

# DEBITTERING OF *Borassus flabellifer* MESOCARP USING NARINGINASE: IMPACT ON THE COMPOSITION, PHYSICOCHEMICAL CHARACTERISTICS, AND FUNCTIONAL PROPERTIES

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**Abstract:** This study explores the physicochemical, composition, and functional properties of the *Borassus flabellifer* mesocarp powder following treatment with naringinase. The debittered mesocarp powder (DMP) has good water-holding (9.4 g/g), swelling (7.8 g/g), and wettability (12.3 s) capacities. Naringinase reduced 33.2% of the DMP particle size, causing an increase in surface area. A larger surface area traps more water/oil molecules, contributing to a higher water/oil capacity. However, the solubility, swelling, and wettability of DMP were markedly decreased following naringinase treatment. Nonetheless, although DMP has higher saponin, phenol, tannin, and DPPH activity contents than the control, it has decreased ferrous-reducing activity.

**Keywords:** Bitterness, dietary fibre, food ingredient and palmyra palm

## 1. Introduction

Palmyra palm (*Borassus flabellifer* Linn) is known as *kelapa laut* in Malaysia. *B. flabellifer* is from the *Arecaceae* family, subfamily *Borassoideae*, and genus *Borassus* (Nesbit, 2005). The palmyra fruit pulp is commercially used for beverages and food products (Chaurasiya et al., 2014; Vijaya Kumara & Prasad, 2015), with a high demand for the latter. Unfortunately, the fruit pulp bitterness is unfavoured by many. The fruit pulp is a part of the annual production of palmyra fruits and is often used as animal feed or discarded (Jansz et al., 2002). Measures to remove or control the bitterness for broader utilisation of the fruit pulp include the addition of pineapple pulp as a masking agent to conceal the bitterness of palmyra pulp (Thanusan et al., 2018) and the removal of bitterness in the tuber flour by soaking at 53 °C for 1 h (Thivya et al., 2018).

Enzymatic hydrolysis is commonly used to remove bitterness and acquire compounds with increased biological activity (Kumar, 2010). Naringinase has been commonly applied as a debittering agent of citrus fruit juice in the fruit juice industry during processing and aids in improving the stability and properties of the juices (Radhakrishnan et al., 2012). Unfortunately, the removal of bitterness using naringinase was more focused on citrus fruit and berry juices, such as kinnow mandarin juice (Puri, 2001), grape juice (Mishra & Kar, 2003), pummelo (*Citrus grandis*) fruit

juice (Ni et al., 2014), and orange juice (Zhu et al., 2017).

To date, reports on the effects of naringinase to remove bitterness from the mesocarp of *B. flabellifer* are scarce. Jansz et al. (1994) successfully attempted to debitter palmyra fruit pulp using naringinase (a mixture of  $\beta$ -glycosidase and  $\beta$ -rhamnosidase), resulting in a beverage with a pleasant mango cordial-like flavour, colour, and texture. A similar attempt was conducted by Ariyasena et al. (2001) to hydrolyse palmyra pulp using naringinase with reduced flabelliferin content without changing its nutritive values. However, the amount of reduced flabelliferin was not reported because the research only used thin-layer chromatography for estimation (only  $R_f$  value was reported).

It is beneficial that removing the bitter compound could control the bitterness of the final product without wastage or adversely affecting other desirable properties of the end product. According to Debenthini et al. (2014), the sensory scores of samples treated at various temperatures were significantly different ( $p < 0.05$ ) from the control based on the bitter taste. The bitterness of the palmyra pulp was eliminated after a 1-h treatment at 60, 80, and 100 °C. Another group demonstrated improved bulk density and increased fibre, starch, and protein contents of the debittered flour of palmyra young shoot using the aqueous extraction technique (Thivya et al., 2018). It is hypothesised that the action of naringinase may influence the chemical and physical properties of the *B. flabellifer* mesocarp powder. Hence, this work investigates the physicochemical, composition, and functional characteristics of debittered *B. flabellifer* mesocarp powders after naringinase treatment.

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## 2. Materials & Methods

### 2.1 Materials

The main materials used in this investigation were as follows: mesocarp of *B. flabellifer*, gallic acid, Trolox, 2,2-diphenyl-1-picrylhydrazyl (DPPH), naringin, and naringinase (EC- 9068-31-9) with the activity of 10,000 units/g.

### 2.2 Sample Preparation for Debittering of *B. flabellifer*

The mesocarp of the immature *B. flabellifer* fruit was cut into smaller pieces, oven-dried (EW-00299-WK, Cole-Parmer, USA) at 50 °C for 24 h and ground (FZ-240, Zhong Xing, Malaysia) before naringinase treatment for the removal of bitterness. The sample was treated using 2.0 g/L of naringinase for 5 h at pH 5.0 and 55 °C. The mixture was then neutralised to pH 7.0 by adding 0.1 M sodium hydroxide (NaOH). The solid sample was collected by filtration through a strainer, and the filtrate was discarded. The sample was washed three times to remove the excess neutralising agent, re-filtered, and oven-dried (EW-00299-WK, Cole-Parmer, USA) at 50 °C for 24 h. The dried sample was ground for 5 min and sieved through a 0.5 mm sieve to obtain the debittered mesocarp powder (DMP). The mesocarp sample without the naringinase treatment was also prepared and used as a control.

### 2.3 Physicochemical Analysis

#### 2.3.1 Proximate Analysis

The proximate composition of the DMP and control samples were analysed using the AOAC (2000) method. Other analyses involved were the moisture content (the oven method), crude protein (Kjeldahl method), crude fat (Soxhlet method), and dietary fibre (using the enzymatic-gravimetric method), as described by AOAC (2005).

### 2.4 Functional Properties of DMP and Control Sample

#### 2.4.1 Solubility and Swelling Power

The solubility and swelling power of DMP and control samples were determined according to Osundahunsi et al. (2003). About 0.35 g of powder sample was mixed with distilled water (12.5 mL) before heating at 60 °C in a water bath for 30 min, with constant shaking. Following centrifugation at 3500 × g for 20 min, the supernatant was decanted into a pre-weighed evaporating dish and dried for 20 min at 100 °C. The solubility was acquired from the difference in the evaporating dish weight, and the weight of the residue after centrifugation was divided over the initial sample weight (dry weight basis) to determine the swelling power.

#### 2.4.2 The Oil-holding and Water-holding Capacities

The oil-holding capacity (OHC) and water-holding capacity (WHC) were measured by mixing 1 g of BMF powder and 20 g of oil (or distilled water) by rigorous vortexing, with 10 min rest periods between each mixing before centrifugation at 3000 × g for 30 min. The remaining materials were weighed after decanting the free oil, and OHC was calculated as g of oil held per g of sample, whereas the WHC was deduced as g of water held per g of sample (Robertson et al., 2000).

#### 2.4.3 The Wettability

The wettability was determined according to Belscak-Cvitanovic et al. (2010), i.e., the time required for 3 g of powder deposited on distilled water surfaces to fully submerge in 100 mL of distilled water at 27 °C.

### 2.5 Physical Analysis

The total soluble solids and pH were ascertained using a refractometer (Mettler Toledo, Schwerzenbach, Switzerland) and a pH meter (HI 221, Hanna, India). The colour of the DMP and control samples were analysed using a chromameter (00328Q, Konica Minolta, Japan), where L\* denotes lightness from 0 (black) to 100 (white), a\* and b\* for redness (+a) to greenness (-a), and yellowness (+b) to blueness (-b; Reddy et al., 2015).

The particle size of each sample was measured using the Mastersizer S (Malvern Instruments Ltd, UK), according to Kelly et al. (2015). The median diameter, i.e.,  $d_{(v0.5)}$ , was chosen to characterise the particle size of the powders. The bulk density was determined according to Samec et al. (2016). Sample powder was poured vertically into a 50-mL measuring cylinder through a cone. The measuring cylinder was subjected to 10 taps before the excess powder on the measuring cylinder was gently scraped off with a steel ruler, and the filled measuring cylinder was weighed.

### 2.6 Determination of Sugar Composition

The sugar composition of the DMP and control samples were determined according to AOAC (1993) and Puwastien et al. (2011). Sugar content was identified and quantified using HPLC (Agilent 1200 Series, USA). Glucose, fructose, mannose, sucrose, and galactose (Sigma-Aldrich, USA) were utilised as the standard sugars. A weight/weight percentage (g/100 g) of each sugar on the sample was calculated after duplicate injections of the samples.

### 2.7 Determination of Lignocellulosic Composition

Acid-insoluble lignin and holocellulose were determined based on the T222 om-88 standard method of TAPPI (Berlin et al., 2006). Meanwhile, cellulose and hemicellulose were measured according to Sabiha-Hanim et al. (2011) with modifications on the chemical concentration used. The composition of hemicellulose in the sample was estimated by the holocellulose and cellulose level differences.

### 2.8 Extraction of Bioactive Compounds

Bioactive compounds were extracted by microwave-assisted extraction technique (ME71K, Samsung, Korea), according to Asma et al. (2016). About 10 g of mesocarp powder was mixed with 200 mL 0.1 M NaOH in a Falcon tube, heated in the microwave at 300 W for 2 min and cooled to room temperature before filtering using a filter paper with a diameter of 150 mm (CHM, Germany). The filtrates were stored at 4 °C for further analysis. A similar procedure was performed for the control sample.

## 2.9 Phytochemical Analysis

Filtrates from the extraction were screened for their bioactive compounds, such as alkaloids, saponins, flavonoids, steroids, cardiac glycosides, tannins, terpenoids, anthraquinone, and flabelliferin (Obadoni & Ochuko, 2002; Obidoa et al., 2010; Aiyegroro & Okoh, 2010). Samples exhibiting positive results from the screening analysis were subjected to quantification.

## 2.10 Quantification of Phytochemical Content

### 2.10.1 Total Phenolic Content Determination

The Folin-Ciocalteu reaction method was utilised to determine the total phenolic content in the extract (Aiyegroro & Okoh, 2010). The standard gallic acid (0–125 µg/mL) was treated similarly to 200 µL of the sample, and the result was expressed as mg of GAE/g sample.

### 2.10.2 Tannin Determination

The method by Eleazu et al. (2012) was employed to evaluate tannin content in the extract. The tannic acid standard curve (0–500 ppm) was used to measure the samples' tannin content, represented as mg of tannic acid equivalence (TAE) per 100 g of dried sample.

### 2.10.3 Saponin Content Determination

Saponin content was determined by the percentage of the sample according to Obadoni and Ochuko (2001).

### 2.10.4 Crude Flabelliferin Determination

The extraction of crude flabelliferin from the mesocarp was adopted from Wickramasekara and Jansz (2003). A mixture of 80 mL of 10% methanol and 20 g of the sample was left at 30 °C for 16 h. The mixture was concentrated at 60 °C in a rotary evaporator after filtering through a filter paper (Whatman No1, U.S.A). The flabelliferin was twice extracted with 50 mL of ethyl acetate before being filtered with a 0.45-µm nylon syringe filter.

The extract was analysed for flabelliferin using the Davis colourimetric method (Puri et al., 2005). This study used naringin as the standard, as flabelliferin is not commercially available. Naringin comprises a sugar complex of α-L-rhamnosides and β-D-glucose, similar to flabelliferin. Naringin was dissolved in warm deionised water to prepare the standard concentration (100–500 ppm). Then, 0.1 mL of the standard solution was added to 5 mL of 90% diethylene glycol, followed by 0.1 mL of 4 N NaOH. The mixture was kept at 30 °C for 15 min. The resultant yellow colour was measured spectrophotometrically at 420 nm. The blank was prepared by treating distilled water similar to the standard procedure. For the sample, 0.1 mL of extract was added instead of the standard solution to measure the concentration of flabelliferin.

## 2.11 Antioxidant Activities

### 2.11.1 Determination of DPPH Radical Scavenging Assay

The free radical scavenging capacity, denoting the antioxidant capacity of the powder samples, was estimated spectrophotometrically using the stable radical DPPH (2,2-diphenyl-1-picrylhydrazyl) based on Chu et al. (2000). Standard Trolox from 100 to 500 µM/mL was prepared, and the results were expressed as mol of Trolox equivalent DPPH radical scavenging activity per g of sample.

### 2.11.2 Ferric Reducing Antioxidant Power (FRAP)

The antioxidant capacity was assessed using the ferric reducing antioxidant power (FRAP) assay described by Langley-Evans (2000), with some modifications. The ferrous sulphate (0.1–1 mM) dilution series was used to obtain the FRAP results, and the antioxidant activity was quantified as mM ferrous per g of sample.

## 2.12 Statistical Analysis

All samples were prepared and tested in triplicate, and the mean and standard deviations were obtained. The data were analysed with Minitab for Windows version 16 (Minitab Inc., Sydney, New South Wales, Australia) using an independent t-test and analysis of variance (ANOVA), and the differences were considered significant at  $p < 0.05$ . The associations between variables were determined using Pearson correlation coefficients, and differences at  $p < 0.05$  were considered significant.

## 3. Results and Discussion

### 3.1 Chemical and Physical Analysis

#### 3.1.1 Proximate Composition

DMP exhibits a reduced pattern in all proximate compositions (Table 1). Protein and fat declined 3.5 and 2.5 times after naringinase treatment, while moisture and ash decreased by 1.1-fold and 1.9-fold to the control. Kelly et al. (2015) reported that moisture level might influence the main functional properties, such as stickiness, wettability, bulk density, and powder flowability. Pectinase and naringinase increase the yield of pummelo juice and eliminate bitterness (naringin, limonin, and nomilin; Ni et al., 2014). Enzymatic debittering processes would likely affect nutrients due to the specification of the enzyme towards bitter compounds. The present results will likely have a similar impact on the proximate composition.

**Table 1.** Phytochemical composition of the debittered mesocarp and the control sample from *B. flabellifer*

Composition (%)	<i>Borassus flabellifer</i>	
	Debittered Mesocarp Powder (DMP)	Control
<b>Proximate (%)</b>		
Protein	1.19±0.04 <sup>b</sup>	4.20 ±0.47 <sup>a</sup>
Fat	0.61±0.03 <sup>b</sup>	1.52 ±0.50 <sup>a</sup>
Moisture	9.35±0.16	10.35±0.22 <sup>a</sup>
Ash	1.88±0.35 <sup>b</sup>	3.66±0.12 <sup>a</sup>
<b>Dietary Fibre (g/100g)</b>		
Total dietary fibre (TDF)	84.420±0.01 <sup>a</sup>	65.970±0.820 <sup>b</sup>
Insoluble dietary fibre (IDF)	79.99 ±0.05 <sup>a</sup>	61.84±1.16 <sup>b</sup>
Soluble dietary fibre (SDF)	4.43 ±0.09 <sup>a</sup>	4.13±0.35 <sup>a</sup>
Ratio IDF/SDF	18.06	14.97
Total soluble solids (%)	0.77±0.06 <sup>b</sup>	2.13±0.06 <sup>a</sup>
pH	5.16±0.01 <sup>a</sup>	3.58±0.08 <sup>a</sup>
<b>Colour</b>		
L*	72.80±0.56 <sup>a</sup>	66.00±0.10 <sup>b</sup>
a*	3.03±0.21 <sup>a</sup>	2.70±0.10 <sup>b</sup>
b*	16.40±0.52 <sup>a</sup>	15.33±0.06 <sup>b</sup>
Particle size (µm)	279.83±8.17 <sup>b</sup>	418.89±23.2 <sup>a</sup>
Bulk density (kg/m <sup>3</sup> )	173.91±4.27 <sup>b</sup>	324.83±0.82 <sup>a</sup>
<b>Lignocellulosic (%)</b>		
Holocellulose	88.10±7.43 <sup>a</sup>	52.52±0.78 <sup>b</sup>
Cellulose	53.76±8.22 <sup>a</sup>	29.03±0.75 <sup>b</sup>
Hemicellulose	34.33±3.73 <sup>a</sup>	23.50±0.02 <sup>b</sup>
Lignin	12.99±0.18 <sup>a</sup>	10.29±0.45 <sup>b</sup>

Results as means from triplicates. a-b Different superscript between debittered mesocarp and control denote significant differences (Independent t-test,  $p < 0.05$ ).

### 3.1.2 Total, Soluble, and Insoluble Dietary Fibre

The total DMP dietary fibre increased 1.3-fold higher than the control (Table 1). The insoluble and soluble dietary fibre of DMP increased significantly ( $p < 0.05$ ) by up to 27.8% and 10.5% after naringinase treatment (Table 1). From the microscope image (image is not shown), the enzymatic treatment caused the fibre-like structure to be broken down into smaller strands, increasing the overall surface area for enzyme penetration and improving enzyme accessibility. Elleuch et al. (2011) reported that enzymatic treatment could alter the proportion of soluble to insoluble fibres, e.g., cell wall treatment with xylanase increased the amount of soluble dietary fibres. The insoluble fibre in DMP is significantly ( $p < 0.05$ ) higher than soluble fibre, with an IDF/SDF ratio of 18.06 (an increase of 17.1% after the naringinase treatment). IDF is the

dominant fibre fraction (about 84.4% of TDF). Thus, DMP may affect insoluble fibre consumption as IDF exerts pronounced effects on intestinal regulation and stool volume (Chau & Uang, 2003). Since IDF absorbs water and expands bolus size, consumption of IDF produces a feeling of satiety. Additionally, it increases the size and weight of the faecal bolus, promoting better digestion and preventing conditions like constipation and colon cancer (Ku & Mun, 2008). Insoluble fibres improve product density and minimise shrinkage, stabilise the food system, and are used as an agent to enhance food appeal and texture (Al-Sheraji et al., 2011).

Thermal processing could alter the amount of total dietary fibre, the ratio of insoluble to soluble fibres, and physicochemical properties (Elleuch et al., 2011). Naringinase treatment in the study was conducted at 55 °C for 5 h before deactivation at 90 °C for 5 min. The temperature employed could be a contributing factor to the outcomes. High temperature and pressure can cause the breakdown of macromolecule covalent bonds, disrupting their physical structures and changing their functional properties (Kim et al., 2006; Singh et al., 2007).

### 3.1.3 Total Soluble Solids, pH, and Sugar Composition

The DMP is less acidic, with less total soluble solids (TSS) than the control (Table 1). TSS is related to the sugar content, and this study found that TSS in DMP was reduced by 63.8% following naringinase treatment, the possible reason for sugar not being detected in the sample. The HPLC analysis revealed that the DMP lacks sugar due to the absence of peaks in the chromatogram. This could be due to the undetectable sugar composition or the absence altogether. Besides, the washing process of DMP before drying could have removed the soluble sugar. In this study, DMP was rinsed three times after the naringinase treatment before drying to eliminate any flabelliferin and naringinase residues, hence the result. De Moraes et al. (2013) confirmed that a washing process before fibre drying from fruit by-products caused sugar removal.

### 3.1.4 Colour

The chromaticity coordinate attributes (L\*, a\*, and b\*) of DMP showed higher values than the control, indicating that the DMP sample is lighter and possesses more red and yellow hues than the control (Table 1). In food drying, colour is one of the vital quality parameters that could lead to darkening (reduced L\* and increased a\*; Garau et al., 2007; Lario et al., 2004). It could be due to the high temperature (90 °C) and the duration (8.3 h) of the drying process, which causes the Maillard reaction (non-enzymatic browning reaction). However, DMP did not turn darker in this study, as the L values increased by 9.34% after the naringinase treatment. Washing the sample before drying could prevent the fibre darkening due to sugar removal (De Moraes et al., 2013). A similar phenomenon was observed when dietary fibre lemon powder from lemon (*Citrus limon* cv. Fino) by-product was washed before drying (Fuentes, 2004). The washing process prevented the fibre from browning due to sugar elimination.

### 3.1.5 Particle Size

The particle size of the mesocarp sample is reduced by 33.2% after naringinase treatment (Table 1). Naringinase could have probably worked on the mesocarp by degrading the fibre strands. The increase in surface area and the rupture of fibre matrix pores could result from reduced particle size, which would also alter the hydration properties (i.e., solubility; Peerajit et al., 2012).

### 3.1.6 Bulk Density

The DMP has a 173.91 kg/m<sup>3</sup> bulk density, 1.9 times lower than the control (Table 2), exhibiting the small amount of packaging materials required. Furthermore, the bulk density and wettability property exhibit a strong negative correlation ( $r = -0.923$ ), i.e., water can easily penetrate the powder surface. The higher bulk density materials indicated lower surface area and lower ability to bind to lipid components (Garcia-Amezquita & Serna-Saldi, 2015).

### 3.1.7 Lignocellulosic Composition

The composition of holocellulose, cellulose, hemicellulose, and lignin of the DMP increases (Figure 1). Naringinase treatment degrades lignin structure, simultaneously allowing the cellulose in the mesocarp fibres to be exposed to enzyme action, represented by increased cellulose and hemicellulose (46.0% and 31.6%). This is likely due to the breakdown of lignin by naringinase, which disrupted the cellulosic crystalline structure of DMP. Lignocellulosic biomass denotes plant biomass with lignin as the recalcitrant outer layer, strongly bound to the complex carbohydrate polymers (cellulose and hemicellulose; Nur Liyana Izyan et al., 2014). The presence of cellulose in the substrate may affect the enzymatic hydrolysis of pretreated lignocellulosic biomass, which is accessible to cellulolytic enzymes or the cellulose surface area of the enzyme (Chandra et al., 2009).

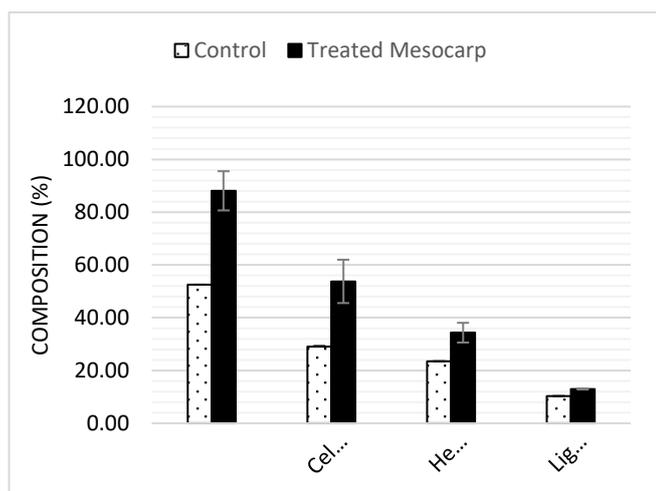


Figure 1. Lignocellulosic Composition of the DMP and Control Sample

### 3.2 Functional Properties

#### 3.2.1 Solubility and Swelling Properties

The solubility of the DMP, performed at 30 and 60 °C, ranged from 4.31% to 4.55% (Table 2). The naringinase treatment affected the solubility of DMP, i.e., reduction by 2-fold and nearly 5-fold, when tested at 30 and 60 °C. The solubility of DMP was lower than dried egg white, buttermilk solids, and non-fat dried milk (18.0%–34.6%; Wong & Kitts, 2003). The solubility property has a strong negative correlation ( $r = -0.907$ ) to the crude fibre content of DMP powder. Kumar et al. (2010) and Selani et al. (2014) also noted a decrease in solubility with increased carrot and pineapple pomace fibre.

The DMP had a swelling capacity value of 7.75 g/g (Table 2), significantly ( $p < 0.05$ ) lower than the control. The swelling capacity of DMP is lower than the apple (12.8 g/g), orange pomace (8.13 g/g; O’Shea et al., 2015), and fibre powder of pink guava by-product (10.9–15.0 g/g; Ibrahim, 2009) but slightly higher and comparable to the dietary fibre powder of citrus by-products (6.11–9.19 g/g; Figuerola et al., 2005). The swelling capacity indicates the extent of the swelling of the fibre upon water absorption due to the amount of soluble dietary fibre (SDF), particularly pectin (López-Vargas et al., 2013). Wuttipalakovorn et al. (2009) demonstrated that the reduced particle size of dietary fibre (63–150 µm) from lime residues resulted in the reduced water retention ability (9.72–11.74 g water/g dry basis) and swelling capacity (10.12–11.86 mL water/g dry basis) of the fibre powder.

Table 2. Functional properties of the debittered mesocarp and the control from *B. flabellifer*

Properties	Debittered mesocarp Powder	Control
Water Holding Capacity (g/g)	9.38±0.39 <sup>a</sup>	7.11±0.29 <sup>b</sup>
Oil Holding Capacity (g/g)	5.76±0.55 <sup>a</sup>	3.33±0.08 <sup>b</sup>
Solubility (%) (Treatment at 30 °C)	4.31±0.24 <sup>b</sup>	8.76±0.99 <sup>a</sup>
Solubility (%) (Treatment at 60 °C)	4.55±0.27 <sup>b</sup>	21.84±2.20 <sup>a</sup>
Swelling Power (g/g d.w.)	7.75±0.86 <sup>b</sup>	9.15±0.11 <sup>a</sup>
Wettability (s)	12.28±0.21 <sup>b</sup>	44.33±1.53 <sup>a</sup>

Results as means from triplicates. <sup>a-b</sup> Different superscripts between debittered mesocarp and control denote significant differences (Independent t-test,  $p < 0.05$ ).

#### 3.2.2 The Water-Holding Capacity (WHC) and Oil Holding Capacity (OHC)

In this investigation, the WHC and OHC of DMP increased after the removal of bitterness via naringinase treatment. Correlation

analysis displayed a robust positive correlation ( $r = 0.948$ ) between WHC and OHC. In another research, Yi et al. (2014) determined the physicochemical properties of dietary fibre of citrus juice by-products after treatment with  $\alpha$ -amylase. The enzyme-treated samples showed higher functional properties such as increased water retention (13.31 mL water/g powder), swelling (8.55 mL/g powder), and oil-holding capacity (8.37 g oil/g powder). This study showed a strong negative correlation ( $r = -0.811$ ) between the particle size and WHC. It indicates that the hydration property of DMP was increased with decreased particle size. According to Raghavendra et al. (2006), the hydration characteristics of coconut fibre increased when the particle size decreased from 1127 to 550  $\mu\text{m}$ .

The WHC of the DMP increased by 24.3% (Table 2), possibly due to the higher content of IDF in the DMP. Zhou et al. (2012) reported that the WHC of Tartary buckwheat (*Fagopyrum tataricum Gaertn*) bran dietary fibre flour was increased to 2.22 g/g after enzymatic treatment with amylase and cellulase due to increased SDF/IDF (0.62%/43.33%) and the decreased size of dietary fibre particles (61.6 nm). Water uptake kinetics were significantly affected by structural characteristics and the chemical composition of fibre, including its component water affinity (Figuerola et al., 2005). The capillary action of water in the fibre is due to its high surface tension. Moreover, different molecular fibre components can interact with water through hydrogen bonding or dipole formation (Martínez et al., 2012). In addition, Chau and Uang (2003) reported that the difference in WHC between the samples is attributed to the number and nature of the water-binding site differences, chemical content, and structure of each sample.

Meanwhile, the OHC of the DMP has increased by 24.25% following naringinase treatment (Table 2). Karaman et al. (2017) revealed that grapefruit seed dietary fibre treated with hesperinidase and naringinase showed enhanced OHC values (3.44 and 4.19 g oil/g fibre). In food applications, the capacity of a sample to retain oil can be crucial, i.e., to prevent fat loss upon cooking (Tosh & Yada, 2010). Meanwhile, from the nutritional aspect, the increased excretion due to the ability to absorb or bind bile acids is associated with reduced plasma cholesterol (Tungland & Meyer, 2002). In addition, high OHC allows high-fat food products and emulsions to stabilise (Elleuch et al., 2011). Hence, DMP may be a potential ingredient for dressings and condiments (such as sauce, ketchup, and mayonnaise) to better emulsion structure and stability. Correlation analysis displayed a robust positive correlation ( $r = 0.948$ ) between WHC and OHC.

### 3.2.3 Wettability

In this study, wettability has increased significantly (3.6-fold,  $p > 0.05$ ) after the naringinase treatment. Very low lipid content prompts the excellent wettability value of the DMP powder (Table 2), which could be supported by the correlation analysis between the fat content and wettability properties, showing a moderate positive correlation for DMP ( $r = 0.327$ ). This finding supported

the findings by Benkovic et al. (2015), where the agglomeration of lower wettability values (6.83 to 60.52 s) of cocoa powder with a high-fat content (16–18 g/100 g) was observed due to the susceptibility of high-fat powder to lipid oxidation, which was less wettable and flowable compared to those with lower free fat levels (Vignolles et al., 2007).

### 3.4 Phytochemical Composition

Table 3 shows that only saponin, tannins, phenol, and flabelliferin are present in DMP. Saponin has increased by 2.4-fold after the naringinase treatment. Saponin is an antioxidant capable of binding to and stopping cholesterol from being absorbed, allowing the body to easily eliminate excess cholesterol. The consumption of saponins is recommended due to their hypocholesterolemic activity, aid in proper immune function, and are important as chemopreventive agents (Abioye, 2018; Emojorho & Akubor, 2016). In contrast, Emojorho and Akubor (2016) reported that the saponin concentration in orange seed flour was gradually reduced following bitterness removal when boiled. In this study, the mesocarp sample was boiled for 5 min at 90 °C to deactivate the naringinase activity upon completion of the treatment. The release of saponin from the DMP was not affected by the thermal effect, possibly due to the shorter exposure to the heat.

**Table 3.** Phytochemical composition and antioxidant activities of the debittered mesocarp and control of *B. flabellifer*

Compounds and antioxidant activities	Debittered Mesocarp Powder (DMP)	Control
<b>Compounds</b>		
Alkaloids (%)	ND	ND
Saponins (%)	41.06±5.21 <sup>a</sup>	16.75± 1.44 <sup>b</sup>
Flavonoids (QE/g)	ND	ND
Steroid (ng/ ml)	ND	ND
Cardiac glycosides (%)	ND	ND
Phenol (GAE/g)	6.080±0.001 <sup>a</sup>	3.230±0.001 <sup>b</sup>
Tannins (TE/g)	3.260±0.020 <sup>a</sup>	0.007±0.005 <sup>b</sup>
Terpenoids (LE/g)	ND	ND
Anthraquinone (%)	ND	ND
Flabelliferin (mg/L)	153.57±2.08 <sup>b</sup>	240.57±23.03 <sup>a</sup>
<b>Antioxidant activity</b>		
FRAP (mM Fe <sup>2+</sup> /g)	126.38±0.03 <sup>b</sup>	157.05±0.08 <sup>a</sup>
DPPH (mM TE/g)	35.93±0.02 <sup>a</sup>	29.90±0.05 <sup>b</sup>

Results as means from triplicates. <sup>a-b</sup> Different superscripts between debittered mesocarp and control denote significant differences ( $p < 0.05$ ). ND: Not detected.

The total phenolic compound (TPC) of DMP was increased almost 2-fold compared to the control. The enhanced TPC may be caused by the release of bound phenolic chemicals from their polymeric or glycosidic precursors (Variyar et al., 2004). A strong negative association was observed between the TPC and DPPH levels. Polymeric phenolic component degradation to simple phenols could cause increased antioxidant activity, increasing

their solubility and capability to interact with DPPH (Kondapalli et al., 2014). The diverse bioactivity of the phenolic compounds, such as antiviral, anti-allergy, anti-mutagenic, and anti-inflammatory properties (Peng et al., 2010), provides an extra benefit to the DMP.

In another debittering process, the phenolic and saponin contents of debittered flour significantly decreased from 320 to 131.651 mg/g and 2.04 to 0.106 mg/g when young shoot palmyra flour was soaked in water at 65 °C for 1 h compared to the raw flour (Thivya et al., 2020). The decreased bioactive compounds in the debittered flour could be attributable to the leaching of soluble phenolics and saponin into water or the chemical oxidation caused by the heat treatment. Similar findings have been documented for the reduced fennel bulb polyphenols after boiling treatment (Rawson et al., 2013). The heating process caused the breakdown of the structure and converted it into a more water-soluble form (Thivya et al., 2020). As previously reported, naringinase action at 55 °C might not be strong enough to leach out the saponin and phenolic contents. However, the deactivation of naringinase activity at 95 °C for 5 min might influence the result.

Likewise, the total tannin content in DMP is higher than in the control. Tannin structure has a significant impact on the qualities of emulsions; the best possibilities are tannins with relatively high molecular weights, which have undergone oxidation processes. Tannins are preferred as stabilisers as they have primary antioxidant capacities, although several natural surfactant agents are currently available (Figuroa-Espinoza et al., 2015).

About 36.2% of residual flabelliferin remained in DMP after the naringinase treatment (unpublished). Despite its contribution to the bitter taste, flabelliferins possess several benefits. The fresh palmyra fruit pulp (PFP) that contained flabelliferins and fibre as the main chemical components caused lower postprandial blood glucose levels in mice due to its hypoglycaemic components (Thabrew & Jansz, 2004). Likewise, Uluwaduge et al. (2005) proved that flabelliferin II might prevent intestinal glucose uptake in mice. It was also reported that the significant inhibitory effects on the absorption of intestinal glucose in mice are mediated by the fraction of flabelliferin (flabelliferin-II) rather than the fibre in PFP (Uluwaduge et al., 2008).

### 3.5 Antioxidant Activities

The DPPH levels of the DMP increased by 1.2-fold higher than the control (Table 3). As reported earlier, the particle size of DMP was reduced after treatment with naringinase. Likely, the smaller size of the fragment is closely related to the size-reduced fibre strand, increasing the DPPH value. Likewise, the superfine dietary fibre of wheat bran had increased antioxidant activities following superfine grinding, including total phenolic content, chelating activity, and reducing power (Zhu et al., 2012).

However, the DMP has a significantly ( $p < 0.05$ ) lower reducing power than the control (Table 3). These results are congruent with Muñiz et al. (2011), who reported that the highest total phenolic content (1227 mg GAE/L) and the lower reducing power (6.3–14.0 mM FeSO<sub>4</sub>) were detected in grapefruit juice following naringinase treatment. The correlation of the particle size with DPPH and FRAP values yielded a moderate negative correlation ( $r = -0.676$ ) and a strong positive correlation ( $r = 0.980$ , Table 3).

## 4. Conclusion

A decline in all chemical compositions of DMP was observed except the dietary fibre. The solubility, swelling, bulk density, and wettability of DMP were markedly decreased, whereas water and oil holding capacities were significantly increased. The low fat content in DMP directly affected solubility, WHC, OHC, and wettability properties because it is less susceptible to lipid oxidation, more wettable, and flowable. The naringinase action increased the lignocellulosic content (mainly the cellulose and hemicellulose) and improved the colour property. DMP also had an enhanced content of saponin, phenol, tannin, and DPPH activity than the control, but the reduced activity of ferrous reducing power. In conclusion, DMP has the potential as a functional ingredient to increase fibre in products that require hydration, low calories, and high fibre, such as noodles, energy bars, and breakfast cereal. DMP with an enhanced content of saponin, phenol, tannin and DPPH activity is also useful for pharmaceutical industries. The results of this study can trigger more investigation into the broader application of *B. flabellifer* in various functional food products.

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