhibition experiment. The mixture was incubated at 37°C for 20 minutes in the dark. The results were expressed as percentage inhibition (%) and the absorbance was measured at 405 nm against a blank. Calculations of the IC_{50} values for acarbose were made as positive controls.

Multiple regression analysis

Statistical Graphics Centurion XV.I software (Statistical Graphics Corp., USA) was used to identify statistically significant variations in the antioxidant capabilities of the CRB extract. The responses used in the multiple regression analysis were TPC and yield extraction. The following equation represents the response surface analysis's second-order polynomial model (quadratic model) (Equation 1):

$$Y = \alpha_0 + \sum \alpha_i X_i + \sum \alpha_{ii} X_i^2 + \sum \alpha_{ij} X_i X_j \quad (1)$$

where

Y is TPC or extraction yield (response variable)

α_0 is the model constant

α_i, α_{ii}, and α_{ij} are linear, quadratic, and interaction effects, respectively

X_i and X_j are the independent variables.

Based on the coeficient of determination (R^2) obtained from the multiple regression analysis, the reference equation was chosen to match the data. A high coefficient of regression ($R^2 > 80\%$) should be present in the chosen reference equation.

RESULTS AND DISCUSSION

According to recent studies, sustainable recovery of valuable chemicals from natural sources can be accomplished using valorization strategies based on green technologies (Chuyen et al., 2018; Perino and Chemat, 2019; Thuy et al., 2022b). Fractions with additional value can be obtained employing green downstream processes that use solvents with increasing polarity. The method of extraction is another important factor in the green extraction process. By applying microwave-assisted extraction, researchers can reduce the usage of solvent, time, and energy (Gilbert-López et al., 2017; Perino and Chemat, 2019; Thuy et al., 2021; 2022b). In this study, microwave-assisted extraction was applied and optimized to identify the most suistable conditions for recovering the highest yield and content of antioxidants from "Cẩm" rice bran.

Multiple regression analysis of TPC

A single dependent variable and a number of independent variables can be analysed using multiple regression, a statistical approach. In order to forecast the value of the single dependent value, multiple regression analysis uses independent variables whose values are known (Moore et al., 2006). The relative contribution of each predictor to the total explained variance as well as the overall model fit can be assessed by multiple regression. Centurion Statgraphics XV.I was used to analyse the data. The quadratic model for the TPC content of CRB extract affected by extraction time and microwave power is presented in Equation 2.

$$\text{TPC (mg GAE/g)} = -30.15 + 0.23\,X_1 + 3.64\,X_2 - 0.00019\,X_1^2 + 0.002\,X_1X_2 - 0.384\,X_2^2 \quad (2)$$

The value of R^2 and adjusted R^2 accounted for 96.77% and 96.10% of the variance, respectively. These values demonstrate that the established model and the results of the multiple regression study fit together effectively. These outcomes support the model's ability to forecast the ideal circumstances required to produce the maximum TPC of the CRB extract. Moreover, the models and effects were significant at the 95.0% confidence level according to an analysis of variance, which revealed that their p-values were less than 0.05 (Table 1).

The contour plot also indicated the effect of microwave levels and extraction time on the content of phenolic compounds in the CRB extract (Fig. 1). An increase in the microwave level led to an increase in the capacity to extract phenolic compounds from the CRB material. Microwave heating produces temperature gradients between the matrix cells and the solvent phase in a solid-liquid system. Consequently, as the microwave level rises, the cell walls of the material become wet and swollen, increasing its surface area and making it easier for the solvent to enter the cells. The internal cell pressure rises as a result, which may lead to the breakdown of the cellular structure and enhance mass transfer towards the solvent phase (Chuyen et al., 2018; Loan et al., 2023b; Sánchez-Mesa et al., 2020; Thuy et al., 2022b). High microwave power typically raises system temperature, which enhances solvent power by lowering viscosity and surface tension, allowing the solubilization of compounds and shortening the extraction time (Sánchez-Camargo et al., 2021). Nonetheless, Figure 1 revealed a drop in

Table 1. Analysis of variance for total phenolic content of CRB by microwave-assited extraction

Source	Sum of Squares	F-Ratio	*p*-value
X_1	105.94	1487.96	0.0000
X_2	10.85	152.38	0.0011
X_1^2	49.02	688.53	0.0001
X_1X_2	2.26	31.79	0.0110
X_2^2	32.39	454.92	0.0002
Blocks	0.0036	0.03	0.9753
Lack-of-fit	5.69	4.21	0.1311
Pure error	0.213		
Total (corr.)	182.855		

R^2 = 96.77%; *R^2 (adjusted for)* = 96.10%; *Standard Error of Est.* = 0.267; *Mean absolute error* = 0.379.

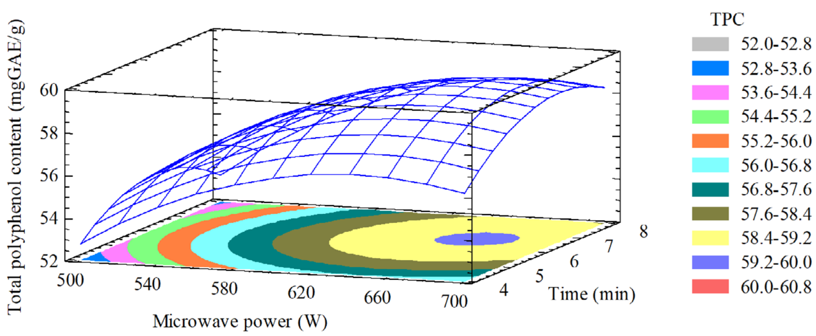

Fig. 1. Response surface plot showing the interaction of microwave power and extraction time on total phenolic content in CRB extract

TPC for values greater than 660 W. The slurry in the extraction vessel reached a high temperature at high microwave power, which may have caused thermolabile chemicals to break down and reduced the TPC (Sánchez-Camargo et al., 2021). In terms of extraction time, extending the time could lead to increaseed extraction efficiency; however, with microwave power ranging from 600 to 700 W, extraction times over 7 min resulted in a decrease in the TPC of the CRB extract. This result is in agreement with the study of Sánchez-Camargo et al. (2021) on mango peel and the study of Solaberrieta et al. (2022b) on tomato seeds. Because the diffusion of solvent into the sample matrix is accelerated with a longer extraction period, the solubility of active chemicals typically increases. This has an effect on the extraction yield. Additionally, by exposing plant tissues to heat for a longer period of time, the cells of the tissues may be damaged, speeding up the penetration of the solvent into the plant material and permitting the release of active compounds that help to raise TPC (Azaroual et al., 2021; Solaberrieta et al., 2022b). On the other hand, an extraction time that is too long could reduce TPC due to oxidation and degradation (Azaroual et al., 2021).

Optimization was carried out to indentify the optimal value. It was observed that the highest TPC content in the CRB extract was 59.27 mg GAE/g, when the microwave power and extraction time were 659 W and 6.6 min, respectively.

Multiple regression analysis of extraction yield

Microwave power and extraction time were two independent variables whose effects on extraction yield were assessed. The following second-order polynomial equation (only significant factors) was used to represent the examined response as a function of independent variables (Equation 3):

$$\text{Extraction yield (\%)} = -14.73 + 0.07\,X_1 + 1.07\,X_2 - 0.00006\,X_1^2 + 0.001\,X_1X_2 - 0.14\,X_2^2 \quad (3)$$

The reliability of the fitted models and the impact of the researched factors on the chosen responses were assessed using an ANOVA (Table 2). The fitted model's accuracy in linking anticipated results to experimental data was demonstrated by the adequate R^2 value and adjusted R^2 value that were achieved (98.00% and 97.58%, respectively). Furthermore, the high p-values for the lack of fit (0.4162 for extraction yield) demonstrated that it was not significant, indicating that the model accurately represented the data. Figure 2 presents the influence of the variables on the extraction yield. It can be observed that it is quite similar to the trend of TPC in CRB extract. As the microwave level was increased, the extraction yield increased. The extraction of CRBs required a lot of microwave power. CRB cells would be destroyed to varying degrees, depending on the microwave strength. On the basis of this, a wide range of microwave power, ranging from 500 W to 700 W, was explored. According to Figure 1, the power range between 620 and 700 W was best for releasing antioxidant chemicals from plant cell matrix. The extraction yields began to slightly decline as the microwave power increased. The reaction and degradation of the delicate active constituents in CRB under increased microwave energy could be connected to the decrease in extraction efficiency.

Table 2. Analysis of variance for extraction yield of CRB by microwave-assited extraction

Source	Sum of Squares	F-Ratio	*p*-value
X_1	10.974	580.08	0.0000
X_2	2.215	117.10	0.0000
X_1^2	4.953	261.81	0.0000
X_1X_2	0.738	39.01	0.0000
X_2^2	4.221	223.14	0.0000
Blocks	0.0402	1.06	0.3619
Lack-of-fit	0.4162		
Total (corr.)	20.822		

R^2 = 98.00%; R^2 *(adjusted)* = 97.58%; *Standard Error of Est.* = 0.14; *Mean absolute error* = 0.09.

As extraction power increased, extraction yield started to decrease. The moist biomass would experience an exothermic reaction in the microwave environment due to the vibration of the extraction solvent molecules (ethanol and water), as well as other polar molecules. The temperature difference between the interior and exterior of the cell wall would be substantial due to the quick rise in temperature. As a result, the internal fluid would exert a great deal of pressure

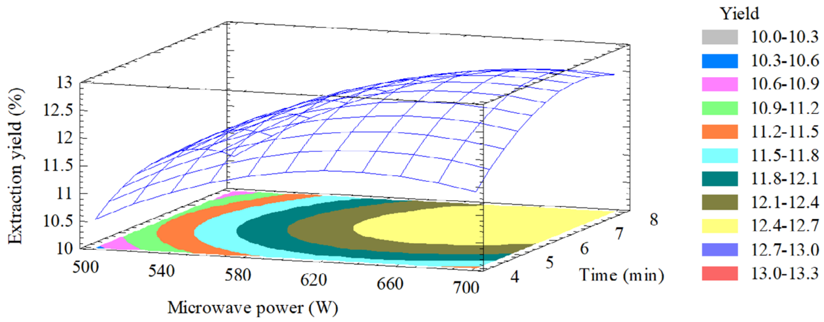

Fig. 2. Response surface plot showing the interaction of microwave power and extraction time on extraction yield in CRB extract

that would act on the cell walls, causing the cells to rupture and their content to spill out. As a result of the disruption of plant matrix and the target constituent hydrogen during the microwave radiation process, the mass transfer process of the target constituents was improved (Mary Leema et al., 2022). Although some sensitive components would deteriorate when the microwave strength surpassed a particular range, this would result in energy waste. With the microwave power increased to roughly 620 W, as shown by the plot, the extraction yields were greatly enhanced. Inadequate microwave power and greater extraction temperatures both had negative effects on the extraction process, which led to a drop in extraction efficiency. The migration of solute molecules may be facilitated by temperature changes, which also ensure that solvent molecules penetrate the extraction matrix for efficient and complete extraction. However, high temperatures could cause some temperature-sensitive components to decompose or change, making them unsuitable for extraction in those conditions (Mai et al., 2020).

During the extraction process, the period of microwave irradiation was crucial. For the study of the impact of extraction time on the extraction yields of the primary bioactive components in CRB, a time range of 4 to 8 minutes was investigated. It was possible to observe that as the radiation period increased from 4 to 6 minutes, the antioxidant constituent extraction yields increased as well. The extraction efficiency of the target chemicals started to fall, though, when the microwave radiation time exceeded 6 minutes. This was because the mass transfer of the target analytes required a lot of time, suggesting that a shorter extraction period can result in subpar extraction. Longer radiation exposure during the MAE process may also result in the breakdown of some sensitive chemicals and excessive consumption of energy (Zhao et al., 2022). The highest extraction percentage was 12.69%, when the extraction was carried out at a microwave power of 665 W for 6.83 min.

Optimization of extraction parameters and model validation

There was some variation across the microwave-assisted extraction experimental settings that maximized TPC and extraction yield. In order to get the best microwave-assisted extraction conditions, which are 6.7 min and 662 W with a desirability value of 0.97, a simultaneous multi-response optimization method was used. This method has been used to successfully determine the optimal conditions with multiple criteria in many processes, such as the extraction of anthocyanin from butterfly pea flower under ultrasound-assisted and microwave-assisted extraction (Thuy et al., 2021), the extraction of polyphenol from *Peristrophe bivalvis* L. *Merr leaf* (Thuy et al., 2022b), and the extraction of phenolic compounds from banana peel (Van Tai et al., 2021). The results are presented in Figure 3. According to the mathematical models, the predicted results were 59.27 mg GAE/g and 12.69%. Verification studies were carried out in triplicate under ideal MAE conditions, and the results showed that the TPC and extraction yield were respectively 60.13 ±0.04 mg GAE/g and 12.42 ±0.2%. These outcomes validate the models used to predict the examined answers.

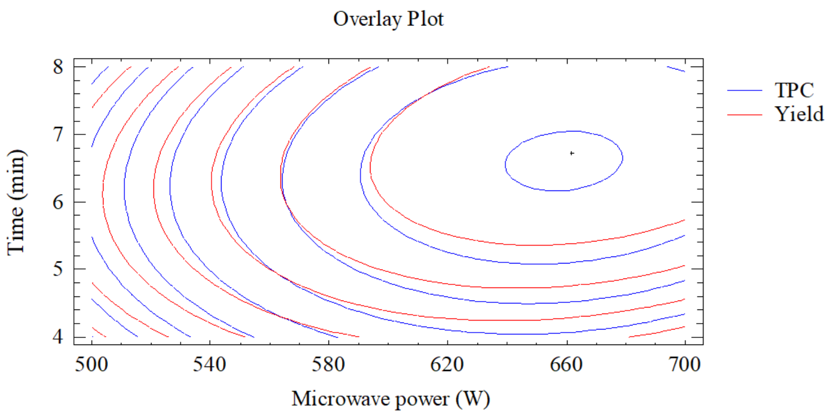

Fig. 3. Desirability value as a function of microwave power and extraction time;
* represents optimal conditions

Antioxidant and anti-diabetic properties of optimized microwave-assisted extracted CRB

CRB extract has high potential antioxidant activity. The % DPPH, FRAP value and ORAC value of CRB extract were 69.4 ±0.75% DPPH, 339.94 ±1.33 µmol Trolox/g dry weight, and 15.23 ±0.98 µmol Trolox/g dry weight, respectively. A positive correlation between antioxidant content and activity was reported by Zhang et al. (2023). Moreover, the recent study of Pokkanta et al. (2022) showed that microwave-assisted extraction could result in higher antioxidant activity and content due to the reduced extraction time. The antioxidant activity of black bean waste could reach 91.37% ABTS and 93.51% DPPH when it was extracted by MAE, which was higher than the levels achieved with traditional extraction methods (Mali and Kumar, 2023). It also could provide many positive health effects (Chen et al., 2022). In the current study, the inhibitory effects of CRB on yeast α-amylase and pancreatic α-glycosidase were analyzed. Acarbose, the positive control, had an IC_{50} value of 0.073 mg/mL and the highest level of α-amylase inhibition (98.4%). RB's IC_{50} value for α-amylase was 0.01 mg/mL, whereas it had moderate (35.2%) inhibitory action. The IC_{50} value of the positive control AC against α-glucosidase was 2.57 mg/mL, which showed reduced inhibitory activity (63.5%). With an IC_{50} of 7.1 mg/mL, CRB showed 32.1% inhibitory efficacy. The reduction of enzyme activity due to the extract's antioxidant properties was observed (Reyes et al., 2023). Mojica et al. (2015) reported 74.2% inhibition for α-amylase from Pinto-Saltillo common bean phenolic extract after heat treatment. Research by Mali and Kumar (2023) has shown that black bean waste extracted by MAE can inhibit the activity of α-amylase by 80.41% and the activity of α-glucosidase by 86.14%. Therefore, it could be expected to have anti-diabetic potential.

CONCLUSION

Microwave-assited extraction was used to successfully recover the antioxidant from "Cẩm" rice bran. The optimum extraction conditions were 662 W of microwave power for 6.7 min. The highest total phenolic compound and extraction yield were obtained at 60.13 ±0.04 mg GAE/g and 12.42 ±0.20%, respectively. Antioxidant activity was evaluated using % DPPH, FRAP, and ORAC values, which reached values of 69.4 ±0.75% DPPH, 339.94 ±1.33 µmol Trolox/g dry weight, and 15.23 ±0.98 µmol Trolox/g dry weight, respectively. CRB extract may also have anti-diabetic potential, according to the results. This research provides information which could be ultilized to further examine the potential use of highly promising waste ingredients from agricutural processes, such as rice bran, as value-added products in Vietnam. These may not only reduce enviromental damage but also create new opportunities for local farmers and businesses in the food industry.

DECLARATIONS

Data statement

All data supporting this study has been included in this manuscript.

Ethical Approval

Not applicable.

Competing Interests

The authors declare that they have no conflicts of interest.

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