# Antioxidant Dietary Fiber from the Bran of Five Philippine Pigmented Rice Varieties

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## ABSTRACT

Pigmented rice varieties are considered rich sources of antioxidant dietary fiber (ADF); consuming them may have health benefits. In this study, bran samples of five Philippine pigmented rice varieties namely, Red-64, Black Rice, Calatrava, Arabon, and Dinorado were analyzed for their ADF contents. The bulk of dietary fiber in the bran of the five varieties is insoluble dietary fiber (IDF), while a very minimal amount is soluble dietary fiber. Calatrava (CL) exhibited the highest total dietary fiber (52.5%), consisting primarily of 50 percent IDF. The CL IDF exhibited the highest total phenolic content (1.8 mg gallic acid equivalents [GAE]/gram dry weight [DW]) and total anthocyanin content (0.524 mg cyanidin-3-glucoside /100 g DW). The CL IDF also displayed the highest antioxidant activity in terms of ferric ion-reducing antioxidant power (17.72 µmol trolox equivalents/gram DW), ABTS*+ radical scavenging activity (33%), and DPPH radical quenching activity (76.7%, 3.9 mg ascorbic acid per gram DW). The majority of the phenolics associated with CL IDF is in the bound form amounting to 6.9 mg GAE/g DW. High-performance liquid chromatography revealed the presence of gallic acid, resorcinol, vanillin, ferulic acid, salicylic acid, and quercetin in the bound phenolic extract. As this study has established that the CL IDF is an ADF believed to possess many health-promoting properties, it can be valorized for potential applications in nutraceuticals and functional foods.

**Keywords:** antioxidant dietary fiber, insoluble dietary fiber, phenolics, pigmented rice bran, soluble dietary fiber
**JEL codes**: I1, Q16



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## INTRODUCTION

Dietary fibers (DF) consist of the edible parts of plants that are resistant to hydrolysis by digestive enzymes (Seregelj et al. 2020). The DF can be classified as soluble or insoluble based on their solubility behavior in water. The soluble dietary fibers (SDFs) include the β-glucan, pectin, gums, and inulin; while the insoluble dietary fibers (IDFs) are represented by cellulose, hemicellulose, lignin, and resistant starches (Mudgil and Barak 2019). The SDFs are associated with the improvement in glycemic status and lipid profile (Barber et al. 2020), reduction in blood pressure and cholesterol levels, prevention of gastrointestinal problems, protection against the onset of various types of cancer, and colonic fermentation (Perry and Wu 2016). On the other hand, the IDFs have been associated with improvements in insulin sensitivity, reduction in the propensity of developing type II diabetes (Barber et al. 2020), as well as an increase in fecal bulk and reduction of transit time through the gut (Vuksan et al. 2008). The wide array of health-promoting benefits of dietary fiber resulted in a substantial rise in the market demand for it lately. In 2023, the global market value of dietary fiber amounted to USD 9.1 billion and is projected to reach USD 16.2 billion by 2032 (Research and Markets 2024).

In 1998, Saura-Calixto proposed the term antioxidant dietary fiber (ADF) to refer to DFs that contain significant levels of natural antioxidants embedded in the DF matrix. As a composite of dietary fiber and antioxidants, ADF represents a powerful duo with enhanced health-promoting benefits. For example, ADF from grapes exhibited greater reducing effects in the lipid profile and blood pressure of human subjects compared with its dietary fiber counterpart (Perez-Jimenez et al. 2008). In another study, IDF rich in polyphenols significantly reduced the total cholesterol and low density lipoprotein (i.e., LDL) of hypocholesterolemic human subjects (Ruiz-Roso et al. 2010).

The Philippines, one of the world's largest rice producers (Sharma 2017; DA 2022), holds a wide range of traditional pigmented rice accessions that can be potential sources of the ADF. For instance, the Philippine Rice Research Institute (PhilRice) houses a core collection of 307 pigmented rice accessions originating from various administrative districts of the country (Mbanjo et al. 2019). Pigmented rice can be brown, red, purple, or black as a result of the presence of various substances, most notably phenolics (Deng et al. 2013). The bran of pigmented rice, which is usually discarded as a by-product of milling, is highly rich in dietary fiber as well as phenolic antioxidants, such as flavonoids and anthocyanins (Bhat et al. 2020), and therefore can be a valuable resource for value-added products such as the ADF.

Exploring into the Philippine pigmented rice as potential source of bran-derived ADF, this study investigated five of its varieties abundantly produced in the Mindanao region for their ADF contents. Specifically, the study aimed to determine the DF profile of pigmented rice bran (PRB) in terms of SDF, IDF, and total dietary fiber (TDF) contents and to characterize these DF fractions for their phenolic content, antioxidant activity, as well as the distribution and identities of phenolics in the free and bound forms.

## MATERIALS AND METHODS

### Procurement of Materials

All chemicals used in this study were either analytical-grade or high performance liquid chromatography (HPLC)-grade. The enzymes for dietary fiber analysis were purchased from Megazyme. Bran from five pigmented rice varieties namely, Red-64, Black Rice, Calatrava, Arabon, and Dinorado were used in this study. They were sourced from PhilRice, Agusan Experimental Station.

### Sample Preparation

The PRB was dried in a convection oven (Vision Scientific, South Korea) at 55°C for 24 hrs. It was then milled to flour, sieved using 100-mesh

screen, and stored in a freezer (Arctiko Ultraflow Freezer, Denmark) at −20°C until use. Before analysis, removal of fat from the bran flour was done following the method of Wang et al. (2016) with some modifications. The PRB was soaked in petroleum ether with a material-to-solvent ratio of 1:6 for 24 hrs. Subsequently, the solvent was removed by heating the sample in an oven at 55°C until a constant weight was obtained. The defatted PRB was kept in a freezer (Arctiko Ultraflow Freezer, Denmark) at −20°C until use.

## Color Profiling of Pigmented Rice Bran Varieties

The color profile of each of the five PRB varieties used in this study was determined using a handheld chromameter (CR-400 Chroma Meter, Konica Minolta, Japan). Approximately 20 g of bran sample was spread on a petri dish. The color was characterized in terms of coordinates as defined by the CIELAB system: L* for lightness/luminosity, a* for redness (+) to greenness (−), and b* for yellowness (+) to blueness (−). The chroma (C*) of the sample was computed using the equation (Zubia et al. 2023):

$$C^* = \sqrt{a^{*2} + b^{*2}}$$

## Determination of SDF, IDF, and TDF in PRB

The determination of SDF, IDF, and TDF in PRB was done following the combined official methods 985.29, 991.42, and 993.19 of the Association of Official Analytical Chemists (AOAC). Briefly, 1 g test portion in 50 mL of 0.08 M phosphate buffer (pH 6.0) was successively incubated with thermostable amylase solution (3,000 U/mL; 0.1 mL) at 98°C for 15 min; protease (50 mg/mL; 0.1 mL; pH 7.5) at 60°C for 30 min; and amyloglucosidase (3,300 U/mL; 0.3 mL; pH 4 – 4.6) at 60°C for 30 min with agitation.

***IDF Determination.*** After cooling the mixture to room temperature, it was subjected to suction filtration using a tared and preweighed fritted glass crucible containing celite. The residue was washed twice with 10 mL water (to remove the SDF), twice with 10 mL 95 percent ethanol, and twice with 10 mL acetone. The residue represented the IDF. The filtrate was saved for SDF determination.

The crucible containing the IDF residue was dried for 5 hrs in a convection oven at 105°C. It was then cooled in a desiccator and weighed to the nearest 0.1 mg. The weight of the crucible containing the celite was subtracted to determine the residue weight. Using one of the replicates, the residue was quantitatively transferred into a digestion flask for analysis of nitrogen content following the AOAC method 960.52 (Kjeldahl Method). The amount of protein in the residue was calculated using N x 6.25 as conversion factor. On the other hand, another replicate sample was incinerated in a furnace for 5 hrs at 525°C. The sample was then cooled in a desiccator and weighed to the nearest 0.1 mg. The mass of the crucible containing the celite was subtracted to determine the ash content of the sample. The percent of IDF was computed using the formula:

$$\%\, IDF = \frac{(Mr - Mp - Ma - Mb)}{Ms} * 100$$

Where: Mr = mass of residue
Mp = mass of protein
Ma = mass of ash
Mb = Mass of blank
Ms = mass of defatted sample

***SDF Determination.*** To determine the SDF, the weight of the combined filtrate and water washing was adjusted to 100 g with water. Four volumes of 95 percent ethanol preheated to 60°C was then added. The mixture was set aside for 60 min to allow the formation of precipitate (SDF). The mixture was then subjected to suction filtration using a tared and preweighed fritted glass crucible containing celite. The residue was washed successively with three 20 mL portions of 78 percent ethanol, two 10 mL portions of 95 percent ethanol, and two 10 mL portions of acetone. The crucible containing the SDF residue was dried for 5 hrs in a convection oven at 105°C.

It was then cooled in a desiccator and weighed to the nearest 0.1 mg. The weight of the crucible containing celite was subtracted to determine the residue weight. On the other hand, one of the replicates was used for the analysis of nitrogen content, while another one was used for ash content determination as described above. The % SDF was computed using the formula as indicated in AOAC Method 991.43:

$$\% SDF = \frac{(Mr - Mp - Ma - Mb)}{Ms} * 100$$

Where: Mr = mass of residue
Mp = mass of protein
Ma = mass of ash
Mb = Mass of blank
Ms = mass of defatted sample

## Preparation of SDF and IDF Powders from the PRB

The preparation of SDF and IDF powders from defatted PRB was done following the method of Nsor-Atindana, Zhong, and Mothibe (2012) and AOAC method 985.29. Briefly, about 50 g of defatted rice bran in 1,500 mL of 0.08 M phosphate buffer (pH 6.0) was successively incubated with thermostable amylase solution (3,000 U/mL; 3.750 mL) at 100°C for 1 hr; protease (50 mg/mL; 1.5 mL; pH 7.5) at 60°C for 1 hr; and amyloglucosidase (3,300 U/mL; 1.75 mL; pH 4 – 4.6) at 60°C for 1 hr with agitation. The suspension was then centrifuged (NovaFuge B115-20R, Senova, China) at 3,000 rpm for 20 min to separate the supernatant from the residue after the enzymatic hydrolysis. The residue was washed twice with hot water (70°C), 95 percent (v/v) ethanol and acetone. The residue obtained represented the IDF. On the other hand, the water washings and the supernatant were pooled together for the isolation of the SDF. Four volumes of 80 percent (v/v) ethanol was added to precipitate the SDF at room temperature for 1 hr. The mixture was centrifuged (NovaFuge B115-20R, Senova, China) at 3,000 rpm for 20 min. The residue was washed twice with 78 percent (v/v) ethanol, 95 percent (v/v) ethanol and acetone. The obtained residue represented the SDF. The wet masses of IDF and SDF were dialyzed using dialysis bags with molecular weight cutoff of 12,000 dalton against water and 78 percent ethanol, respectively, for 48 hrs at 25°C. They were then lyophilized using a freeze-dryer (Labconco, USA). The lyophilized IDF and SDF were stored in a freezer (Arctiko Ultraflow Freezer, Denmark) at −20°C until use.

## Analysis of Phenolics Associated with the SDF and the IDF from the PRB

### *Extraction of Soluble Phenolics*

Total phenolic content (TPC) associated with the IDF and the SDF of the PRB was determined following the method of Deng, Penner, and Zhao (2011). The soluble phenolics were extracted using the solvent mixture consisting of 0.1 percent HCl / 70 percent acetone / 29.9 percent water (v/v/v) at a material to solvent ratio of 1 g : 4 mL. The suspension was placed in an ultrasonic bath for 1 hr at room temperature, making sure that the temperature of the ultrasonic bath did not exceed 45°C. The mixture was then centrifuged (NovaFuge B115-20R, Senova, China) at 10,000×g for 15 min. The extraction process was done thrice for every sample. The supernatant from the three extractions were pooled together, then concentrated on a rotary evaporator (Yamato RE200, Japan) at 40°C under vacuum. The volume was brought to 50 mL with distilled water. The extract was stored in a freezer (Arctiko Ultralow Freezer, Denmark) at −20°C until use.

### *Determination of Total Phenolic Content*

The TPC associated with the SDF and the IDF of the PRB was determined following the Folin-Ciocalteu (FC) reagent-based colorimetric assay as described by Singleton, Orthofer, and Lamuela-Raventos (1999) and Deng, Penner, and Zhao (2011). The TPC was expressed as milligram gallic acid equivalent (GAE) per gram dry weight (DW) of the sample.

### *Determination of Total Flavonoid Content*

Total flavonoid content (TFC) associated with the SDF and the IDF in the PRB was determined following the method of Lopez-Vargas et al. (2013). The TFC was expressed in terms of mg quercetin equivalents/g of the sample.

### *Determination of Total Anthocyanin Content*

Total anthocyanin content (TAC) associated with the SDF and the IDF in the PRB was determined using a pH differential method with a two-buffer system (0.025 M potassium chloride buffer, pH 1.0 and 0.4 M sodium acetate buffer, pH 4.5) following the method of Rafi et al. (2018). In order to quantify the anthocyanin content, the absorbance (A) was calculated:

$$A = [(A_{510}, \text{pH } 1 - A_{700}, \text{pH } 1)] - [(A_{510}, \text{pH } 4.5 - A_{700}, \text{pH } 4.5)]$$

The TAC was expressed as cyanidin-3-glucoside (% w/w) equivalents as follows:

$$[\text{Anthocyanin in mg/L}] = [A \times MW \times DF \times 1000] / (\varepsilon \times 1)$$

Where A = absorbance, MW = molecular weight (449.2 g/mol), and DF = dilution factor, ε = extinction molar coefficient (26,900 L/cm mol). The final anthocyanin content was expressed as cyanidin-3-glucoside equivalent (CYE) in 100 g of dried powder (mg CYE/100 g dried powder).

## Determination of Antioxidant Activities In Vitro

### *Assay for Ferric Ion-Reducing Antioxidant Power (FRAP Method)*

The ability of the phenolics in the SDF and the IDF from the PRB to reduce ferric ion ($Fe^{+3}$) to the ferrous ion ($Fe^{+2}$) state was determined following the method of Spiegel et al. (2020) and Bolanos-Dela Torre et al. (2015). The total equivalent antioxidant capacity (TEAC) was expressed in terms of μmol trolox equivalent (TE) per gram DW of the sample.

### *ABTS•+ Scavenging Assay*

The ABTS•+ scavenging activity of the SDF and the IDF from the PRB was measured following the method of Li et al. (2012) and Meera, Siyakumar, and Sisay (2019). It was calculated as:

$$ABTS\ Scavenging\ Activity\ (\%) = \frac{(Abs\ of\ control - Abs\ of\ sample)}{Abs\ of\ control} * 100$$

### *PPH Free Radical Quenching Assay*

The DPPH free radical quenching ability of the SDF and the IDF from the PRB was assessed following the method of Zheng et al. (2020). The DPPH radical quenching activity was expressed in terms of milligram ascorbic acid per gram dry weight of the sample. In addition, the percent DPPH radical quenching activity was computed as:

$$DPPH\ Radical\ Quenching\ Activity\ (\%) = \frac{(Abs\ of\ control - Abs\ of\ sample)}{Abs\ of\ control} * 100$$

The bran variety with the highest dietary fiber content, TPC, TFC, TAC, and antioxidant ability was chosen and used for further analysis and characterization.

## Profiling of Free and Bound Phenolics Associated with Calatrava IDF

### *Extraction of Free and Bound Phenolics*

The free phenolics associated with Calatrava IDF was extracted following the method of Kotásková et al. (2016). The residue obtained was subsequently used for extracting the bound phenolics following the method. The free and bound phenolic extracts were stored in a freezer (Arctiko Ultralow Freezer, Denmark) at −20°C until use.

### *Quantification of Free and Bound Phenolics*

The free and bound phenolics associated with Calatrava IDF were quantified using the FC method as described above.

### *Identification of Free and Bound Phenolics by HPLC*

Free and bound phenolic compounds associated with Calatrava IDF were identified using high performance liquid chromatography coupled with a photodiode array (PDA) detector, HPLC-PDA (LCMS 2020 Shimadzu Prominence, Japan) following the method of Lopez-Vargas et al. (2013). Briefly, 20 µL each of the free and bound phenolic extracts was injected into the HPLC instrument equipped with a $C_{18}$ column that was controlled thermostatically at 40°C. A solvent system consisting of one percent acetic acid in water (solvent A) and acetonitrile (solvent B) was used as the mobile phase to carry out gradient elution as follows: 0 min (5% B); 0→15 min (25% B); 15→30 min (50% B); 30→40 min (70% B); 40→50 min (95% B); 50→60 min (95% B). Sample components were detected by measurement of absorbance at 254 nm. Phenolic compounds present in the free and bound forms from Calatrava IDF were identified based on the availability of the following phenolic standards: gallic acid, catechin, quercetin, vanillin, resorcinol, ferulic acid, p- nitrophenol, and salicylic acid.

### *Statistical Analysis*

All the data gathered were analyzed through one-way analysis of variance (ANOVA, $p \leq 0.05$) followed by Tukey post hoc test using the JASP Computer Software Version 0.16.2 of the University of Amsterdam.

## RESULTS AND DISCUSSION

### Color Profile of Pigmented Rice Bran Varieties

There is evident variation in the color of the bran samples considered in this study (Figure 1) presumably because of their unique pigment composition. The pigments in rice bran are primarily composed of hydrophilic and semi-hydrophilic compounds such as anthocyanins and polyphenols, as well as lipophilic compounds such as carotenoids, tocopherols, tocotrienols, and γ-oryzanol (Seechamnanturakit et al. 2018). These pigments add value to the bran because of their many reported health-promoting properties such as hypolipidemic, hypoglycemic, anti-inflammatory, anticancer, and antioxidant, among others.

The color profile based on the SCIELAB system of each PRB is shown in Table 1. All five varieties manifested significant differences in the degree of color lightness as evidenced by their differing L* values. Dinorado had the lightest bran color while Calatrava exhibited the darkest color. Both Red-64 and Dinorado had the most reddish and yellowish color as manifested by their a* and b* values, respectively. Moreover, both exhibited the greatest color intensity as indicated by their high C* values. The color of rice bran is largely influenced by its predominant pigment.

**Figure 1. Representative images of the bran of five Philippine pigmented rice varieties used in the study**

**Table 1. Color analysis of pigmented rice bran varieties**

| Variety | L* | a* | b* | Chroma (C*) |
|---|---|---|---|---|
| Red-64 | 49.4 ± 0.09$^{e}$ | 8.9 ± 0.02$^{c}$ | 14.5 ± 0.5$^{d}$ | 17.0 ± 0.43$^{c}$ |
| Black rice | 36.5 ± 0.17$^{b}$ | 6.3 ± 0.02$^{b}$ | 5.4 ± 0.03$^{b}$ | 8.3 ± 0.03$^{b}$ |
| Calatrava | 33.6 ± 0.24$^{c}$ | 6.8 ± 0.04$^{ab}$ | 4.2 ± 0.04$^{c}$ | 8.0 ± 0.05$^{b}$ |
| Arabon | 37.6 ± 0.11$^{a}$ | 6.9 ± 0.11$^{a}$ | 6.5 ± 0.03$^{a}$ | 9.5 ± 0.09$^{a}$ |
| Dinorado | 55.1 ± 0.17$^{d}$ | 8.6 ± 0.44$^{c}$ | 14.8 ± 0.06$^{d}$ | 17.1 ± 0.17$^{c}$ |

Note: Values represent mean ± SD (n = 3). Within a column, values bearing the same letter superscripts are not significantly different.

**Table 2. Dietary fiber profile of the bran of pigmented rice varieties**

| Variety | % IDF | % SDF | % TDF |
|---|---|---|---|
| Red-64 | 48.34 ± 0.56$^{ac}$ | 0.58 ± 0.12$^{a}$ | 48.91 ± 0.68$^{ab}$ |
| Black Rice | 44.13 ± 0.38$^{a}$ | 0.90 ± 0.06$^{ab}$ | 45.03 ± 0.44$^{a}$ |
| Calatrava | 50.25 ± 2.96$^{c}$ | 2.24 ± 1.13$^{b}$ | 52.50 ± 3.30$^{b}$ |
| Arabon | 47.06 ± 1.62$^{ac}$ | 0.33 ± 0.13$^{a}$ | 47.39 ± 1.49$^{a}$ |
| Dinorado | 38.06 ± 0.87$^{b}$ | 0.55 ± 0.03$^{a}$ | 38.61 ± 0.85$^{c}$ |

Note: Note: Values represent mean ± SD. Within a column, values bearing the same letter superscripts are not significantly different.

Huang and Lai (2016) reported that red rice bran is primarily rich in proanthocyanidins but low in anthocyanins. In contrast, the black rice bran is low in proanthocyanidins but rich in anthocyanins.

## Dietary Fiber Profile of Pigmented Rice Bran Varieties

The dietary fiber profile of the bran of each pigmented rice variety was analyzed in terms of percent SDF, percent IDF, and percent TDF (Table 2). The percent TDF values ranged from 38.6 percent for Dinorado to 52.5 percent for Calatrava. No significant differences were noted for the TDF of Arabon, Black Rice, and Red-64, in which levels were intermediate between those of Calatrava and Dinorado.

Table 2 further shows the relative amounts of the SDF and IDF fractions of the bran of the five pigmented rice varieties evaluated in this study. The IDF fraction is significantly higher than the SDF counterpart in all the five varieties. While the IDF fraction amounted to almost 50 percent, the SDF barely reached one percent in all the varieties except for Calatrava where the SDF was 2.2 percent. Thus, it can be said that the IDF constitutes the bulk of the TDF in PRB, and that, these bran varieties are poor sources of the SDF. Calatrava exhibited the highest percent IDF amounting to 50 percent (Table 2). The percent IDFs of Arabon, Black Rice, and Red-64 were not significantly different ($p > 0.05$) from each other and were the second highest among the varieties evaluated. Dinorado registered the lowest amount of IDF. The same dietary fiber profile was obtained by Abdul-Hamid and Luan (2000) for brown rice bran (25% IDF and 2% SDF) and by Daou and Zhang (2014) for another rice bran variety (30% IDF and 2.7% SDF). However, it must be noted that the reported TDF values in those studies were lower than the TDF of the bran of the five pigmented rice varieties obtained in this study presumably owing to varietal differences. Considering the purported health benefits of dietary fiber, the five varieties used in this study show promise in terms of bran valorization as their dietary fiber content is relatively higher than those of the other rice bran varieties reported so far.

It is apparent from the results that PRB is rich in IDF but not in SDF. Thus, in future

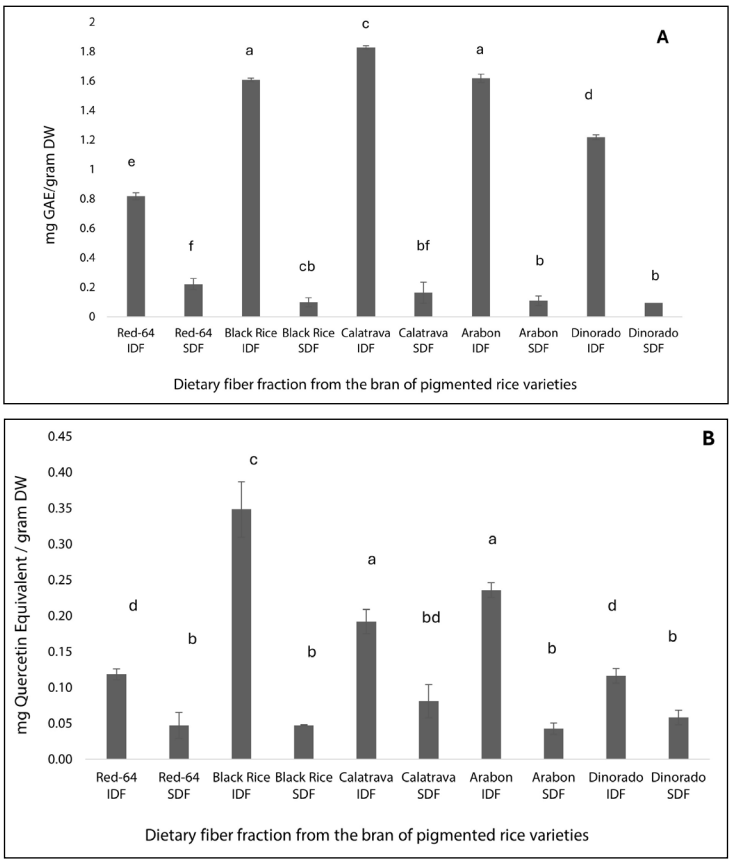
investigations on dietary fiber, the bran from Calatrava, Arabon, Black Rice, and Red-64 can be considered as good candidates for the extraction of IDF. It is important to note that SDF and IDF have different physico-chemical properties. They also impart varying physiological functions and health benefits (Vuksan et al. 2008; Perry and Wu 2016; Barber et al. 2020). Thus, the results of this study can influence future valorization efforts that can be done for these PRB varieties.

## Analysis of Phenolics Associated with the IDF and the SDF in the PRB

### *Total Phenolic Content*

ADF pertains to dietary fiber with associated antioxidant components in the fiber matrix. In a review article by Das et al. (2020), the antioxidant activities of the ADF from 36 plant sources were ascribed to the presence of various phenolic compounds. Since phenolics are ubiquitous among plant species, it is not remote that they are also present in the dietary fiber fraction of PRB. Figure 2A shows that for the bran of each pigmented rice variety analyzed in this study, the total phenolics is concentrated in the IDF fraction at 0.8–1.8 mg GAE/gram DW while the TPC in SDF is significantly lower ($p < 0.05$) and did not even amount to half of the TPC in the IDF fraction. Essentially, the same observation was reported by Zhao et al. (2018) for the TPC profile of the SDF and the IDF from defatted rice bran. Among the IDF samples analyzed in this study, the highest TPC was observed in Calatrava while the lowest was observed in Red-64. This study showed

**Figure 2. Total phenolic content (A) and total flavonoid content (B) of the IDF and SDF bran fractions of pigmented rice varieties**




Note: Bars bearing the same letters are not significantly different.

that Calatrava IDF is a promising source of phenolics for various potential nutraceutical and functional food applications.

### *Total Flavonoid Content*

Flavonoids are the most abundant phenolics accounting for two-thirds of dietary phenolic compounds (De la Rosa et al. 2018). Figure 2B shows a comparison of the TFC of the IDF and SDF fractions of the bran of five pigmented rice varieties evaluated in this study. Consistent with the profile for total phenolics, the TFC is also significantly higher ($p < 0.05$) in the IDF fraction than in the SDF counterpart. This trend is similar to that obtained by Zhao et al. (2018). The TFC values of the SDF fraction of the five varieties amounted to more or less 0.05 mg quercetin equivalent per gram DW of SDF, and they were not significantly different from each other. On the other hand, among the IDF fractions, Black Rice gave the highest TFC at 0.35 mg quercetin equivalent per gram DW. Arabon and Calatrava also registered high TFC but significantly lower than that of Black Rice. The relatively low TFC of Dinorado and Red-64 is reflective of their low TPC. The study of Zhao et al. (2018) indicated that the TFC of rice bran IDF amounted to 2.7 mg catechin equivalent per gram DW, which consists of 1.1 mg and 1.6 mg catechin equivalent of free and bound flavonoids, respectively.

### *Total Anthocyanin Content*

The anthocyanins represent a subclass of flavonoids. Results of this study indicated that anthocyanins were detected only in the IDF fractions of the bran of Calatrava, Black Rice, and Arabon. Table 3 shows that Calatrava IDF significantly ($p < 0.05$) contained the highest TAC. The TAC of Arabon IDF and Black Rice IDF did not differ significantly from each other but were significantly lower than that of Calatrava IDF. It is interesting to note that these three bran varieties were dark in color (Figure 1), while both Red-64 and Dinorado had the most reddish tinge (Table 1). The results obtained in this study agree with that of Huang and Lai (2016), which reported significant amounts of anthocyanins in black rice bran samples but not in red rice bran varieties.

**Table 3. Total anthocyanin content (TAC) of the bran of pigmented rice varieties**

| Variety | TAC (mg cyanidin-3-glucoside / 100 g DW) |
|---|---|
| Arabon IDF | $0.110 \pm 0.048^{a}$ |
| Black Rice IDF | $0.139 \pm 0.127^{a}$ |
| Calatrava IDF | $0.524 \pm 0.048^{b}$ |

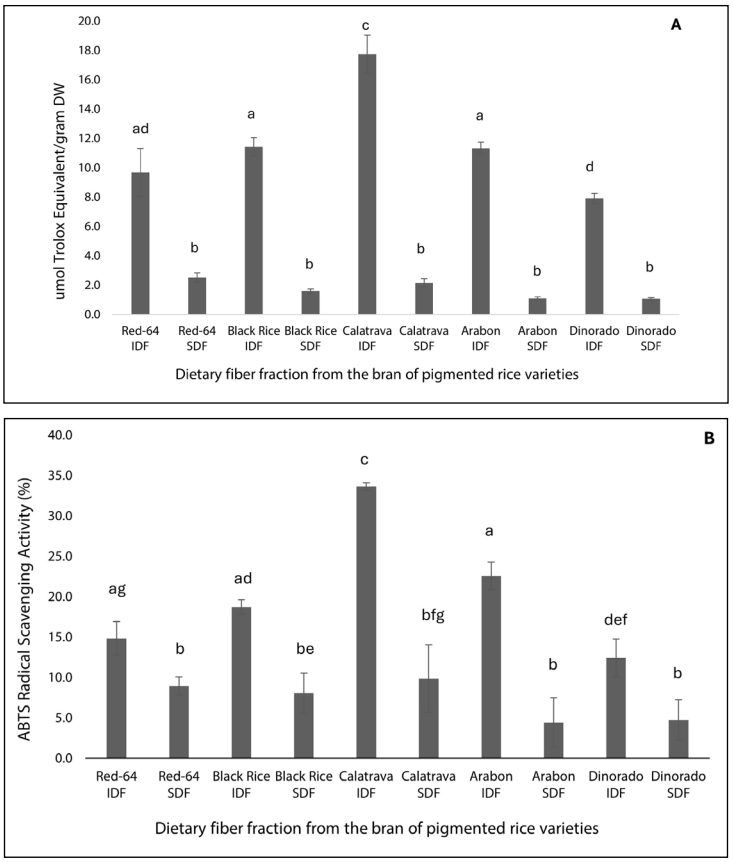
Note: Values represent mean ± SD. Values bearing the same letter superscripts are not significantly different.

By far, studies on anthocyanins from rice bran IDF and SDF are limited. To our knowledge, this is the first report on the TAC contents of the SDF and IDF obtained from the bran of the pigmented rice. However, many studies reported the presence of anthocyanins in rice bran but not in the specific fiber fractions (Kapcum et al. 2016; Ilmi, Pratiwi, and Purwestri 2018; Apridamayanti et al. 2018).

### *Total Equivalent Antioxidant Capacity*

Phenolic compounds are gaining a great deal of attention because of their antioxidant ability, which could alleviate oxidative stress and attenuate various adverse physiological conditions. As can be seen in Figure 3A, the trend in the TPC is essentially the same as that for the antioxidant capacity whereby the IDF fractions displayed significantly higher ($p < 0.05$) values relative to the corresponding SDF fractions. All the SDF fractions displayed a TEAC of about 2 μmol TE/g DW of SDF. On the other hand, among the IDF fractions, Calatrava displayed the highest TEAC at 17.72 μmol TE/g DW. Dinorado and Red-64 displayed the lowest antioxidant capacity among the IDF samples. Zhao et al. (2018) also used the FRAP method to compare the TEAC of the IDF and SDF fractions of defatted rice bran. Basically, the same antioxidant profile between the IDF and the SDF was obtained, but the values of μmol TE/g DW were higher (8.7 for SDF and 31.6 for IDF) relative to the values obtained from this

**Figure 3. Total equivalent antioxidant capacity (A) and ABTS radical-scavenging ability (B) of the IDF and SDF bran fractions of pigmented rice varieties**




Note: Bars bearing the same letters are not significantly different.

study. Though the rice variety is an integral factor that can affect the antioxidant ability of dietary fiber, other external factors are equally important, such as the extraction solvent used and the particle size of the fiber, among others. These factors may be further explored in future studies.

The FRAP method measures the reducing ability of antioxidants as evidenced in the conversion of the $Fe^{+3}$ ion to $Fe^{+2}$. Essentially though, there are other mechanisms for the antioxidant ability of substances, which include scavenging/quenching of free radicals, and metal chelation, among others (Shahidi and Zhong 2015). By far, no single universal assay can accurately reflect the mechanism of action of all antioxidants (Karadag, Ozcelik, and Saner 2009). In addition, the various assays also differ in their target antioxidants depending on the hydrophilicity or lipophilicity of the latter (Floegel et al. 2011). Hence, in practice, multiple antioxidant assays are necessary to assess more comprehensively the antioxidant activity. In this study, the results obtained through the FRAP method for the antioxidant activity of the SDF and the IDF fractions of the five varieties of PRB were supplemented using the ABTS and the DPPH assays.

### *ABTS*⁺ Radical Scavenging Ability*

The ABTS (2,2'-azino-bis[3-ethylbenzothiazoline-6-sulfonate]) assay is based on the ability of antioxidants such as the phenolics to scavenge the ABTS cation radical $ABTS^{*+}$ either through single electron transfer or hydrogen atom transfer (HAT) mechanism (Santos-Sánchez et al. 2019). Figure 3B shows the $ABTS^{*+}$ scavenging activity

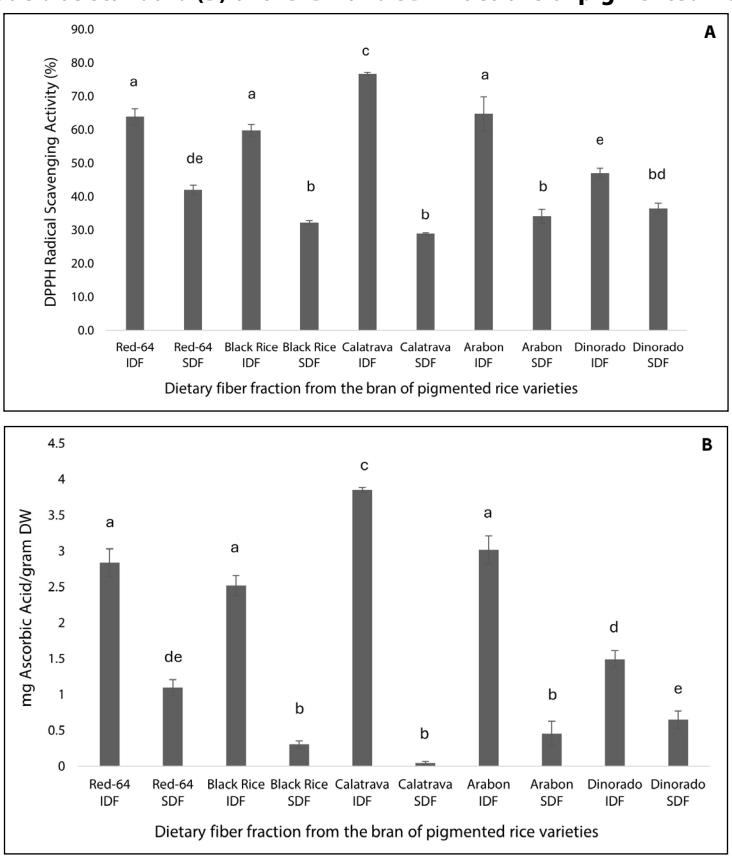
of the SDF and IDF fractions of the bran of five pigmented rice varieties tested in this study.

It is noteworthy that the trend for antioxidant activity closely resembled those obtained using the FRAP method (Figure 3A). For all the varieties tested, the IDF fraction displayed significantly higher ($p < 0.05$) ABTS*+ scavenging activity relative to the SDF fraction. Undoubtedly, this is due to the higher phenolic content of the former. Among the fractions tested, Calatrava IDF manifested again the highest antioxidant activity at 33 percent ABTS*+ scavenging activity. This value is almost twice as high as the ABTS*+scavenging activity of the rice bran IDF obtained by Qiao et al. (2021). The activity of the IDF fractions of Arabon, Black Rice, and Red-64 did not differ significantly from each other, and they ranked next to Calatrava IDF. Dinorado IDF exhibited the lowest scavenging activity among the IDF samples. On the other hand, for all the SDF fractions, the ABTS*+ scavenging activity did not differ significantly ($p > 0.05$) from each other, which amounted to less than 10 percent only. Hydrophilic antioxidants are better reflected by the ABTS assay (Floegel et al. 2011). Hence, it is highly probable that among the SDF and IDF fractions from the five PRB tested in this study, Calatrava IDF is the most abundant of hydrophilic antioxidants.

### *DPPH Radical Scavenging Ability*

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is based on the ability of the antioxidants to scavenge the DPPH radical, DPPH*, through HAT mechanism. Figure 4A shows a comparison

**Figure 4. Percent DPPH radical scavenging ability (A) and DPPH radical scavenging ability relative to ascorbic acid as standard (B) of the IDF and SDF fractions of pigmented rice varieties**




Note: Bars bearing the same letters are not significantly different.

of the DPPH radical scavenging ability of the SDF and IDF fractions of the bran of the five pigmented rice varieties considered in this study. In all these varieties, the IDF fraction manifested significantly higher ($p < 0.05$) percent DPPH radical scavenging ability relative to the corresponding SDF fraction. This can be correlated to the previous findings that the phenolics are concentrated in the IDF fraction of the bran while only minimal amount could be detected in the SDF fraction. Essentially the same trend was obtained by Daou and Zhang (2011), whereby the IDF from defatted rice bran also showed higher DPPH scavenging ability of about 80 percent compared with SDF having 60 percent scavenging activity. In this study, the highest percent DPPH radical scavenging ability was from Calatrava IDF, which amounted to as high as 76.7 percent. Arabon IDF, Black Rice IDF, and Red-64 IDF ranked next to Calatrava, while Dinorado had the lowest percent scavenging among the IDF fractions. On the other hand, among the SDF fractions, both Dinorado and Red-64 manifested the highest percent radical scavenging ability at about 40 percent, while the other three varieties (Arabon, Black Rice, and Calatrava) displayed only about 30 percent scavenging ability.

Using ascorbic acid, a commonly used antioxidant standard, Calatrava IDF manifested a DPPH radical scavenging activity equivalent to 3.9 mg ascorbic acid per gram dry weight as shown in Figure 4B. The IDF fractions of Arabon, Black Rice, and Red-64 also showed high radical scavenging activity equivalent to 2.5 to 3 mg ascorbic acid per gram dry weight. All the SDF fractions scavenged DPPH radical equivalent to only less than 1 mg ascorbic acid per gram dry weight except for Red-64 SDF, which showed a scavenging ability equivalent to 1 mg ascorbic acid/gram dry weight.

The DPPH assay can evaluate both hydrophilic and lipophilic antioxidant compounds (Charles 2013). Hence, it can be said that among the dietary fiber fractions tested in this study, Calatrava IDF is associated with the highest amount of antioxidants. This is further corroborated with its superior phenolic content as presented in Figure 3A.

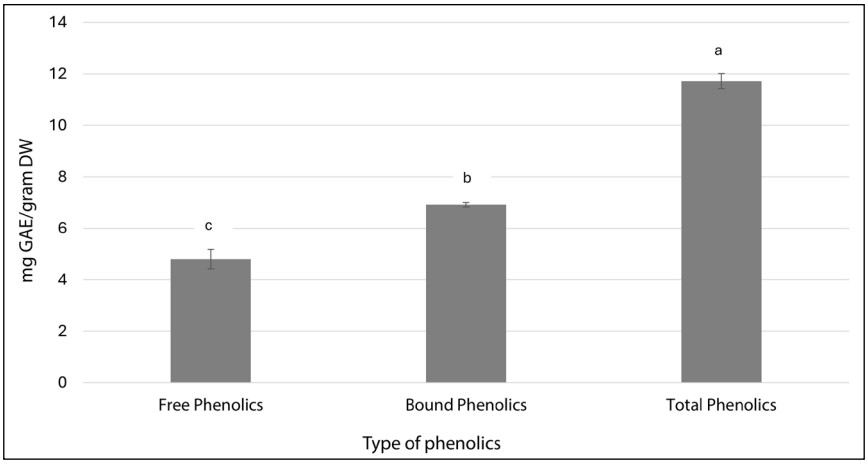
## Phenolics Profile of Calatrava IDF

Since Calatrava IDF was superior consistently in terms of the amount of dietary fiber, total phenolics, total anthocyanins, as well as antioxidant activity using three different assays (FRAP, ABTS and DPPH), it was characterized further in terms of the distribution and identity of the phenolics present.

The phenolics associated with dietary fiber can exist either in free or bound form. The latter is covalently bonded to other molecules such as cellulose, hemicellulose, pectin, and structural proteins (Alves et al. 2021). While the free phenolics are readily extractable using solvents such as alcohol, the bound phenolics need more drastic extraction condition such as acid, alkali, or enzymatic hydrolysis (Wang et al. 2019). In this study, the phenolics profile of Calatrava IDF was explored as this information will be useful in future valorization efforts for PRB. Studies have shown that the free and bound phenolics differ in their relative amounts in plant sources, which largely depend on the plant matrix. For example, in fruits and vegetables, majority of the phenolics exists in the free form, while for cereal-based matrices like rice, majority of the phenolics is in the bound form (Acosta-Estrada, Gutiérrez-Uribe, and Serna-Saldívar 2014).

This results in variation in the antioxidant ability, with the bound phenolics usually demonstrating a significantly higher antioxidant activity in many in vitro assays (Acosta-Estrada, Gutiérrez-Uribe, and Serna-Saldívar 2014; Wu et al. 2021). Also, some studies indicate that the bound phenolics have greater hypolipidemic effect (Zhao et al. 2022) and greater inhibitory activity than the free form against the carbohydrate-digesting enzyme alpha-amylase (Hu et al. 2013; Santos-Zea, Villela-Castrejon, and Gutierrez-Uribe 2018; Zheng et al. 2020). Thus, knowledge on the phenolics profile of Calatrava IDF will serve as baseline data for its eventual utilization in the future. In this study, exhaustive methanol extraction and alkali hydrolysis were done to separate the free and bound phenolics, respectively.

**Figure 5. Distribution of phenolics in Calatrava IDF**




Note: Bars bearing different letters are significantly different

Figure 5 shows that the amount of bound phenolics in Calatrava IDF was significantly higher ($p < 0.05$) than that of the free phenolics. The bound form amounted to 6.9 mg GAE/g DW while the free form amounted to only 4.8 mg GAE/g DW. Essentially the same phenolics profile was obtained by Harukaze, Murata, and Homma (1999) for the bran and polished grain of 21 Japonica rice cultivars; and Pang et al. (2018) for white, red, and black rice varieties.

There have been several attempts to study the physiological importance of the free and bound phenolics in rice bran. Zhao et al. (2022) reported on the hypolipidemic effect of hydrolyzed bound phenolics from PRB. Moreover, Wu et al. (2018) reported on the antidiabetic potential of the phenolics from rice bran through the increase in the α-amylase inhibitory activity of the bound phenolics and increase in the α-glucosidase inhibitory activity of the free phenolics.

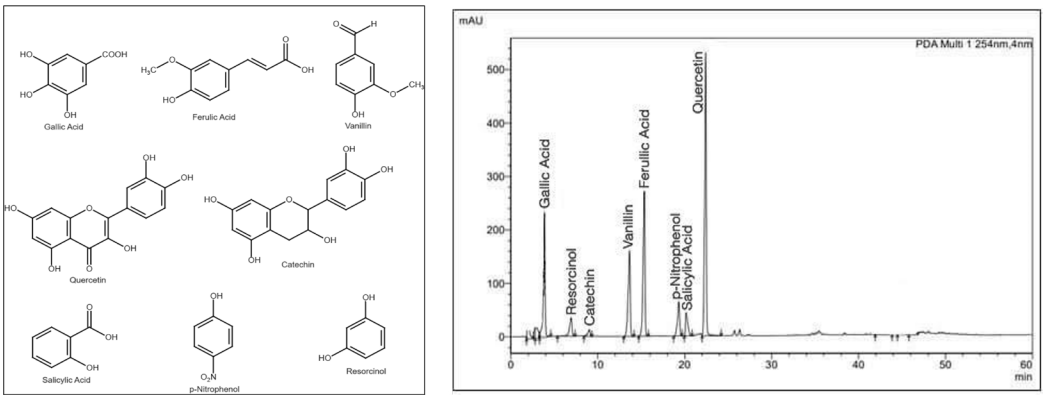
## HPLC Analysis of the Phenolics

To further study the phenolics profile of Calatrava IDF, qualitative analyses were done using HPLC equipped with $C_{18}$ column, PDA detector, and a mobile phase consisting of one percent acetic acid (A) and acetonitrile (B) to carry out gradient elution. Eight phenolic standards (Fig 6A) were used in this study in order to identify the free and bound phenolics of Calatrava IDF. Figure 6B shows the chromatogram of the mixed phenolic standards from HPLC analysis as measured at a wavelength of 254 nm.

Corresponding extracts from Calatrava IDF containing free and bound phenolics were also analyzed by HPLC using the same parameters used for the phenolic standards. Figure 7A shows the chromatogram of the bound phenolics extracted from Calatrava IDF. It is evident that the bound phenolics is a composite of numerous phenolic compounds that eluted at varying times within a 60-min elution period. Comparison of their respective retention time with those of the phenolic standards showed the presence of gallic acid, resorcinol, vanillin, ferulic acid, salicylic acid, and quercetin in the bound phenolic extract of Calatrava IDF. In a related study by Zhao et al. (2018), 10 monomeric compounds were identified in the bound phenolic fraction of the IDF from defatted rice bran, of which, ferulic acid and p-coumaric acid were detected in highest amounts.

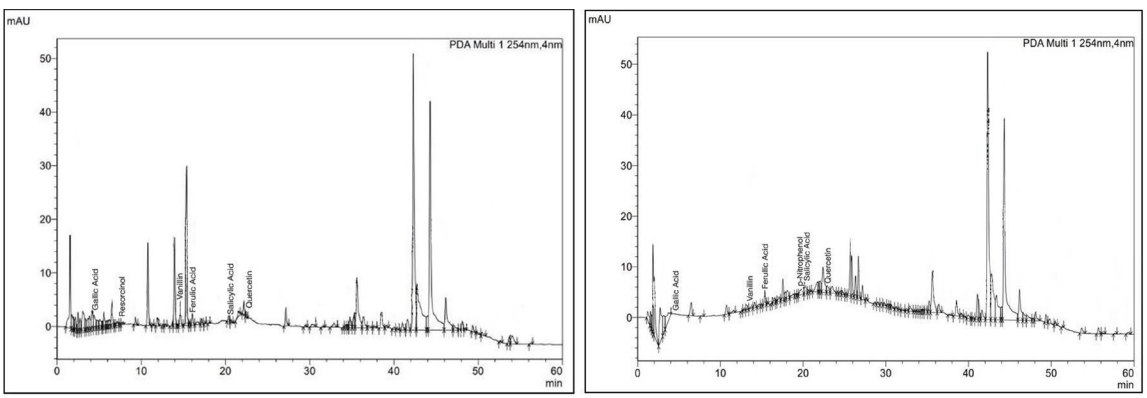
On the other hand, Figure 7B shows the HPLC chromatogram of the free phenolics extracted from Calatrava IDF. As with the bound phenolics, numerous phenolic compounds also constituted the free phenolic fraction of Calatrava IDF. In comparison with the retention time of the phenolic standards, the following compounds

**Figure 6. Structures of phenolic standards (A) and the chromatogram (B) obtained from HPLC analysis**




A B

**Figure 7. HPLC chromatogram of bound (A) and free (B) phenolics from Calatrava IDF**




A B

were tentatively identified to be present in the free phenolic fraction of Calatrava IDF: gallic acid, vanillin, ferulic acid, p- nitrophenol, salicylic acid, and quercetin. Zhao et al. (2018) identified six monomeric compounds in the free phenolic fraction of IDF from defatted rice bran, of which, vanilline was present in predominant amount. Future investigations may delve on identifying and quantifying the unknown peaks in the chromatograms of both the bound and free phenolic fractions of the IDF of Calatrava rice bran.

## CONCLUSION

All the bran samples from five Philippine pigmented rice varieties that were considered in this study are potential sources of ADF. This is owing to their high TDF content associated with significant amounts of phenolic antioxidants. Calatrava variety manifested the highest percent TDF amounting to 52.5 percent, consisting mainly of IDF fraction. Calatrava IDF displayed the highest TPC, TAC, and antioxidant ability in terms of ferric ion reducing antioxidant power, ABTS radical scavenging and DPPH radical quenching. Most of the phenolics associated with Calatrava IDF is in the bound form. HPLC analysis validated the presence of gallic acid, vanillin,

ferulic acid, salicylic acid, and quercetin in both free and bound phenolic fractions of Calatrava IDF. Additionally, resorcinol was present in the bound fraction while p-nitrophenol was present in the free form. Future research efforts can be done to further identify the other phenolic compounds in Calatrava IDF to provide information for future valorization as possible component of functional foods and nutraceuticals.

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