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RESEARCH ARTICLE

Flabelliferin removal by sodium salts and sodium hydroxide: Pretreatment in *Borassus flabellifer* mesocarp

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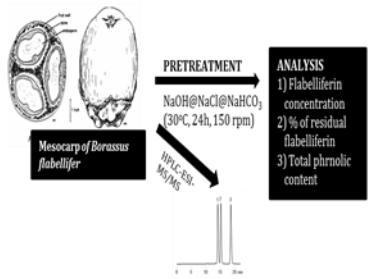
Graphical abstract



The mesocarp of *Borassus flabellifer* has been proven to be a good potential as a food ingredient; however, the presence of bitterness in it is a deterrent to its acceptable application. Therefore, this study was aimed to determine the effectiveness of sodium hydroxide, sodium chloride, and sodium bicarbonate at 1% to 3% concentrations in reducing the bitter component, flabelliferin, in the mesocarp of *B. flabellifer* and how the pretreatment could affect the reduction of flabelliferin and phenolics contents. Eleven compounds were identified in the mesocarp by high-performance liquid chromatography-electrospray ionisation mass spectrometry (HPLC-ESI-MS/MS), which consisted of seven types of phenolics, two types of antocyanidins, and two types of steroidal saponins. The highest reduction at 46.2% of flabelliferin was obtained by 3% sodium hydroxide treatment. However, the removal of the bitter component was also concurrently resulted in a decrease of the total phenolic contents (0.16 mg GAE/g) of the sample. Mild treatment such as enzymatic treatment should be employed as the alternative method to preserve the antioxidant content in the mesocarp of *B. flabellifer* in order to promote its application in the food industry as a potential food ingredient.

Keywords: Bitterness, *Borassus flabellifer*, flabelliferin, mesocarp and steroidal saponin

INTRODUCTION

The bitter taste is an outstanding issue in the food and pharmaceutical industries because of its adverse hedonic effect upon ingestion (Drewnoswki, 2001; Drewnoswki & Gomez-Carneros, 2000). Bitter tastes are desirable only in a small set of foods at reasonable levels and commonly, preference for these foods must be learnt with children and infants being particularly opposed towards bitter tastes (Steiner, 2001). However, in an uncommon situation, consumers prefer a strong bitter taste for food and beverages, e.g., in black or green tea, black coffee, red wine, grapefruit products, beer, or bitter lemon. In most different cases, the bitter taste is not desirable and has to be removed from or masked in the product. For instance, most legumes, fruits, and staple foods are broadly optimised by utilising breeding and cultivation technology to become less bitter, astringent, or sour variations throughout time (Ley, 2008) or more advanced processing methods to additionally decrease the bitterness (e.g. treatment of orange juice with naringinase; Coupland & Hayes, 2014), application of strong flavours or tastants (e.g. salt, sweeteners, acid, and intense fruit flavours; Ley, 2008). Sodium salts such as NaCl, NaAcetate, NaGluconate and others have been shown to be potent inhibitors for some bitter compounds (Keast *et al.*, 2001). Soaking the vegetables in sodium chloride (NaCl) solution followed by the blanching process can minimize the bitter taste (Din et al. 2011; Krawinkel and Keding 2006). Sodium hydroxide (NaOH) can also be applied in the removal of the bitterness in olives. Olives are processed using a series (3–5) of sodium hydroxide treatments (~0.5 M NaOH) for several hours per treatment. NaOH penetrates the olive flesh before hydrolyzing the olive phenolics and debittering the olives (Johnson and Mitchell, 2018).

Borassus flabellifer is a palm tree grown in tropical Asian countries such as Thailand, Bangladesh, India, Myanmar, Sri Lanka, Malaysia, etc. (Jansz *et al*., 1994; Ariyasena *et al*., 2000, 2001). It is well known as palmyra palm. Many studies reported that some food and beverage products are prepared from the palmyrah fruit pulp for commercial use in the developing countries like Bangladesh, India, Pakistan, and Sri Lanka. Even though the demand for the food products is significantly high, some consumers despise the bitterness of the fruit pulp, which leads to the unacceptable quality. Due to this reason, the more significant part of the annual production of palmyrah fruits is either to be discarded or utilised as animal feed (Jansz *et al*., 2002). Besides that, the disposal of abundant residues from the skin of immature *B. flabellifer* fruit, which are significant by-products generated during the preparation of natural drink, has become an enormous problem and this issue has often been overlooked. The previous study proved that the skin, mainly the mesocarp and exocarp, contained a considerable number of bioactive compounds (tannin, saponin, and phenol), dietary fibre, and antioxidant properties (Rodiah *et al.*, 2017) which give promises for the utilization of this by-product as a food ingredient.

Generally, many techniques like chemical methods and heat treatments are in practice to decrease the bitterness of the food and beverages of the palmyrah pulp. The bitter compound presents in the palmyrah fruit pulp is flabelliferin (Fii), which is identified as a

tetraglycoside (Nikawela *et al*., 2000; 2011). These bitter compounds are steroidal saponin and the term flabelliferin is coined from the specific name flabellifer (Jansz *et al*., 2002). Flabelliferins or borassoside found in palmyrah is usually contained β-glucose as the first sugar moiety attached to the aglycone (Nikawala *et al*., 2000; Ariyasena *et al.,* 2002; Yoshikawa *et al*., 2007). The flabelliferin II (Fiiɪ) can be hydrolysed into rhamnose and glucose molecules, but although 40% of the Fii bitterness can be hydrolysed by α-amylase, some of it is not susceptible to α-amylase (Jansz *et al.,* 2002; Zhang *et al.,* 2007). Ariyasena *et al.* (2002) found six types of flabelliferin in the palmyra fruit pulp, i.e. the FF (flabelliferin monoglucoside, M.W. 576), FD (flabelliferin diglucoside, M.W. 722), FE (flabelliferin diglucoside, M.W. 734), FB (branched flabelliferin triglucoside, M.W. 868), FC (linear flabelliferin monoglucoside, M.W. 868), and Fii – flabelliferin (tetraglycoside, M.W. 1030). It will be desirable to reduce the concentration of these bitter compounds to a point where even the most sensitive individuals will not be aware of their presence (Soares & Hotchkiss, 1998).

To the best of our knowledge, there is a significant lack of research concerning the exploitation of the Borassus fruit co-products such as the mesocarp during the immature stage and the study of the removal of the bitterness from it. Thus, it is necessary to study these co-products to identify alternatives for processing and reusing the co-products that are formed, overcoming environmental issues, and adding value to these products. Therefore, the removal of bitterness in immature *B. flabellifer* mesocarp using chemical treatment using sodium hydroxide, sodium chloride or sodium bicarbonate was investigated. The identification of the bioactive compounds available in the immature *B. flabellifer* mesocarp using chromatography was also conducted. This study was deemed necessary to determine if the debittering procedure could produce an anti-nutritional effect in the end product in order to enhance the commercial use of the immature *B. flabellifer* mesocarp.

EXPERIMENTAL

Sample preparation

The immature fruit of *B. flabellifer* was obtained from Cameron Highlands, Pahang, Malaysia. Samples with similar maturity stage (age: 3–4 weeks) were sorted before the analysis. The fruits were cleaned of dirt and soil and kept in an insulated box (4°C) before being transported to the laboratory. Upon arrival, the mesocarp was then separated from the exocarp, cut into smaller pieces and dried at 50°C for 24 h in an oven. The samples were ground using a grinder (FZ-240, Zhong Xing, Malaysia), sieved through a 0.5 mm sieve, and placed in a tightly sealed plastic container at room temperature (30°C) away from light and oxygen to prevent oxidation for further analyses.

Preparation of crude extract

Extraction of bioactive compounds from the mesocarp was adopted from Ariyasena *et al.* (2001) as well as Wickramasekara and Jansz (2003). Twenty g of mesocarp was mixed with 10% methanol (80 mL) and left for 16h at room temperature (30°C). The mixtures were then filtered through filter paper (Whatman No1, U.S.A) before being concentrated in a rotary evaporator at 60°C. The concentrate was extracted with ethyl acetate (2 ×50 mL) and then filtered with 0.45µM nylon syringe filter before injecting into the high-performance liquid chromatography-electrospray ionisation mass spectrometry (HPLC-ESI-MS/MS; Ariyasena *et al.,* 2001; Wickramasekara & Jansz, 2003).

HPLC-ESI-MS/MS

The identification of bioactive compounds was performed according to the methods described by Schutz, Persike, Carle and Schieber (2006). The analysis was carried out using Sciex 3200 QTRAP linear ion trap quadrupole mass spectrometer (AB Sciex, Canada) fitted with Turbo V ESI source operating in positive mode and coupled with Perkin Elmer Flexar FX15 Ultra HPLC system. The Analyst 1.5.2 software was used for instrument control, data acquisition, and mass processing. The mobile phase was prepared from water with 0.1% formic acid as solvent A, and acetonitrile with 0.1% formic acid as solvent B. The gradient program was 5% B to 100% B from 0.01 min to 10.0 min in 10 min, held for 2 min before adjusted to 5% B in 0.1 min, and re-adjusted for 3 min. The flow rate was in the range of 0.25 to 0.4 mL/min with a total run time of about 15 min.

Positive-ion mass spectra of the column eluent were recorded in the range of m/z 50–1500. Purified nitrogen gas was used as the drying gas and nebulising gas, at a pressure of 40 psi. The nebuliser temperature was set at 500 °C and a potential of 4500 V was used on the capillary. Purified nitrogen was used as the collision gas for collision-induced dissociation (CID).

Effects of chemical treatment in the removal of mesocarp bitterness

an amount of 10g mesocarp powder was mixed with 200 mL of sodium hydroxide at a variable concentration (1%, 2%, and 3%), followed by incubation at 30°C for 24h with an agitation speed of 150 rpm in an incubator shaker (Thermo Scientific, USA). The mixture was then filtered with membrane filter paper (150 mm, CHM, Germany) and the residue (solid) was further dried at 45°C overnight in an oven (EW-00299-WK, Cole-Parmer, USA). The dried sample was stored in a gas-tight container prior to further analysis, while the remaining filtrate was kept for flabelliferin determination. The similar procedure was conducted as the previous treatment using the sodium bicarbonate and sodium chloride. A control sample was also prepared using the same procedures, but the distilled water was used instead of the chemical solution. Samples were prepared in triplicate.

The extraction method of debittered mesocarp

The dried sample or known as ‘debittered mesocarp powder’ and the control samples from the previous analysis were extracted using microwave-assisted extraction, which was performed in an experimental microwave oven (ME71K, Samsung, Korea). About 2g of the debittered sample was transferred into a conical flask containing 40 mL of 0.1 M sodium hydroxide (ratio of 1:20). The mixtures were than heated at a microwave power of 300W for 2 min (Rodiah *et al*., 2015). After microwave heating, the mixture in the conical flask was allowed to cool down at room temperature and filtered using filter paper (150 mm, CHM, Germany). All filtrates were kept at 4°C in the dark prior to analysis of residual flabelliferin and total phenolic content.

Overall, the performances of pretreatments were evaluated based on the total concentration of flabelliferin released from the mesocarp during the pretreatment, the percentage of residual flabelliferin and total phenolic content in debittered mesocarp after the pretreatments. All analyses were performed as follows:

Analytical Method

Determination of flabelliferin

The concentration of flabelliferin released from the mesocarp was analysed using Davis colourimetric method (Puri, Banerjee, & Banerjee, 2005). In this study, naringin was used as the closest standard for the determination of flabelliferin compound. Naringin was dissolved in warm deionised water to prepare the standard concentration ranging from 100 to 500 ppm. Then, 0.1 mL of the standard solution was added to 5 mL of 90% diethylene glycol, followed by the addition of 0.1 mL of 4N sodium hydroxide. Samples were kept at room temperature (28 °C) for 15 min. The intensity of the resultant yellow colour was measured at 420 nm using a spectrophotometer. A volume of 0.5 mL of deionised water was added instead of the standard to prepare the blank. Then, 0.1 mL of filtrate from the previous pretreatment was added instead of the standard solution to measure the concentration of flabelliferin. The sample was prepared in triplicates

Percentage of residual flabelliferin

The determination of residual flabelliferin in the debittered mesocarp was modified from the method of Kumar (2010). The decrease in flabelliferin content was directly correlated to the

reduction in bitterness. To determine the percentage of residual flabelliferin after the pretreatment, the flabelliferin content of fresh mesocarp without any treatment was also quantified. From the amount of flabelliferin presented in the fresh mesocarp, the percentage reduction in the bitterness of the debittered mesocarp was calculated. The formula for the percentage of residual flabelliferin was measured as follows;

$$\text{Percentage of residual flabelliferin} = \frac{\text{Flabelliferin in fresh mesocarp} - \text{flabelliferin in debittered mesocarp}}{\text{Flabelliferin in fresh mesocarp}} \times 100$$

Determination of total phenolic content

The effect of chemical pretreatment on the total phenolic content of the debittered mesocarp extract was also measured. The amount of phenol in the extract was determined by the Folin-Ciocalteu reagent method (Aiyegroro & Okoh, 2010). Approximately, 200µL of sample was added to 1.5 mL of diluted Folin-Ciocalteu reagent (1:10, v/v) and incubated for 5 min at room temperature. A volume of 1.5 mL of 0.566 M Na_2CO_3 was added to the mixture. The absorbance of the mixture was measured at 725 nm using a spectrophotometer (Genesys 20, USA) after 90 min of incubation. Standard Gallic Acid within the range of 0–125 µg/mL was treated similarly as the 200 µL samples stated above. The result was expressed as mg of GAE per amount of sample in g. Three replicates of each sample were made for each analysis.

Statistical analysis

Each measurement was done in triplicate and the means and standard deviations (SD) were computed. The results were then expressed as mean ±SD. The gathered data was statistically analysed using the Minitab 16th version software using one-way ANOVA for significance ($p \leq 0.05$) and Turkey pair wise comparison where required.

RESULTS AND DISCUSSION

Peak Identification and assignment

The ESI-MS base peak chromatogram from the mesocarp showed a relatively complex mixture containing peaks of phenolic acids, anthocyanidin, and steroidal saponin. Identification and peak assignment of compounds in the mesocarp were based on a comparison of their mass spectral data with those of the standards and published data (Table 1; Fig. 1). In the analysed mesocarp extracts, 7 peaks were confirmed as phenolic compounds. Peak 1 had the same molecular weight (m/z 153) as peak 3, but with a slightly shorter retention time (2.20 vs. 2.51). This compound was similar to that found in dried plum (Nianbai Fang) and *B. flabellifer* leaves (Saravanan *et al*., 2016); thus this peak was tentatively identified as protocatechuic acid. The peak 4, 5, and 6 yielded the main product ion with m/z 179.01, 336.10, and 193.02, and characterised as caffeic acid, 5-0-caffeoyl shikimic acid and ferulic acid, respectively. Another three phenolic compounds were found in the *B. flabellifer* mesocarp and they were identified as kaempferol, sakuranetin, and genkwanin by comparing their MS data to standards and previous data (Adetunji, Duodu, & Taylor, 2015). Peak 7, which was identified as kaempferol, had the molecular ion [M]- at 534 while peak 8 and 11 produced a fragment ion m/z at 285.09 and 283.65, suggesting sakuranetin and genkwanin, respectively. In this context, the presence of phenolic compounds in *B. flabellifer* mesocarp has proven its potential use as a healthy food ingredient as Dagnon *et al*., (2012) reported that the effects of plants on health were often attributed to their polyphenolic components.

Two anthocyanidins were detected and the MS data of peak 2 (m/z 271.09) and peak 10 (m/z 271.09) indicated that this compound was identified as pelargonidin and malvidin 3-(6"-p-coumarylglucoside), respectively. In this study, the identification of anthocyanidin in mesocarp was in agreement with published literature that examined the anthocyanidin profile in blueberry, concord grape and red grape (Wu & Prior, 2005). The *B. flabellifer* mesocarp was also found to contain three types of steroidal saponins, which were flabelliferin I, flabelliferin II, and flabelliferin B. The MS data of peak 9 ([M]- at m/z 1061.43) and peak 12 ([M]- at m/z 1030.97) indicated that these compounds were identified as flabelliferin I and flabelliferin II, respectively. An identical molecular mass of 1062 and 1030 was obtained by Jansz *et al.* (2002), which also confirmed the presence of these compounds in the fruit pulp of *B. flabellifer*. Flabelliferin II was found to be a tetraglycoside (M.W. 1030) with a rhumnosyl terminus having two glucosyl and two rhamnosyl residues in its carbohydrate moiety (Jansz *et al.,* 2002).

The MS data of peak 13, 14, 15, and 16 yielded the main product ion relatively at m/z 868, but slightly different MS/MS ions (m/z 721.50, m/z 721.56, m/z 723.01, and 721.48 m/z, respectively). Fragmentation of the $[M]^+$ at *m/z* 868 at Peak 13-16 had one mass fragment ion which was detected by MS/MS analysis at *m/z* 721 (neutral loss of 146 Da) due to the elimination of one molecule of rhamnose moiety. An identical molecular mass of 868 was obtained by Nikawela et al. (1998), which confirmed the presence of flabelliferin B (FB) in the *B. flabellifer* fruit pulp. Flabelliferins also known as Borassoside, could be found in *B. flabellifer* and it usually contained β-glucose as the first sugar moiety attached to the aglycone (Nikawala et al., 2000). This finding has an agreement with the previous studies, which reported that *B. flabellifer* was consisted of steroidal saponins, dioscin, steroidal glycosides (one of them are β-sitosterol 3-O- β-Dglucopyranoside) and three known steroids (one of them are β-sitosterol) (Suthar and Kumar, 2014). In addition, the methanolic extract from the male flowers of *B. flabellifer* contained spirostane-type steroid saponins, borassosides A–F (1–6; Yoshikawa & Pongpiriyadacha, 2007). Even though FB as the major flabelliferin available in Borassus mesocarp, but it could be confirmed from the human and animal studies that the use of FB ointment extracted from *B. flabellifer* on infected wounds has no adverse effect. The application of the ointment on wounds was appeared to be an effective treatment used in the hospital and full-scale clinical trial (Attanayaka *et al.*, 2008).

Table 1 Mass spectrometric data and identification of bioactive compounds in the mesocarp of *B. flabellifer*.

Peak No	t_R	[M]+ (*m/z*)	MS/MS (*m/z*)	Compounds
1	2.20	152.99	108.99, 90.96	Protocatechuic Acid
2	2.40	271.09	241.04	Pelargonidin
3	2.51	153.00	108.98	Protocatechuic Acid
4	2.98	179.01	134.93	Caffeic acid
5	3.15	336.10,	292.17,180.07	5-0-caffeoylshikimic acid
6	3.48	193.02	164.88	Ferulic acid
7	3.50	534	504.16, 396.10	Kaempferol
8	4.11	285.09	270.06	Sakuranetin
9	4.91	1061.43	1038.58	Flabelliferin I
10	5.90	639.28	593.30,431.25	Malvidin 3-(6"-p-coumarylglucoside)
11	6.07	283.65	268.33	Genkwanin
12	6.38	1030.97	869.82, 725.54	Flabelliferin II (F II)
13	6.99	867.52	721.50	Flabelliferin B (F_B)
14	7.00	867.48	721.56, 572.52	Flabelliferin B (F_B)
15	7.25	868.55	723.01	Flabelliferin B (F_B)
16	7.76	867.44	721.48	Flabelliferin B (F_B)

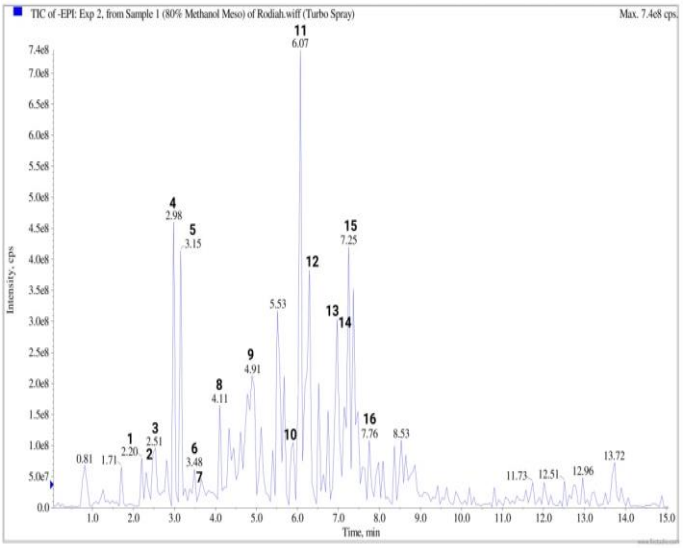


Fig 1 Separation of bioactive compounds in the *B. flabellifer* mesocarp. The number of each spectrum was corresponded to the peak number in Table 1.

The flabelliferin concentration released from mesocarp during pretreatment

It could be seen that the liberation of flabelliferin was significantly increased ($p<0.05$) when pre-treating with sodium salt and sodium hydroxide (Table 2). The highest removal was obtained by pre-treatment in the concentration of 3% for all chemicals used. By increasing all chemical concentrations from 1% to 3%, significant effect on flabelliferin liberation by 1.45 to 1.54-fold was obtained. The pre-treatment with 3% sodium hydroxide yielded the highest total flabelliferin content released during the de-bittering process and this result indicated that plant cell was more vulnerable when using sodium hydroxide compared to sodium chloride or sodium bicarbonate. Similar action was also found in removal of olive bitterness when using the sodium hydroxide treatment, resulting in the softening towards the olive cell walls and texture (Johnson and Mitchell, 2018). Muhammad (2012) also reported that a diluted (2-5%) aquoues solution of sodium hydroxide could enable to remove the bitterness and increase the permeability of the cell wall.

The percentage of residual flabelliferin in debittered mesocarp after pre-treatment

The reduction in bitterness was demonstrated based on the amount of residual flabelliferin retained in the debittered mesocarp. As solution concentration (1%, 2%, and 3%) for all treatments was increased, the removal of flabelliferin was concomitantly increased (Table 2). There was about 34.87% to 46.25% of flabelliferin removal observed when mesocarp sample was treated with 3% solution from each treatment. Significant differences were detected when the concentrations of up to 3% were used for all treatments. The removal of bitterness using 3% of sodium hydroxide was more effective than 3% of sodium chloride, followed by sodium bicarbonate. Sodium hydroxide is an alkaline reagent which is very effective to treat a variety of lignocellulosic feedstocks. It has been reported that *Borassus flabellifer* mesocarp was contained with cellulose (29.03%), hemicelluloses (23.50%) and lignin (10.29%) (unpublish data). Therefore, the use of alkaline solutions such as sodium hydroxide could help in removing of the lignin barrier, reducing the cellulose crystallinity, disrupting of structural linkages and decreasing the polymerization degree of carbohydrates (Xu *et al*., 2010), which would influence the liberation of the flabelliferin from the mesocarp.

Table 2 Flabelliferin content during the chemical pre-treatment and the percentage of residual flabelliferin after the chemical pre-treatment of the *B. flabellifer* mesocarp.

Sample	Flabelliferin content (mg/L)	Percentage of residual flabelliferin (%)
Sodium hydroxide		
1%	60.65^{b}±12.01	68.27^{a} ±6.28
2%	69.90^{b}±5.83	63.43^{a} ±3.05
3%	88.40^{a}±5.80	53.75^{b} ±3.03
Sodium chloride		
1%	43.15^{b}±7.97	77.43^{a} ±4.17
2%	51.40^{b}±3.87	73.11^{a} ±2.02
3%	66.65^{a}±7.97	65.13^{b}±4.17
Sodium bicarbonate		
1%	56.40^{b}±9.88	70.49^{a} ±5.17
2%	61.15^{b}±8.22	68.01^{a} ±4.30
3%	83.15^{a}±7.09	56.50^{b} ±3.71
Control	37.90±7.16	80.17±3.74
Fresh sample (without any treatment)	191.15±17.03	-

Values are the mean of triplicate experiments. $^{a–b}$ Different superscripts between concentrations of chemical for each treatment denote significant differences ($p < 0.05$).

The effect of chemical treatment on the total phenolic content (TPC)

As reported earlier, the highest removal of flabelliferin for all treatments was obtained from the sodium salts and sodium hydroxide at the concentration of 3%. However, the results in Table 3 revealed that all treatments at the concentration of 3% have a reduction of TPC (87.28% to 98.62%) in the debittered mesocarp. The TPC was significantly ($p<0.05$) decreased when sodium hydroxide concentration was increased from 1% to 3% and the TPC in the debittered mesocarp was prominently decreased 72.5 times compared to the fresh sample. Nevertheless, the TPC was decreased by only about 7.86 times for the mesocarp pre-treated with 3% of sodium chloride. Recently, Rashima et al. (2017) also reported that no significant difference ($p>0.05$) was observed in the total phenolic content of the bitter gourd juice either treated with or without the NaCl solution. However, an unfortunate relationship was still existed between TPC of the debittered mesocarp and the level of bitterness based on the result obtained. This might be caused by the action of alkaline treatment that broken the ester bonds cross-linking lignin and xylan (Saifuddin *et al.,* 2013), resulting in the increase in the porosity of mesocarp and the release of the phenolic compound from the sample.

The present finding demonstrated that chemical treatments using sodium hydroxide, sodium chloride and sodium bicarbonate were not feasible and practical for debittering the *B. flabellifer* mesocarp. Since removing flabelliferin to improve its taste would eliminate its potential health benefit, enzymatic treatment to remove the bitterness might be an alternative to improve acceptability while preserving this health-promoting compound. Enzymatic hydrolysis was a possible method to remove the bitterness and could acquire compounds with enhanced biological activities (Kumar, 2010).

Table 3 Total phenolic content of *B. flabellifer* mesocarp during the chemical treatment.

Sample	Total phenolic content (mg GAE/g sample)
Sodium hydroxide	
1%	0.62[a] ±0.05
2%	0.39[b] ±0.06
3%	0.16[c] ±0.06
Sodium chloride	
1%	2.61[a] ±0.42
2%	2.10[ab] ±0.20
3%	1.49[b] ±0.31
Sodium bicarbonate	
1%	1.41[a]±0.17
2%	1.08[b] ±0.10
3%	0.91[b] ±0.04
Control	2.64±0.28
Fresh sample	11.68±0.41

Values are the mean of triplicate experiments. [a–b] Different superscripts between concentrations of chemical for each treatment denote significant differences ($p < 0.05$).

CONCLUSION

Eleven compounds were identified in the *B. flabellifer* mesocarp by HPLC-ESI-MS/MS, which consisted of seven types of phenolics, two types of antocyanidins, and two types of steroidal saponins. Steroidal saponin or Flabelliferin B (FB) was the bitter compound comprised of the most compound detected in the mesocarp and caused the bitterness to this fruit part. The progressive increase in the flabelliferin concentration was released from the mesocarp during pre-treatment and the decrease in the percentage of residual flabelliferin in debittered mesocarp proved that chemical pre-treatment did affect the debittering process. The removal of bitterness using 3% of sodium hydroxide was more effective than 3% of sodium chloride and sodium bicarbonate. However, about 87.28% to 98.62% of the total phenolic content in the mesocarp sample was reduced due to the action of these pre-treatments. This finding revealed that suppressing or removing bitterness with sodium hydroxide, sodium chloride or sodium bicarbonate was not feasible and practical concerning the reduction of the total phenolic content (the health-promoting compound) in the debittered mesocarp. Thus, other mild methods such as enzymatic treatment using naringinase should be selected as the alternative method to remove the bitterness from the *B. flabellifer* mesocarp as to enhance its quality especially the antioxidant content to allow its applications in the food industry as a potential ingredient in food products.

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REFERENCES

Adetunji, A. I., Duodu, K. G., Taylor, J. R. N. 2015. Inactivation of tannins in milled sorghum grain through steeping in dilute NaOH solution. *Food Chemistry*, *175*, 225–232.

Aiyegroro, O. A., Okoh, A. I. 2010. Preliminary phytochemical screening and in vitro antioxidant activities of aqueous extract of Helichrysum Longifolium DC. *BMC Complementary and Alternative Medicine*, *10*(21), 1-8.

Ariyasena, D. D., Jansz, E. R., Jayesekera, S., Abeysekara, A. M. 2000. Inhibitory effect of bitter principle of palmyrah (Borassus Flabellifer L.) fruit pulp on the growth of mice: Evidence using bitter and non-bitter fruit pulp. *Journal of the Science of Food and Agriculture*, *80*, 1763-1766.

Ariyasena, D. D., Jansz, E. R., Abeysekera, A. M. 2001. Some studies directed at increasing the potential use of palmyrah (Borassus flabellifer L) fruit pulp. *Journal of the Science of Food and Agriculture*, *81*(14), 1347–1352.

Attanayaka, K., Mendis, S., Jansz, E., Ekanayake, S., Perera, A. 2008. A pilot study on wound healing using an antibacterial steroidal saponin. *International Journal of Biological and Chemical Sciences*, *2*(3), 299–305.

Castillo, E., Hadi, A. S., Balakrishnan, N., Sarabia, J. M. 2005. *Extreme Value and Related Models with Applications in Engineering and Science*. New Jersey: Wiley.

Coupland, J. N., Hayes, J. E. 2014. Physical approaches to masking bitter taste. *Lessons from Food and Pharmaceuticals*, 2921–2939.

Dagnon, S., Ivanov, I., Bojilov, D., Docheva, M., Statkova, S. 2013. Evaluation of the main polyphenolic compounds in aromatic plants of asteraceae and solanaceae families of bulgarian origin. *Journal of Pharmacognosy and Phytochemistry, 1*, 76–84.

Din, A., Aftab, S., Bukhari, H., Salam, A., Ishfaq, B. 2011. Development of functional and dietetic beverage from bitter gourd. *Food Technology, 13,* 355–360.

Drewnoswki, A. 2001. The science and complexity of bitter taste. *Nutrition Reviews*, *59*(6), 163–169.

Drewnowski, A., Gomez-Carneros, C. 2000. Bitter taste, phytonutrients, and the consumer: A review. *The American Journal of Clinical Nutrition, 72*(6), 1424–1435.

Jansz, E. R., Nikawela, J. K., Gooneratne, J. 1994. Studies on the bitter principle and debittering of Palmyrah fruit pulp. *Journal of the Science of Food and Agriculture*, 65, 185-189.

Jansz, E. R., Nadi T., W., Sumuduni, K. A. V. 2002. A review of the chemistry and biochemistry of seed shoot flour and fruit pulp of the palmyrah palm, *Journal of the National Science Foundation of Sri Lanka, 30*, 61–87.

Johnson, R. L., Mitchell, A. E. 2018. A Review: Reducing phenolics related to bitterness in table olives. *Journal of Food Quality*, 1-12.

Keast, R., Gary Beauchamp, K., Breslin, P. 2001. Suppression of bitterness using sodium salts. CHIMIA International Journal for Chemistry, 55, 441-447.

Krawinkel, M. B., Keding, G. B. 2006. Bitter gourd (*Momordica Charantia*): A dietary approach to hyperglycemia. *Nutrition Reviews, 64*, 331–337.

Kumar, V. V. 2010. Comparative studies on inducers in the production of naringinase from *Aspergillus Niger*MTCC 1344. *African Journal of Biotechnology, 9*(45), 7683–7686.

Ley, J. P. 2008. Masking bitter taste by molecules. Chemosensory Perception, 58–77.

Muhammad S. 2012. *Tropical and Subtropical Fruits: Postharvest Physiology, Processing and Packaging*. USA: John Wiley & Sons.

Nikawala, J. K., Jansz, E. R., Abeysekera A. M., Wijeyaratne, S. C., Gamage, U. C. 1998. Studies on chemistry and bioactivity of the flabelliferins, steroidal saponins from palmyrah *Borassus flabellifer* L.) fruit pulp. *Chemistry in Sri Lanka*, 15(1), 6-7.

Nikawala, J. K., Jansz, E. R., Baeckstrom, P., Abeysekera, A. M., Wijeyaratne, S. C. (2001). Flabelliferins of naringinase debittered palmyrah fruit pulp. *Vidyodaya Journal of Science, 9*, 81-88.

Nur, H., Hayati, F., Hamdan, H. 2007.On the location of different titanium sites in ti-oms-2 and their catalytic role in oxidation of styrene. *Catalysis Communications, 8*, 2007-2011.

Nur, H., Guan, L. C., Endud, S., Hamdan, H. 2004. Quantitative measurement of a mixture of mesophases cubic MCM-48 and hexagonal MCM-41 by ^{13}C CP/MAS NMR. *Materials Letters, 58*, 12-13.

Puri, M., Banerjee, A., Banerjee, U. C. 2005. Optimization of process parameters for the production of naringinase by *Aspergillus niger* MTCC 1344. *Process Biochemistry*, *40*, 195–201.

Rodiah M. H., Jamilah B., Russly A. R., Sharifah Kharidah S. M. 2017., Chemical composition of mesocarp and exocarp from *Borassus flabellifer*, *Proceedings of the International Food Research Conference 2017*, pp 371-375, Faculty of Food Science and Technology, Universiti Putra Malaysia. 25-27 July 2017.

Saifuddin M. N., Refal H., Kumaran P. 2013. Microwave-assisted alkaline pretreatment and microwave assisted enzymatic saccharification of oil palm empty fruit bunch fiber for enhanced fermentable sugar yield. *Journal of Sustainable Bioenergy Systems,* 3, 7-17.

Saravanan, K., Pushpesh, K. M., Girendra, K. G. 2016. Isolation and characterization of procatechuic acid, gallic acid and 10-Octadecenoic acid, methyl ester from methanolic extract. *European Journal of Pharmaceutical and Medical Research, 3*(12), 263–266.

Schutz, K., Persike, M., Carle, R., Schieber, A. 2006. Characterization and quantification of anthocyanins in selected artichoke (*Cynara scolymus L.*) cultivars by HPLC–DAD–ESI–MS. *Analytical and Bioanalytical Chemistry*, 384, 1511-1517.

Soares, N. F. F., Hotchkiss, J. H. 1998. Naringinase immobilization in packing films for reducing naringin concentration in grapefruit juice. *Journal of Food Science, 63*, 61–65.

Steiner, J. E., Glaser, D. Hawilo, M. E., Berridge, K. C. 2001. Comparative expression of hedonic impact: Affective reactions to taste by human infants and other primates. *Neuroscience & Biobehavioral Reviews*, *25*(1), 53–74.

Suthar, S., Kumar, (2014). Evaluation of anti-inflammatory activity of *Borassus flabellifer* root ethanolic extract. *International Journal of Research in Pharmaceutical Sciences*, *5*(3), 27-29.

Wickramasekara, N. T., Jansz, E. R. 2003. The range of steroidal saponins of palmyrah flour: could they contribute to toxic effect on consumers. *Journal of Science, Eastern University Sri Lanka, 3*, 11-18.

Wu, X., Prior, R. L., Nutrition, H. 2005. Systematic Identification and characterization of anthocyanins by HPLC-ESI-MS/MS in common foods in the United State: Fruits and berries. *Journal of Agricultural and Food Chemistry*, *53*, 2589−2599.

Xu, J., Cheng, J. J., Sharma-Shivappa, R. R., Joseph, C. B. 2010. Sodium Hydroxide pretreatment of switchgrass for ethanol production. *Energy Fuels*, 24, 2113–2119.

Yoshikawa, M., Pongpiriyadacha, Y. 2007. Medicinal flowers. XII.1) new spirostane-type steroid saponins with antidiabetogenic activity from *Borassus flabellifer*. *Chemical and Pharmaceutical Bulletin,* 55(2) 308-316.