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Investigating the role of solvent type and microwave selective heating on the extraction of phenolic compounds from cacao (*Theobroma cacao* L.) pod husk

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ABSTRACT

Cacao pod husk (CPH) is a primary waste in the cacao industry that contains favourable natural antioxidants based on phenolic compounds. This study reported an investigation of the effect of extraction parameters to maximise the bioactive yields of CPH extract. The preliminary extraction was focused on high total phenolic content and continued to maximise the total monomeric anthocyanin and antioxidant activity that have the potential to be applied as food additives. The solvent selection and particle size were the key parameters for extraction to reach the maximum phenolic yield (100.4 ± 0.5 mg GAE/g dw). It was confirmed that 50% (v/v) aqueous ethanol was the most appropriate solvent, either based on experimental results or Hansen Solubility parameter prediction. At the same time, the comparison of microwave and conventional heating suggested that Microwave-assisted Extraction was the best method to get high phenolic content due to its selective heating effects. The results showed that the maximum bioactive yields were 0.37 ± 0.0 mg Cy_3GE/g dw of anthocyanin and 3.36 ± 0.02 mg TE/g dw of antioxidants obtained under 50 °C and 5 min extraction time. Gallic acid, catechin, epicatechin, coumaric acid and quercetin were identified in CPH extract using High-Performance Liquid Chromatography.

1. Introduction

Theobroma cacao, known as cacao, is one of Indonesia's primary export commodities, contributing 12.7% of the world's total cocoa production. In the last ten years, Indonesia produced around 683-kilo tonnes of cacao per year (Ministry of Agriculture, 2021). Cacao is usually exported in the form of processed products, such as fermented cacao beans, cocoa butter, cocoa powder, cocoa cake, cocoa paste, chocolate, and other food preparations containing cocoa. During cocoa processing, cacao pod husks (CPH) (Fig. 1) are discarded as a primary waste, accounting for 76–86% of the total weight of

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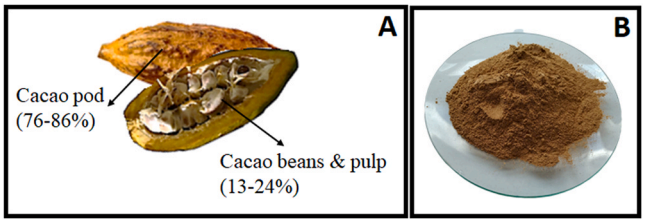

Fig. 1 – CPH Samples: A. Cacao fruit; B. Dried cacao pod husk powder.

cacao fruit. Thus, it can be estimated that approximately ± 540 thousand tonnes of CPH are discarded per year. Only a small portion of this waste is utilised either directly as fertiliser and animal feed or after being processed into products such as soap and biogas. Meanwhile, the rest of the waste is left on the cacao plantation, which can initiate black pod rot disease and foul odour problems. Rather than being wasted, the unexploited CPH has a high potential to be valorised as it contains protein, carbohydrate, lipid, lignocellulosic compounds, pectin, alkaloid, and phenolic compounds, including anthocyanidins and pigment (Campos-Vega et al., 2018; Lecumberri et al., 2007; Nguyen, 2015; Nguyen et al., 2021; Valadez-Carmona et al., 2017; Vriesmann et al., 2012, 2011). Therefore, various studies have provided evidence of the potential for CPH to be valorised as adsorbents (Rachmat et al., 2018; Tsai et al., 2020), bioenergy (Adjin-Tetteh et al., 2018; Kilama et al., 2019), anti-biofouling agent (Wibisono et al., 2021), and food additives: antioxidant (Karim et al., 2014a, 2014b; Martínez et al., 2012; Teboukeu et al., 2018; Valadez-Carmona et al., 2017; Yapo et al., 2013), colourant pigment (Nguyen, 2015), pectin-emulsifying and gelling agents (Vriesmann and Petkowicz, 2013, 2017; Yapo and Koffi, 2013) based on their bioactive compounds.

In recent years, phenolic compounds (polyphenol) have garnered significant attention for their ability to prevent lipid oxidation on food products. For example, the application of phenolic compounds from mango pulp and yerba mate extract as an additive in antioxidant food packaging films for palm oil (Reis et al., 2015), the addition of rosemary extract to hemp seed oil (Moczkowska et al., 2020), chestnut hull extract to beef patties (Zamuz et al., 2018), or CPH extract in palm olein (Teboukeu et al., 2018) to prevent lipid oxidation and extend the shelf life of food products. The phenolic extract from CPH was efficient in delaying palm olein oxidation during heating at 180 °C for 1–7 days of treatment (3 h heating per day) (Teboukeu et al., 2018). Thus, current research suggests that phenolics extracted from biomass have a high potential to be applied as a natural antioxidant. In addition, the demand for antioxidants in 2018 accounted for 18–20% of the global artificial preservative market, where 7.25% was contributed by phenolic compounds (Global Market Insights, 2019). The phenolic antioxidants market was valued at USD 1370.8 million in 2020 and has the potential to increase by 2.9% to reach USD 1725.4 million by the end of 2027.

Nguyen (2015) reported the presence of pigment in CPH but has not mentioned its pigment compounds. Vriesmann et al. (2011) reported that the phenolic compounds in cacao were flavan-3-ols (epicatechin, catechin, and procyanidin) with small amounts of anthocyanin; they were usually accumulated in the outermost layer, such as shells, husks, and skin. Anthocyanins are a class of phenolic compounds promising as natural food colourants with antioxidant activity and low to no toxicity (Khoo et al., 2017). It can be applied as a food colourant in producing purple-coloured jam, yogurt drink, or mixed fruit juice. The importance of anthocyanin attracts our interest to determine the anthocyanin content in phenolic extract from CPH. Based on all previous studies, the extraction of the phenolics that might also be useful for antioxidant and food colourants could provide a viable route on the valorisation of cacao pod waste.

Phenolics, including pigment compounds, could be recovered from biomass using solvent extraction techniques. Therefore, selecting an extraction solvent is critical because the appropriate solvent can dissolve the phenolic compounds without dissolving undesired compounds. Teboukeu et al. (2018) showed that solvent was the most significant factor influencing the phenolic content and antioxidant activity in CPH extraction. To improve solvent selectivity in the extraction process, the Hansen solubility parameter (HSP) can predict the most appropriate solvent to extract the phenolic compounds from CPH. The HSP theory was developed by Hansen (1967) based on "like dissolving like" principle. The more similar the solubility parameters of two materials, the more likely they dissolve each other. Thus, HSP prediction could be used to select the appropriate solvent for phenolic extraction from CPH by solvent extraction method.

Various solvent extraction methods have been reported to extract the phenolic compounds from CPH, as shown in Table 1. As the simplest extraction method, maceration shows a lower yield and longer extraction time than other methods. While sequential conventional solvent extraction (CSE) can achieve a higher yield than maceration, it requires a sequential process and a high solvent loading. Microwave-assisted extraction (MAE) was reported by Nguyen et al. (2020) to obtain ~29% higher phenolic content than conventional extraction. Others reported that MAE performed phenolic extraction in a shorter time, at low temperature, and produced a high yield (Dahmoune et al., 2015; Li et al., 2017; M'hiri et al., 2015).

The literature, therefore, indicates that CPH contains phenolic compounds that may be of value as antioxidants and/or pigments, but there is no report on anthocyanin pigment in CPH extract. Thus, these extracts' phenolics, anthocyanin content, and antioxidant activity will be investigated in this study. Work needs to be done to understand the best process to extract them and the effect of extraction parameters on the extraction yields. Although references indicate that MAE has the potential to extract phenolic compounds, how this interacts with the other system variables to maximise the potential benefits of microwave heating in CPH extraction has not been investigated. To address this challenge, a systematic study of different extraction parameters and the extracts' yields, functionality, and composition is required to understand the extraction of bioactive compounds from CPH. Therefore, our aim is to investigate the effect of extraction parameters on the phenolic extraction from CPH to understand how the processing parameters affect extraction yields and functionality. The objectives are to (1) evaluate the appropriate solvent for selective phenolic extraction based on HSP estimation; (2) investigate the effect of extraction method (conventional and microwave) on the yield and functionality of the extract; and (3) evaluate the effect of extraction parameters: particle size, temperature and time, and solvent concentration on yield and functionality of the extract. Total phenolic content (TPC) and total monomeric anthocyanin (TMA) content are used to assess the extract yields; the antioxidant activity (AOA) is measured to indicate

Table 1 – Summary of solvent extraction for phenolic compounds from CPH.

Extraction methods	Extraction time (min)	TPC*	AOA**	References
Maceration	1440 (24 h)	2.1 mg GAE/g	18.4 μM TE/g	(Martínez et al., 2012)
	60	18.9 ± 1.4 mg GAE/g dw	70.8 ± 14 μM TE/g	(Valadez-Carmona et al., 2017)
CSE	300	98 mg/g dw	NR	(Vriesmann et al., 2011)
	30	47.6 ± 3.2 mgGAE/g extract	77.6% scavenging EC_{50} = 45.3 mg/mL	(Karim et al., 2014a)
	334	150.9 ± 0.5 mg GAE/g extract	97.6%	(Teboukeu et al., 2018)
	90	12.2 mg GAE/g dw	5.8 mg TE/g dw	(Nguyen et al., 2021)
Four sequential different-solvent CSE	320	68.9 ± 5.6 mg GAE/g dw)	85.4 ± 1.6% EC_{50} = 25.0 ± 1.0 g/g	(Yapo et al., 2013a)
Supercritical Fluid Extraction (SFE)	150	13.0 mg GAE/g extract	NR	(Valadez-Carmona et al., 2018)
MAE	5 s microwave radiation and 30 min maceration	11.0 mg GAE/g dw	121.5 mg/g	(Nguyen et al., 2020)

*Folin-ciocalteu method.
**DPPH (2,2-Diphenyl-1-picrylhydrazyl) method.
NR: not reported.

functionality; the composition is determined via High-Performance Liquid Chromatography (HPLC).

2. Materials and methods

2.1. *Plant material*

CPH was collected from Malang, Indonesia, in November 2018. Soon after receiving, CPH (moisture content 86.7% w.b) was then sliced into small size (2–4 cm) and dried at 50 °C in a forced air dryer to final constant weight. The dried CPH sample was prepared in two forms: CPH powder (with size reduction) and CPH chip (without size reduction). To prepare the CPH powder, dried CPH was ground and sequentially sieved onto 150, 125, 90, 63, and 38-micron sieves. The moisture content of dried CPH was determined by drying the CPH in a hot-air oven at 105 °C to a constant weight. The moisture content of dried CPH was 9.25% (w.b).

2.1.1. *Brunauer-Emmett-Teller (BET) surface area analysis of CPH*

The surface area and pore characteristics of CPH were measured by nitrogen adsorption/desorption isotherms at – 195.85 °C using an automated Surface Area and Porosity Analyser (Micromeritics ASAP 2420, USA). Prior to the measurements, 2 g of CPH was degassed at 90 °C for 24 h. The isotherm was measured over the relative pressure range (P/P_0) from 0.001 to 0.998 and back. The nitrogen adsorption/desorption data were analysed using the BET (Brunauer-Emmet-Teller) model for specific surface area (between 0.05 and 0.20 P/P_0). Pore volume and size distribution were calculated using t-plot (Harkins-Jura correction) and BJH (Barrett-Joyner-Halenda, Harkins-Jura correction) models.

2.2. *Solvent extraction procedure*

Approximately 1 g of dried CPH sample was put in a borosilicate flask (Pyrex) and mixed with a solvent. The flask was covered with a rubber stopper. Extractions were carried out for required conditions under stirring at 1200 rpm. Each extract was then separated by vacuum filtration using a paper filter of Whatman no 1 (pore size 11 μm). All extraction experiments and their extract analysis were carried out in triplicate. The detailed conditions for each extraction parameter studied can be seen in Fig. 2 and Table 2. The schematic design of reflux, MAE and CSE can be seen in Section S.1 in Supplementary data.

2.2.1. *Reflux*

The mixture of dried CPH and solvent was loaded into a 250 mL three-neck borosilicate glass round bottom flask. Reflux was carried out for 60 min at the solvent's boiling point.

2.2.2. *Maceration*

The dried CPH was mixed with solvent in an 80 mL-Pyrex TE cavity flask (inner dia. 39 mL, height 70 mm) covered with a rubber stopper. Maceration was carried out for 60 min at 20 °C.

2.2.3. *Microwave-assisted extraction (MAE)*

The MAE was performed using a microwave system of a Miniflow 200SS (Sairem, France) batch reactor at 120 W and 2.45 GHz, as explained in the previous report (Dewi et al., 2021). CPH powder (1 g) and ethanol/water solvent were put in an 80 mL-Pyrex reactor within a TE_{10n} single-mode cavity in WE340 waveguide terminating in a short circuit. The bulk temperature inside the reactor was measured and controlled using a temperature optical fibre. At the bottom of the microwave reactor, an external magnetic stirrer (IKA magnetic stirrer) was placed to stir the CPH sample mixture.

2.2.4. *Conventional solvent extraction (CSE)*

CSE was firstly set up using a water bath (WB) and ethylene glycol bath (EgB) to understand their heating profile. Both bulk heating profiles were then compared to that of MAE. As seen in Fig. 3, CSE using EgB has the same bulk heating profile as MAE. Therefore, the CSE experiment was designed to replicate the MAE conditions by immersing the sample in an 80 mL-Pyrex TE cavity flask (inner dia. 39 mL, height 70 mm) in 120 °C ethylene glycol bath (EgB) until reached the set temperature before transferring it to a water-bath (WB) at the desired temperature. An alcohol thermometer was used to measure the temperature of the sample inside the reactor.

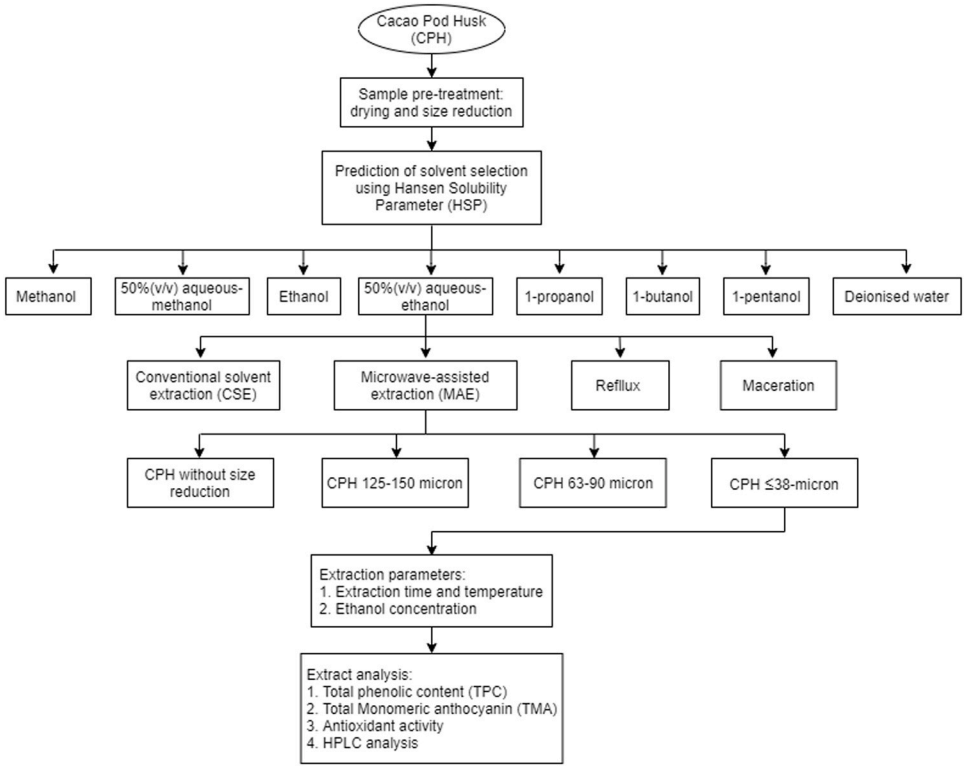

Fig. 2 – Schematic of bioactive extraction from cacao pod husk (CPH).

2.3. *Extract analysis*

2.3.1. *Determination of total phenolic content (TPC)*

The total phenolic content (TPC) of each CPH extract was determined by the colourimetric method using Folin-ciocalteu reagent, according to Galan et al. (2017). Firstly, each CPH extract was diluted using deionised water with a dilution factor of 20. Then, 7.5 mL of ultrapure water (Millipore Mili-Q Plus Water Purifier, France), 0.5 mL of Folin-Ciocalteu reagent 1 N (Sigma Aldrich), and 0.5 mL of the sample were put into a 20 mL bottle and stirred at 300 rpm for 3 min. After mixing, 1.5 mL of sodium carbonate solution 200 g/L (Honeywell Fluka, Germany) was added into the mixture and kept in the dark at room temperature for 1 h. Finally, the absorbance was measured at 760 nm using UV/Vis Spectrophotometer (Cecil CE 1020 Series, UK). The maximum wavelength was chosen based on the spectrum of gallic acid from 300 to 1000 nm, shown in Fig. S.2 Section S.2. Supplementary data. The concentration of the extract was calculated according to a calibration curve of gallic acid standards between 0 and 200 mg/L (Fig. S.3 in Section S.2 Supplementary data). All analysis was carried out in

Table 2 – Conditions for extraction of phenolic compounds from CPH.

Extraction parameter	CPH sample (particle size)	Extraction method	Solvent	Temperature (°C)	Time (min)
Various solvent	≤ 150-micron	Reflux	Methanol, ethanol, 1-propanol, 1-butanol, 1-pentanol, deionised water, 50% (v/v) aqueous-methanol, 50% (v/v) aqueous-ethanol Solvent to feed (S/F) ratio: 40/1 (mL/g)	Boiling point (bp) of solvent	60
Various method	≤ 150-micron	• CSE • MAE • Reflux • Maceration	50% (v/v) aqueous-ethanol S/F ratio: 40/1 (mL/g)	• CSE: 70 °C • MAE: 70 °C • Reflux: bp of solvent • Maceration: 20 °C	60
Particle size	0.5 × 0.5 cm 125–150 µm 63–90 µm ≤ 38-micron	MAE	50% (v/v) aqueous-ethanol S/F ratio: 40/1 (mL/g)	60 °C	30
Extraction time and temperature	≤ 38-micron	MAE	50% (v/v) aqueous-ethanol S/F ratio: 40/1 (mL/g)	50, 60, 70 °C	1, 5, 10, 15, 30
Ethanol concentration	≤ 38-micron	MAE	Deionised water, 10%, 30%, 40%, 50%, 60%, 70%, 90%, and 100% (v/v) ethanol/water	50 °C	5

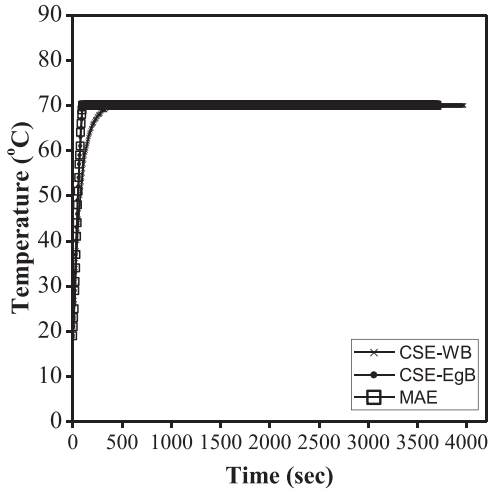

Fig. 3 – Comparison of bulk temperature profile for MAE and CSE experiments at 70 °C for 60 min.

triplicate. The TPC of each extract can be calculated as follows the Eq. (1):

$$TPC = c \times \frac{V_{extract}}{W_{sample}} \quad (1)$$

Where TPC is total phenolic content in mg GAE/g dry weight; c is the concentration of gallic acid equivalent (GAE) in mg/L; V is the volume of extract in litre; W is the weight of dry CPH in gram. The extracts' TPC was expressed as milligram gallic acid equivalent per gram dry weight (mg GAE/ g dw).

2.3.2. *Determination of total monomeric anthocyanin (TMA)*

Total monomeric anthocyanin (TMA) was determined using pH differential method (Lee et al., 2005) with modification. This method is based on the structural change of the anthocyanin chromophore between pH 1.0 and 4.5 at 520 and 700 nm (Fig. S.4 Supplementary data). Monomeric anthocyanins undergo a reversible structural transformation as a function of pH (coloured oxonium form at pH 1.0 and colourless hemiketal form at pH 4.5). A pH 1.0 buffer solution was prepared by dissolving 1.864 g potassium chloride (KCl) (SLS lab, UK) in 960 mL ultrapure water. The pH of the solution was adjusted to pH 1.0 by adding HCl. To prepare a buffer solution of pH 4.5, sodium acetate ($CH_3COONa\ 0.3\ H_2O$) (SLS lab, UK) (32.814 g) was diluted in 960 mL ultrapure water and adjusted to a pH of 4.5 by adding HCl. For analysis of the TMA, each extract (0.5 mL) was dissolved in a 4.5 mL buffer solution and stirred at 300 rpm for 2 min, then kept in the dark for 50 min. Afterwards, the absorbance of each sample was measured by UV/Vis Spectrophotometer (Cecil CE 1020 S, UK) at 520 nm and 700 nm. The difference in absorbance at 520 nm is proportional to the concentration of monomeric anthocyanin; the reason for measuring the absorbance at 700 nm is to correct for haze (Fig. S.5 Supplementary data). All analyses were carried out in triplicate. The difference in absorbance was measured as follows the Eq. (2).

$$A = (A_{520} - A_{700})_{pH\ 1.0} - (A_{520} - A_{700})_{pH\ 4.5} \quad (2)$$

Total monomeric anthocyanin (TMA) can be calculated as follows the Eq. (3):

$$TMA = \frac{A \times MW \times df \times 10^3}{\varepsilon \times l} x \frac{V_{extract}}{W_{sample}} \quad (3)$$

Where, A is difference absorbance of each sample; molecular weight (MW) for Cyd-3-glu = 449.2 g/mol; df is diluting factor (df=10); molar extinction coefficient (ε) of 26,900 L/cm.mol and l is the pathlength in cm; 10^3 is the factor for conversion from g to mg; V is the volume of extract in litre; W is the weight of dry CPH in gram. The TMA of the extracts were expressed as milligram cyanidin-3-glucoside per gram dry weight (mg Cy_3GE/ g dw).

2.3.3. *Determination of antioxidant activity (AOA)*

The antioxidant activity of each phenolic extract was determined using DPPH radical scavenging assay as described by Brand-Williams et al. (1995) with modification. DPPH is a stable free radical with a delocalised electron. When DPPH free radical is mixed with antioxidant, the antioxidant will donate a proton to DPPH free radical (purple colour), thus forming the reduced DPPH-H (yellow colour) (Fig. S.6 Supplementary data). Firstly, 24 mg of 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (Sigma Aldrich, USA) was dissolved in 100 mL of ethanol to prepare a DPPH stock solution. To reach an absorbance of 1.1 ± 0.05 at 517 nm, 25 mL of ethanol was added to 10 mL of DPPH stock solution. The extract (0.1 mL) was added to 3.9 mL of DPPH working solution and then stored in the dark for 30 min. UV/Vis Spectrophotometer (Shimadzu UV-1280, Japan) measured the free radical scavenging activity at 517 nm. The incubation time (30 min) and maximum wavelength (517 nm) were decided based on the preliminary results in Figs. S.7 and S.8 in Supplementary data, respectively. The concentration of the DPPH was calculated according to a calibration curve of Trolox standards between 0 and 120 mg/L (Fig. S.9 Supplementary data). All analyses were carried out in triplicate. The AOA of each extract can be calculated as follows Eq. (4).

$$AOA = \frac{C \times V_{extract}}{W_{sample}(g)}$$
$$C = \frac{(A_{DPPHcontrol} - A_{sample}) - b}{a} \quad (4)$$

Where C is concentration of DPPH with sample in mg/L; V is the volume of extract in litre; W is the weight of dry CPH in gram; $A_{DPPHcontrol}$ is absorbance of DPPH working solution; A_{sample} is the absorbance of DPPH with sample; a and b are slope and intercept of Trolox calibration curve, respectively. The antioxidant activity (AOA) was expressed as mg Trolox equivalent per gram dry weight (mg TE/g dw).

2.3.4. *Identification of individual phenolic compound*

The phenolic compound of the extract was identified using High-Performance Liquid Chromatography (HPLC) as described by Gottumukkala et al. (2014) with modification. Two millilitres of CPH extract were filtered through a 0.2 µm syringe filter (Regenerated Cellulose Membrane, Sartorius Minisart). HPLC analysis was carried out using Agilent HPLC series 1260 Infinity II (Agilent Technologies Co, Ltd, USA) with a variable wavelength detector (HPLC-VWD). Ten-microliters of each sample was individually injected by an automatic injector into a C18 reverse-phase column (Waters Sunfire, 250 ×4.6 mm i.d., 5 µm particle size), which was maintained at 30 °C by a column oven. The analysis was run with a flow rate of 1 mL/min for 50 min using 0.01% orthophosphoric acid in ultrapure water (A) and acetonitrile (B) mobile phases under the following gradient program: 0.01 min 11% B, 30 min 25% B, 35 min 100% B, and finally after 40 min, 11% B phase was reached. The UV detector was set at 280 nm.

Table 3 – Hansen solubility parameter (HSP) of solvents and phenolic compounds (gallic acid).

Solvent	Temp (°C)*	HSP of Solvent ($MPa^{1/2}$)				HSP of Gallic Acid (phenolic compound exemplar standard)				Ra
		δ_d	δ_p	δ_h	δ_t	δ_d	δ_p	δ_h	δ_t	
Methanol	60	18.2	10.8	23.8	31.8	21.4	8.8	22.3	32.1	6.9
Ethanol	72	17.2	8.6	19.1	27.1	21.8	8.8	22.8	32.7	9.9
1-propanol	88	17.2	7.2	16.2	24.7	22.3	8.9	23.6	33.7	12.7
1-butanol	105	17.7	6.6	17.6	25.8	22.8	9.0	24.4	34.6	12.5
1-pentanol	124	18.3	6.1	16.4	25.3	23.4	9.0	25.3	35.6	13.8
Deionised water	93	19.6	13.3	27.5	36.3	22.4	8.9	23.8	33.9	8.0
50% (v/v) aqueous methanol	72	18.8	12.0	25.4	33.8	21.8	8.8	22.8	32.7	7.3
50% (v/v) aqueous ethanol	77	18.2	10.9	23.0	31.3	21.9	8.8	23.1	33	7.7

*Boiling point of the solvent (based on the experiment)

Identification of the phenolic compounds was shown based on the retention time of standard solutions: gallic acid, catechin, (-)-epicatechin, p-coumaric acid, and quercetin (Sigma Aldrich) at concentrations ranging from 50 to 400 µg/mL for quercetin and 20–150 µg/mL for others.

2.4. *Hansen solubility parameter (HSP) prediction*

Hansen solubility parameter (HSP) defines the miscibility behaviour of solvents in three components: δ_d representing the influence of dispersion (related to van der Waals), δ_p representing polar effects (related to dipole moment), and δ_d representing hydrogen bonding contributions (Gao, 2014), where can be expressed by Eq. (5).

$$\delta_t^2 = \delta_d^2 + \delta_p^2 + \delta_h^2 \quad (5)$$

HSP can be predicted using HSPiP Sofware 4.1.07 by entering the SMILES (Simplified Molecular Input Line Entry Syntax) of the target molecular, resulting in the cohesion energy in terms of dispersion, polar, and hydrogen bonding. The cohesive energy is how much energy is required to overcome the intermolecular force between two molecules. The HSP value of several solvents used for extractants and gallic acid (as phenolic compound standard) were predicted using HSPiP, and their values were represented in Table 3.

3. Results and discussion

3.1. *Part I: Screening experiments to identify the best solvent type, extraction method, and CPH particle size*

Screening for phenolic extraction from CPH was assessed based on the TPC yield of extract. Reflux was first chosen as the extraction method because it works with repeatable solvent evaporation and condensation at constant boiling temperature and ambient pressure without losing a large amount of solvent. It is widely used because it is a simple method and more efficient than maceration (Zhang et al., 2018).

3.2. *Phenolic extraction using various solvent*

Selecting an appropriate solvent with high selectivity is crucial in the extraction process because the extraction yield is directly attributed to the solute's solubility in the solvent. Teboukeu et al. (2018) reported that solvent was the most significant factor influencing the phenolic content and antioxidant activity in CPH extraction. Thus, as phenolic compounds are known as polar compounds, the use of deionised water and the following group of alcohols (both as individual species and alcohol mixtures) was initially investigated as solvents to extract the phenolics from CPH: methanol, ethanol, 1-propanol, 1-butanol, and 1-pentanol. It can be assumed that the solubility of phenolic compounds from 1-pentanol to methanol would increase with the increasing alcohol relative polarities, which are 0.568, 0.586, 0.617, 0.654, and 0.762, respectively (Reichardt, 1984).

The experimental results presented in Fig. 4 show that the solubility of phenolic compounds was affected by the polarity of the solvent; it decreased from methanol to 1-pentanol and increased with the addition of water into the pure solvent. Adding water to pure alcohol solvent (ethanol and methanol) to form a 50:50% (v/v) mixture was shown to produce a synergic extraction effect, increasing the TPC yield compared to both the pure alcohol solvent deionised water alone. The maximum TPC was obtained by 50% (v/v) aqueous-ethanol, 72.2 ± 0.47 mg GAE/g dw. The higher solubility of the phenolic in 50% (v/v) aqueous-ethanol was rationalised by using the HSP value in Table 3. Valadez-Carmona et al. (2017) proved that CPH has the following phenolic constituents: 36% catechin, 21% quercetin, 21%

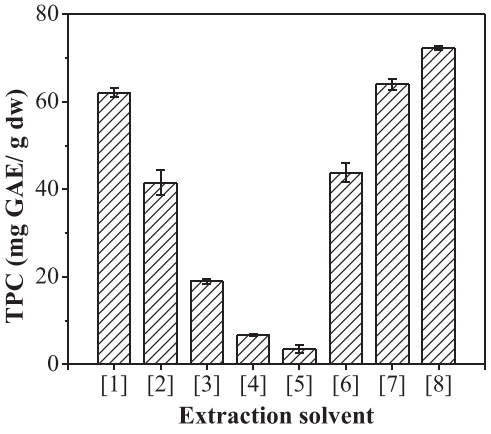

Fig. 4 – Effect of solvent type on TPC yield (Reflux extraction, CPH with particle size ≤150-micron, S/F ratio 40/1 mL/g, stirring rate 1200 rpm, 60 min extraction time): [1] methanol; [2] ethanol; [3] 1-propanol; [4] 1-butanol; [5] 1-pentanol; [6] deionised water; [7] 50% (v/v) aqueous-methanol; and [8] 50% (v/v) aqueous-ethanol solvents; mean ± S.D (n = 9, triplicate extraction and triplicate analysis).

(-)-epicatechin, 11% gallic acid, 7% coumaric acid, and 5% protocatechuic acid. Based on those contents, it can be predicted the polar part (δp) and hydrogen part (δh) of HSP values for the total phenolic compounds in CPH are 10.2 and 20.7. To define if this trend in solvents was predicted by the HSP method, a single component, gallic acid was used as an exemplar material, and the HSP distance between two molecules, conventionally called Ra, was calculated. The Ra is a measure of how alike molecules are and the smaller Ra, the more likely they are to be compatible. It is calculated using Eq. (6), and the results are shown in Table 3.

$$Ra^2 = 4(\delta_{d1}-\delta_{d2})^2 + (\delta_{p1}-\delta_{p2})^2 + (\delta_{h1}-\delta_{h2})^2 \quad (6)$$

Table 3 shows the hydrogen bonding part (δ_h) of gallic acid (as phenolic standard compound) at 77 °C is close to the part of 50% (v/v) aqueous-ethanol. Thus, as the principal intermolecular force in the acidic reagent will be hydrogen bonding, it is likely to have a dominant influence over this extraction process. Thus, the similarity in these values indicated the potential comparability between the solvent system and the reagent. The trends in the calculated Ra values corrected for the temperature at which the extraction was conducted broadly agreed with the extraction yields achieved, with those alcohols/mixtures exhibiting a Ra of 8 or below presenting good extraction yields. Thus, based on the predicted solubility (HSP) and experimental results, the data suggests that 50% (v/v) aqueous-ethanol was the preferred extraction solvent for the subsequent experiments, even though the effect of ethanol/water concentration still needs to be investigated for anthocyanin and antioxidant yields. In addition, ethanol is GRAS (Generally Recognised as Safe) solvent for human consumption and is easily mixed with water; it can also be recovered by reduced pressure distillation.

3.3. *Comparison of various extraction methods*

Choosing an effective extraction method to attain the highest phenolic yield while considering minimal phenolic degradation is also essential. Therefore, MAE and several conventional methods: CSE, reflux, and maceration, were evaluated to extract the phenolic compounds from CPH. Maceration is extraction at room temperature without any heating. It may prevent the phenolic degradation but does not produce a high yield, while reflux is heating the plant material and solvent at the solvent's boiling point, which may degrade the extracted phenolic. Otherwise, conventional solvent extraction (CSE) is extraction heating at the desired temperature, so the phenolic degradation can be prevented by reducing extraction temperature, but it still may also reduce the yield. MAE has been proposed to produce a higher yield than conventional methods (Li et al., 2017; Nguyen et al., 2020) due to its volumetric and selective heating. However, there has not been a systematic comparison of MAE with conventional extraction methods to determine whether it is the selective and/or volumetric heating of microwaves that leads to the observed improved processing outcomes. Volumetric heating can provide rapid heating throughout the material, providing excellent control of temperature profiles and minimising thermal degradation of the target extracts. Selective heating can lead to temperature gradients within the extraction mixture, enhancing mass transfer via Temperature-Induced Diffusion (Mao et al., 2021). Therefore, to understand the volumetric heating of MAE, the bulk heating rate between MAE and CSE was compared, as shown in Fig. 3. The heating time for MAE to reach 70 °C set point was fourfold faster (100 s) than that of CSE-WB (375 s), so any differences in extraction outcomes could be due to volumetric or selective heating. To decouple selective and volumetric heating, the CSE experiments (CSE-EgB) were designed to replicate the bulk heating rate of the MAE experiment; this negates the effect of bulk heating rate and therefore any differences seen between MAE and CSE-EgB experiments can be attributed to selective microwave heating effects.

The extraction methods comparison is presented in Fig. 5 , showing the significant differences in TPC among the four methods. MAE performed the highest TPC (81.61 ± 3.35 mg GAE/g dw) compared with all conventional methods. As explained above, we believe it is because of the volumetric and selective heating on MAE. Compared to CSE using the same heating profile, the TPC yield in MAE increased by 15%; this means the selective heating effect on MAE leads to a rise in the TPC yield. The result was in line with a previous study by Nguyen et al. (2020), representing that MAE yielded ~29% higher TPC than CSE. Galan et al. (2017) also reported that MAE produced 8% higher TPC than CSE on phenolic extraction from sea buckthorn leaves. They found that the plant matrix had been selectively heated at and above 60 °C. In addition, maceration revealed the lowest TPC because there is no heating involved. It was proven that heating enhances extraction rates and yields by increasing diffusion rates and solubility. Therefore, MAE was chosen for the subsequent experiments to maximise the yield on phenolic extraction from CPH.

3.4. *Effect of particle size on TPC yield*

Another essential aspect that must be considered in phenolic extraction is the particle size of biomass. Veggi et al. (2013) stated that finer particle size improves the contact surface area in extraction, that would increase the extraction efficiency. The phenolic compounds are located in the cell wall (Hutzler et al., 1998) and vacuole of plant cells (Ferreres et al., 2011), so we hypothesised that size reduction (grinding) could break the plant cell wall and increase the particle surface area, so the bioactive compounds are more accessible to extract. Therefore, it is interesting to study the effect of particle size of CPH on phenolic extraction.

The results (Fig. 6) showed that reduction in particle size increased the TPC yield and reached a maximum by the smallest particle size (≤ 38-microns), that was 101.8 ± 1.3 mg GAE/g dw. The size reduction is then subjected to the BET analysis to understand the changes in surface area, pore volume, and pore size. These changes due to size reduction can be seen in Table 4. From the data on average pore diameter (19–26 nm) in Table 4, it can be clearly understood that CPH powder is mesoporous material. The adsorption-desorption isotherm graphs shown in Section S.5 in Supplementary data represent close to Type IV behaviour (Fig. S.11 Supplementary data), as expected for mesoporous material. A “knee” point was observed around P/Po 0.01–0.06, yet the “knee” points were not sharp; thus, the isotherm may be in border Type V behaviour (Fig. S.11 Supplementary data) of mesoporous material with weak adsorbent-adsorbate interactions. Monolayer-multilayer adsorption may begin at low relative pressure followed by pore condensation; the

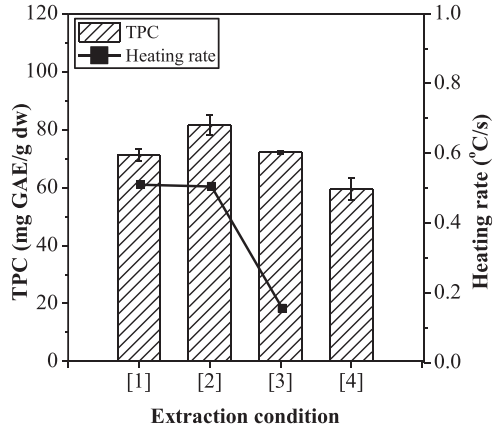

Fig. 5 – Phenolic extraction by using a number of extraction methods (CPH with particle size ≤150-micron, 50% (v/v) aqueous-ethanol, S/F ratio 40/1 mL/g, stirring rate 1200 rpm, 60 min extraction time): [1] CSE-EgB at 70 °C; [2] MAE at 70 °C; [3] Reflux at 77 °C; [4] Maceration at room temperature (20 °C); mean ± S.D (n = 9, triplicate extraction and triplicate analysis).

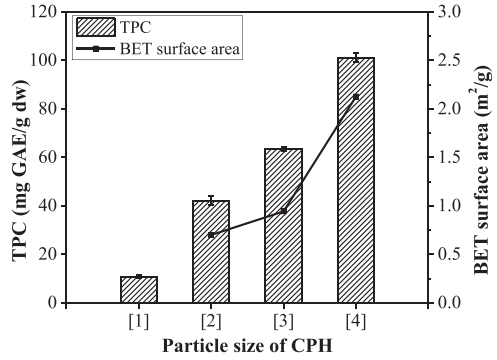

Fig. 6 – Effect of CPH particle size on TPC yield (MAE, 50% (v/v) aqueous-ethanol, S/F ratio 40/1 mL/g, stirring rate 1200 rpm, 30 min extraction time): [1] CPH with size 0.5 × 0.5 cm (without size reduction); [2] CPH with particle size 125–150 µm; [3] CPH with particle size 63–90 µm; [4] CPH with particle size ≤ 38-micron; mean ± S.D (n = 9, triplicate extraction and triplicate analysis).

hysteresis loops observed in the isotherms show the Type H4 (Fig. S.12 Supplementary data) that is often found in micro-mesoporous carbons (Thommes et al., 2015).

Based on data in Table 4, size reduction on CPH from 150-micron to 38-micron has increased the surface area, micropore and mesopore volumes. The surface area and micropore volume have improved 2.5 times from 0.77 to 1.96 m^2/g and 0.22–0.56 mm^3/g, respectively, while the mesopore volume increased twofold from 1.54 to 3.35 mm^3/g. As a result, the TPC yield increased 2.5 times from 40.3 to 101.8 mg GAE/g dw. An increase in the contact surface area could ease the interaction between CPH matrix and extraction solvent; the data suggest that as the particle size decreases, micropore volume and surface area increase, improving the extraction rate of phenolic from CPH and finally increasing the TPC yield. It could be stated that size reduction is also essential for phenolic extraction. Therefore, CPH with particle size ≤ 38-micron was chosen for the following experiments.

3.5. *Part II: maximisation of the extraction process and extract characterisation*

After the screening process, the next step is maximising the extraction process. In Part I, the screening process is based on the TPC value, that is a rough guide to yield phenolic compounds, even though it has been reported that CPH extract has antioxidant activity that can be applied. In addition, it was noticed that CPH contains anthocyanin pigment, yet there was no report about the amount of it in the CPH extract (Nguyen, 2015; Vriesmann et al., 2011). Therefore, in this part, not only the TPC yield would be used as a parameter of extract quality, but also their total monomeric anthocyanin and antioxidant activity were evaluated. Several extraction parameters on MAE: extraction time, temperature and ethanol concentration, were investigated to maximise the extraction yield. The outcomes are intended to be used as helpful inputs into a decision-making process about scaling the process up.

3.6. *Effect of extraction time and temperature*

Solvent extraction relies on a series of mass transfer steps, including penetration of the solvent into the biomass, diffusion through the biomass, solubilisation of target extracts into the solvent, diffusion to the biomass surface, and external transfer to the bulk solution (Flórez et al., 2015). Each step takes time, with diffusion generally considered the rate limited step. However, once solubilised, the target compounds are also subject to degradation. Increasing extraction time increases extract yield and allows more time for degradation of the target extracts. Likewise, elevated temperature increases the rate of the mass transfer steps and increases the solubility and therefore yield, but also expedites degradation of the thermolabile phenolic compounds. It is important to investigate the temperature and time to maximise yields and minimise degradation, and this information can be used to support scale-up design. Fig. 7 shows the effect of extraction temperature and time on the extraction yields of total phenolics and anthocyanin (TPC and TMA) alongside the extracts' antioxidant activity (AOA). In general, extraction yields increased from 1 min and reached a maximum at 5 min. Extending extraction time from 10 to 30 min led to a gradual decrease in yields of the extract and its antioxidant functionality. This is consistent with previous studies, which have observed that long

Table 4 – BET surface area, pore volume of CPH with different particle size.

CPH particle size	BET Surface Area (m^2/g)	Micropore volume (mm^3/g)	Mesopore volume (mm^3/g)	Total pore volume (mm^3/g)	Average pore diameter (4 V/A) (nm)
125–150 µm	0.77 ± 0.07	0.22 ± 0.01	1.54 ± 0.08	5.00 ± 0.53	26.12 ± 5.05
63–90 µm	0.94 ± 0.03	0.26 ± 0.00	1.87 ± 0.10	6.20 ± 0.87	26.30 ± 2.93
≤ 38-micron	1.96 ± 0.25	0.56 ± 0.02	3.35 ± 0.73	9.39 ± 2.61	19.71 ± 3.95

mean ± S.D (n = 2, duplicate analysis)

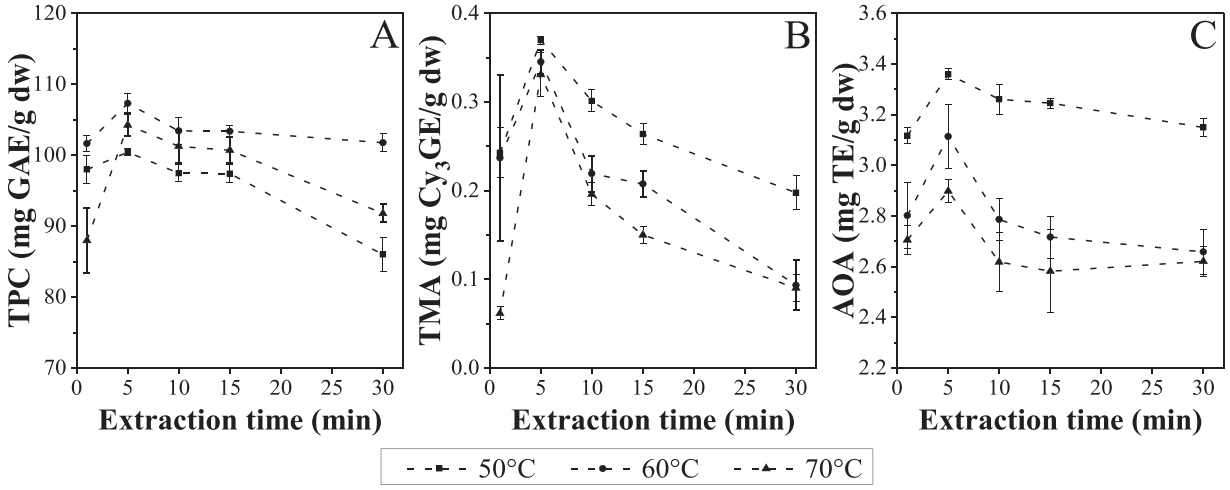

Fig. 7 – Effect of extraction time and temperature on extraction yields (MAE, CPH with particle size ≤38 µm, 50% (v/v) aqueous-ethanol, S/F ratio 40/1 mL/g, stirring rate 1200 rpm): A. Total phenolic content (TPC); B. Total monomeric anthocyanin (TMA); C. Antioxidant activity (AOA); mean ± S.D. (n = 9, triplicate extraction and triplicate analysis).

extraction time at high temperatures could decrease TPC caused by hydrolysation and oxidation of some phenolic compounds (Galan et al., 2017).

On the other hand, the temperature had variable effects on TPC, TMA and AOA. The amount of phenolics (TPC) increased when the temperature increased from 50° to 60°C, then slightly decreased at 70 °C. However, both TMA and AOA decreased with increasing temperature from 50° to 70°C. This indicates that using TPC only to assess extraction efficiency is insufficient. The active compounds appear to be more thermolabile than TPC measurement; the conditions under which the maximum yield of TPC is attained do not necessarily yield the highest quality antioxidant. The increase in temperature may enhance the phenolics' solubility, but at the same time, a high temperature can cause degradation of some extracted phenolic compounds. Liazid et al. (2007) reported that epicatechin and p-coumaric acid in grape skin extract decreased when MAE was conducted above 50 °C; the epicatechin decreased about 4% at 75 °C of MAE process. In addition, Volf et al. (2014) also stated that catechin and gallic acid from grape seed experienced thermal degradation when heated at a temperature of 60, 80 and 100 °C; the degradation rate of catechin was about 13% at 60 °C and increased to 25% at 100 °C. Yu et al. (2016) stated that temperatures above 50 °C in the MAE process resulted in anthocyanin degradation of extracts which the lowest degradation occurred at 53.6 °C. Therefore, some phenolic compounds such as anthocyanin, epicatechin, p-coumaric acid, catechin or other thermolabile phenolics, which are responsible to antioxidant activity, may be degraded above 50 °C. As a result, the TMA and AOA yields decrease.

Overall, the highest amount of TPC was shown at 60 °C (107.3 ± 1.4 mg GAE/g dw), while the highest anthocyanin (TMA) and AOA were reached at 50 °C and all in 5 min (0.370 ± 0.0 mg Cy_3GE/g dw and 3.36 ± 0.02 mg TE/g dw, respectively). Therefore, 5 min extraction time and 50 °C were chosen as the set temperature and extraction time for the next experiments due to high yields (anthocyanin and antioxidant activity) and lower energy considerations. Our results are in accordance with Gharekhani et al. (2012) and Lovrić et al. (2017), who demonstrated that 5 min was the most suitable time for phenolic extraction by MAE.

3.7. *Effect of ethanol concentration*

In Section 3.1, we have reported that ethanol/water mixture is preferable as an extraction solvent for phenolic compounds. Therefore, the effect of ethanol/water concentration is interesting to study. Fig. 8 presents that ethanol concentration significantly affected the extraction yields. Both the TPC and TMA showed a clear maximum at 50% (v/v) aqueous-ethanol solvent, being 100.4 ± 0.5 mg GAE/g dw and 0.37 ± 0.0 mg Cy_3GE/g dw, respectively; decreasing the ethanol concentration led to significant decreases in yields of TPC and TMA. This finding is consistent with the MAE of phenolics from sea buckthorn leaves (Galan et al., 2017), *Eucalyptus camaldulensis Dehn* leaves (Gharekhani et al., 2012), blackthorn flowers (Lovrić et al., 2017), and pomegranate peels (Kaderides et al., 2019), who reported that the appropriate solvent to reach maximum phenolic compounds from the plant was 50% (v/v) aqueous-ethanol. That maximum TMA yields correspond with maximum TPC yields is expected, given that anthocyanins are a class of phenolic compounds.

Compared to TPC and TMA, the antioxidant activity was stable at around 3.3 mg TE/g dw between 0% and 50% ethanol, then increased with increasing ethanol concentration up to 4.6 ± 0.01 mg TE/g dw at 100% ethanol. This was not expected and has not been reported previously in the literature; Chew et al. (2011) reported the behaviour of AOA was correlated with TPC in different ethanol concentrations. TPC and AOA reached a maximum of 60% ethanol, and both decreased up to 100% ethanol. We then expected to see the antioxidant activity correlate with TPC and TMA yields. Following this unexpected result, we hypothesised that there may be one or more compounds that were extracted at high ethanol concentrations that were potent antioxidants. To investigate this hypothesis, we carried out HPLC analysis to identify the individual compounds present in the extracts.

Extraction yields achieved at 50 °C and 5 min using 50% (v/v) aqueous-ethanol were 100.43 ± 0.5 mg GAE/g dw of TPC and 0.37 ± 0.0 mg Cy_3GE/g dw of TMA, with an AOA of 3.36 ± 0.02 mg TE/g dw of AOA (~30.2 µM TE/g; 75.4% scavenging). The individual phenolic compounds in that extract are presented in the HPLC chromatogram in Fig. 9. The

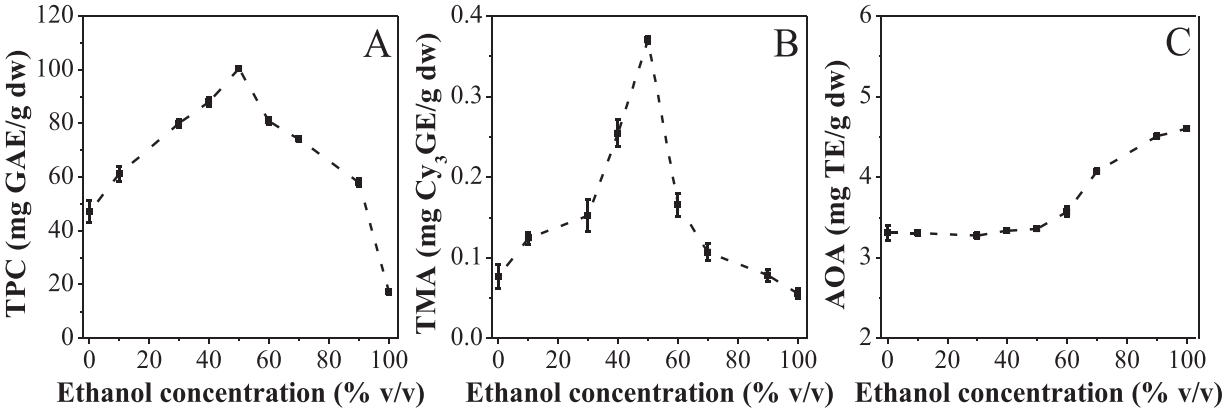

Fig. 8 – Effect of aqueous-ethanol concentration on extraction yield (MAE, CPH with particle size ≤38-micron, S/F ratio 40/1 mL/g, stirring rate 1200 rpm, temperature 50 °C, 5 min extraction time): A. Total phenolic content (TPC); B. Total monomeric anthocyanin (TMA); C. Antioxidant activity (AOA); mean ± S.D. (n = 9, triplicate extraction and triplicate analysis).

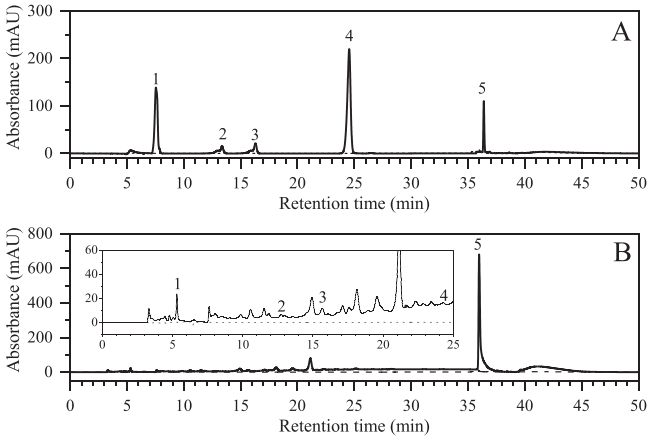

Fig. 9 – HPLC chromatograms of phenolic compounds at 280 nm: A. standard compounds; B. CPH extracts in 50% (v/v) aqueous-ethanol by MAE at 50 °C for 5 min: [1] gallic acid; [2] catechin; [3] (-)-epicatechin; [4] p-coumaric acid, [5] quercetin; the red line () is baseline solvent.

chromatogram shows that the CPH extract contains gallic acid, catechin, (-)-epicatechin, quercetin, and p-coumaric acid at the retention time of 7.54, 13.36, 16.30, 24.55, and 36.38 min, respectively. This data is in accordance with a study by Valadez-Carmona et al. (2017) that reported the presence of gallic acid, catechin, (-)-epicatechin, coumaric acid, quercetin, and protocatechuic acid in CPH extract. Nguyen et al. (2021) also identified some phenolic compounds in CPH extract, such as gallic acid, theobromine, theophylline, (+)-catechin, (-)-epicatechin, (-)-epigallocatechin, and (-)-epigallocatechin gallate. Table 5 shows the comparison of individual phenolic compound concentrations of CPH extract in water, 50% aqueous-ethanol and 100% ethanol; these concentrations were all highest in the 50% (v/v) ethanol/water extract, followed by CPH aqueous extract and alcoholic extract (100% (v/v) ethanol/water). Those results are in accordance with the TPC and TMA yields (Figs. 8A and 8B). Catechin was not found in the alcoholic extract, while p-coumaric acid was detected in all extracts but the concentration was below the concentration standard. The detailed HPLC chromatogram for all extracts and solvents (as baseline) can be seen in Section S.6 (Fig. S.13 and S.14) in Supplementary data while the calibration curve to calculate the amount of individual phenolics are shown in Section S.7 Supplementary data. These findings indicate that CPH is rich in phenolic compounds that have potential as antioxidants. However, some peaks were not identified, including anthocyanin pigment. Likewise, the increase in antioxidant activity with increasing ethanol concentration has not been explained. Therefore, further analysis using more sensitive instruments, such as LCMS, is needed to determine the individual peaks in CPH extract, especially those that are responsible for antioxidant activity.

Our result showed a higher TPC yield compared to previous studies reported by Yapo et al. (2013), Karim et al. (2014a), Valadez-Carmona et al. (2017), Vriesmann et al., (2011) and Nguyen et al. (2021, 2020) even only required shorter extraction time (5 min) and lower extraction time (50 °C). However, its AOA yield was lower than that of their reports. While the anthocyanin yield in CPH extract is relatively low compared to purple corn cob (Lao and Giusti, 2018) and blueberries (Brito et al., 2014; Yuan et al., 2020), it is still higher than that in jamun fruit pulp (Maran et al., 2015). The HPLC represented some bioactive phenolics: gallic acid, catechin, (-) epicatechin, p-coumaric acid and quercetin, but anthocyanin was not detected. The differing amounts of extraction yield between this study and others may be attributed to several factors: (1) different clones of CPH; (2) different extraction solvents may extract different compounds; (3) extraction method and/or conditions affected the different yields.

The work presented in this paper can be used as information for future engineering design in cacao pod valorisation. The use of "greener" solvents such as 50% (v/v) aqueous-ethanol will be preferable for industry; in addition, reduced extraction time (5 min) and lower extraction temperature (50 °C) will also be profitable in industry. However, to scale up the microwave process, the processing conditions must be further identified based on techno-economic considerations. The application of MAE in large-scale extraction industry should consider the high production cost. Galan et al. (2017) stated that scale-up must focus on evaluating the processing conditions that maximise the selective heating as well as offering economic advantages: 1) operating in the temperature range of selective heating; 2) improving the yield by optimising power delivery to plant materials via electromagnetic design based on information from selective

Table 5 – HPLC phenolic compounds profile of CPH extract in different solvents (μg/g dry CPH).

Compound name	Phenolic concentration (μg/g dry CPH)		
	Deionised water	50%ethanol	100%ethanol
Gallic acid	35.9 ± 6.9	76.4 ± 1.6	16.2 ± 1.9
Catechin	27.1 ± 7.9	112.4 ± 1.7	0
(-)-Epicatechin	48.2 ± 5.6	332.0 ± 15.3	16.0 ± 2.5
p-Coumaric acid	nd	nd	nd
Quercetin	3696.7 ± 242.1	15,505.5 ± 253.5	3085.4 ± 426.6
Total	3807.9	16,026.3	3117.6

mean ± S.D (n = 3, triplicate extraction)
nd: not defined

heating mechanism; 3) understanding the penetration depth limitation in the design, and 4) understanding theoretical energy requirements of the system. In addition to information on extraction parameters, therefore, the dielectric properties and penetration depth of solvent and plant material are also essential to designing a proper reactor for more effective extraction. Identification of process parameters to form the basis of design for a scaled up process, thus enabling a techno-economic assessment of MAE to valorise cacao waste for phenolic production, is the focus of our ongoing work.

4. Conclusion

Cacao pod husk reveals antioxidant properties with a promising amount of phenolic and anthocyanin. The extraction solvent and particle size are critical parameters for phenolic extraction from cacao pod husk. Prior to the extraction process, the appropriate solvent can be predicted using Hansen solubility parameters. Comparison of microwave and conventional heating indicates that Microwave-Assisted Extraction (MAE) has greater potential to extract bioactive compounds from CPH due to the volumetric and selective heating. Three MAE parameters, extraction time, temperature and ethanol concentration, significantly affected the phenolic compounds and their functionality. The highest TPC was found at 60 °C, but the highest anthocyanin and antioxidant yields were obtained at 50 °C, which were 0.37 ± 0.0 mg Cy_3GE/g dw of TMA and 3.36 ± 0.02 mg TE/g dw of AOA. However, more research is needed to evaluate the extraction conditions in large-scale experiments and determine their economic effect to enable economically viable production. Gallic acid, catechin, epicatechin, coumaric acid, and quercetin were identified in 50% (v/v) aqueous-ethanol extract. However, further analysis of individual compounds in CPH extract needs to be addressed to identify the other peaks found and the anthocyanin in the extract. Finally, the results presented in this paper are meaningful information for future work in preliminary engineering design and techno-economic assessment for the development of the industrial extraction process of cacao pod husk to obtain the highest extraction yields and lower the production cost. This study will positively impact the environment to reduce CPH waste and produce CPH extracts for possible use as an antioxidant source in the food industry. Our future work will investigate the technical, economic and sustainability aspects of the implementation of Microwave-Assisted Extraction as part of a cacao waste biorefinery.

CRediT authorship contribution statement

Shinta R Dewi: Conceptualisation, Methodology, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Lee A. Stevens**: Methodology, Investigation (BET, TGA), Data curation, Writing – review & editing, Funding acquisition, Supervision. **Amy E. Pearson**: Methodology, Investigation (HPLC). **Rebecca Ferrari**: Conceptualisation, Writing – review & editing, Funding acquisition, Supervision. **Derek J. Irvine**: Conceptualisation, Writing – review & editing, Funding acquisition, Supervision. **Eleanor R. Binner**: Project administration, Funding acquisition, Conceptualisation, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fbp.2022.05.011.

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