Extraction of bioactive compounds from cacao pod husks (CPH)

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ABSTRACT

In Indonesia, approximately 505-kilo tonnes of fresh cacao pod husk (CPH) are discarded annually as a primary waste by-product during cacao production. Although they can be directly applied as fertiliser or animal feed, most of them are left on farms, causing environmental issues that could reduce cacao productivity. In the meantime, CPH contains bioactive compounds, including phenolics and anthocyanins, which have promising antioxidant activity. Therefore, recovering bioactive compounds from CPH by solvent extraction is a promising sustainable way to both avoid waste and valorise it as a new renewable resource. The aim of this research was to study how the processing parameters can maximise the extraction yields and consider process efficiency when designing a flowsheet of CPH valorisation. A systematic extraction research was studied to understand the influence of material pretreatment (drying and size reduction), solvent type, and different heating methods (conventional and microwave) on extraction yields. How other system variables (extraction time and temperature, solvent concentration and solvent-to-feed ratio) interact within conventional and microwave heating was also investigated to maximise yields.

The work presented in this thesis can first demonstrate that CPH contained up to 107.3 mg GAE/g dw of phenolics including 0.37 mg Cy_3GE/g dw of anthocyanin with good antioxidant activity (up to 4.6 mg TE/g dw or ~ 94% radical scavenging). Phenolic and anthocyanin compounds were concentrated in CPH epicarp layer, while antioxidant was found maximum in CPH endocarp layer. Solvent type and material pretreatment (size reduction and drying) were very influential in maximising the extraction yields. Size reduction enhanced the phenolic and anthocyanin yields significantly but had no significant effect on the antioxidant activity of extract. Phenolic compounds, including anthocyanins, were highly extracted in aqueous ethanol (50%

(v/v) ethanol/water) due to their similar solubility based on Hansen Solubility Parameter (HSP) value. However, the highest antioxidant activity was found in the ethanolic extract (100% (v/v) ethanol) due to protic solvent effect. In terms of the heating method, microwave has been regarded as a promising extraction method due to its volumetric and selective heating, which allows for rapid heating and increased yield.

A comparison of MAE and CSE at a similar heating rate (by neglecting microwave volumetric heating) demonstrated no differences in optimum extraction time, solvent concentration and solvent-to-feed (S/F) ratio for both methods but a difference in extraction yields. The best time to extract bioactive compounds was 5 min because a longer extraction time resulted in lower bioactive yields. In contrast, extraction temperature had varying effects: increasing temperature can increase the phenolic yields while decreasing anthocyanin and antioxidant yields. MAE at 60 °C had 5% higher phenolic yield than CSE, which was attributed to a selective heating effect. Meanwhile, extracting anthocyanin and antioxidant compounds was favoured at low temperature (50 °C) to prevent degradation despite no selective heating effect.

Additionally, the CPH solid residue from the extraction process still has a potential to be valorised into other valuable products, such as bio-oil, non-condensed gases and activated carbon, due to its proximate and lignocellulosic contents. This study, therefore, can be used as input data in the preliminary engineering design of CPH valorisation flowsheet to select an efficient process and assess its viability. The usage of CPH as new resource material for the production of high-value products has the potential to increase its economic value while reducing waste.

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LIST OF ABBREVIATIONS AND NOMENCLATURE

Abbreviations

ABTS	2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid
AOA	Antioxidant activity
AP	Absorbed power
ASTM	American society for testing and materials
BET	Brunauer-Emmett-Teller (BET)
CPH	Cacao pod husk
CSE-EgB	Conventional solvent extraction with ethylene glycol bath before transfer to water bath (and hereafter referred to as CSE)
CSE-WB	Conventional solvent extraction with water bath
CT	Condensed tannin
CUPRAC	Cupric Reducing Antioxidant Capacity
DF	Dilution factor
DPPH	1,1-Diphenyl-picrylhydrazyl
DTG	Differential thermogravimetric
DUAE	Direct ultrasound-assisted extraction
FC	Fixed carbon
FP	Forward power
FRAP/ FRP	Ferric reducing antioxidant power / Ferric antioxidant power
FTE	Freeze-thawing extraction
FUTE	Freeze-ultrasonic thawing technology
GRAS	Generally Recognised as Safe
HAE	Heat-assisted extraction
HHV	High heating value
RP-HPLC	Reverse phase – High performance liquid chromatography
HPLC-VWD	HPLC with variable wavelength detector
HbSP	Hildebrand solubility parameter
HSP	Hansen solubility parameter
IUAE	Indirect ultrasound-assisted extraction
LC-MS	Liquid chromatography-Mass spectrometry
MAE	Microwave-assisted extraction

MAHE	Microwave-assisted hydro-distillation extraction
MC	Moisture content
mg AAE/g	Milligram ascorbic acid equivalent per gram sample
mg CE/g dw	Milligram catechin equivalent per gram dry weight
mg Cy_3GE/g dw	Milligram cyanidin-3-glucoside equivalent per gram dry weight sample
mg EE/g dw	Milligram escin equivalent per gram dry weight sample
mg GAE/g dw	Milligram gallic acid equivalent per gram dry weight sample
mg QE/g dw	Milligram quercetin equivalent per gram dry weight sample
mg RE/g extract	Milligram rutin equivalent per gram extract
mg TE/g dw	Milligram Trolox equivalent per gram dry weight sample
μM TE/g	Micromolar Trolox equivalent per gram sample
MUAE	Microwave-ultrasound assisted extraction
OEC	Overall extraction curve
PLE	Pressurised liquid extraction
PTFE	Polytetrafluoroethylene
RP	Reflected power
RSA	Radical scavenging activity
RSM	Response surface methodology
RT	Room temperature
SC	Saponin content
SEM	Scanning electron microscope
S/F	Solvent-to-feed
SMILES	Simplified Molecular Input Line Entry System
SFE	Supercritical fluid extraction
TAC	Total antioxidant capacity
TCC	Total catechin content
TDF	Total dietary fibre
TEAC	Trolox equivalent antioxidant capacity
TFLC	Total flavonol content
TFC	Total flavonoid content
TGA	Thermogravimetric analysis

TID	Temperature-Induced Diffusion
TMA	Total monomeric anthocyanin
TPC	Total phenolic content
UAE	Ultrasound-assisted extraction
VM	Volatile matter

Nomenclature

A	Absorbance
c	Concentration, mg/L
α_{def}	Electric polarizability of molecules
Δ	Delta
Δe	Cohesive energy, cal/mol
Δv	Molar volume, cm^3/mol
ΔH	Heat of vaporisation, cal/mol
δ	Solubility parameter, $cal^{1/2}/cm^{-3/2}$, $Mpa^{1/2}$
δ_t	Solubility parameter total / Hansen solubility parameter, $Mpa^{1/2}$
δ_d	Dispersion part of Hansen solubility parameter, $Mpa^{1/2}$
δ_p	Polar part of Hansen solubility parameter, $Mpa^{1/2}$
δ_h	Hydrogen bonding part of Hansen solubility parameter, $Mpa^{1/2}$
ε	Molar extinction coefficient, L/cm.mol
ε^*	Complex permittivity
ε'	Real part of the complex permittivity (dielectric constant)
ε''	Imaginary part of the complex permittivity (dielectric loss factor)
$tan\ \delta$	Loss tangent (dissipation factor)
Dp	Penetration depth, cm
F	Local electric field strength
G	Weight of sample, gram
λ_o	Free space microwave wavelength, for 2.45 GHz, λ_o = 12.2 cm
λ	Wavelength, nm
I	Intensity
I_o	Initial intensity
l	Path length, cm
MW	Molecular weight, g/mol
π	Pi constant = 3.14
p	Induced dipole moment
P	Partial pressure, Pascal
P_o	Saturated pressure, Pascal
R	Universal gas constant= 1.987 cal/K.mol
S_{BET}	BET surface area, m^2/g

T	Temperature, °C or K
V	Volume, mL
V_m	Molar volume, cm^3/mol

LIST OF PUBLICATIONS AND PRESENTATIONS

Publications:

1. Dewi S.R., Stevens, L., Irvine, D., Ferrari, R., Binner, E., 2021, Microwave-Assisted Extraction of Phenolic from Cacao Pod Husks-an Alternative for Valorisation. Paper Conference in 18th International Conference on Microwave and High-Frequency Applications: AMPERE 2021, Gothenburg, Sweden. https://doi.org/10.5281/zenodo.5645565
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Presentations:

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CHAPTER 1. INTRODUCTION

1.1 Introduction

Cacao (*Theobroma cacao)* is one of Indonesia's main traded commodities, which supplies 12.7% of global cocoa production. Over the past decade, cacao production in Indonesia has reached 683-kilo tonnes per year (Ministry of Agriculture, 2021). Cacao is typically traded as processed products (fermented cacao beans, cocoa butter, cocoa powder, cocoa paste, chocolate and other cocoa-containing food preparations) which only employs the cacao bean and leaves the rest as waste. Cacao pod husks (CPHs), which account for 74% (w/w) of the total weight of cacao fruit, are disposed as a major by-product (L. C. Vriesmann et al., 2011); for every tonne of dry cacao beans produced, ten tonnes of wet CPH are wasted (Campos-Vega et al., 2018). Thus, it is approximated that Indonesia annually produces about approximately 505 thousand tonnes of wet CPH, with little of it being processed into goods, such as soap and biogas, or directly applied as fertiliser or animal feed. Meanwhile, most CPH waste is just left around the cacao farms, which over time can lead to environmental issues, including black pod rot disease and unpleasant odour, reducing cacao productivity.

Instead of being wasted, there is great potential for valorising CPH into more valuable products due to the presence of precious compounds, such as protein, carbohydrate, lipid, fibres (cellulose, hemicellulose, and lignin), pectin, alkaloid, and phenolic compounds, including anthocyanidins and pigment contents (Campos-Vega et al., 2018; Nguyen, 2015; Nguyen et al., 2021; Valadez-Carmona et al., 2017; L. . Vriesmann et al., 2011; Vriesmann et al., 2012). Various studies reported potential CPH for pulp and paper products (Daud et al., 2013), bioenergy (Adjin-Tetteh et al., 2018; Kilama et al., 2019), adsorbent (Rachmat et al., 2018; Tsai et al., 2020), anti-biofouling

agents (Wibisono et al., 2021) and food additives, including antioxidant (Martínez et al., 2012; Teboukeu et al., 2018; Valadez-Carmona et al., 2017; Yapo et al., 2013), colourant (Nguyen, 2015), and pectin-emulsifying and gelling agents (Vriesmann and Petkowicz, 2013, 2017; Yapo and Koffi, 2013).

The capability of phenolic compounds (polyphenols) as antioxidants in preventing lipid oxidation for food products has attracted much interest in recent years. Several following examples showed this antioxidant ability; phenolic compounds derived from mango pulp and yerba mate extract have been successfully tested as an additive in antioxidant food packaging films for palm oil (Reis et al., 2015). In other works, introducing rosemary extract to hemp seed oil could inhibit lipid oxidation and prolong the shelf life of those food products (Moczkowska et al., 2020). While oxidation of palm olein was effectively delayed by the CPH phenolic extract during seven days of heating treatment (3 hours of heating per day) at 180 °C (Teboukeu et al., 2018). That research indicates that phenolic compounds from biomass have a promising possibility for use as a natural antioxidant. Additionally, in 2018, the antioxidants demand in the worldwide market for artificial preservatives was about 18-20%, with phenolic compounds contributing for 7.25% (Global Market Insights, 2019). The market for phenolic-antioxidants was worth at USD 1370.8 million in 2020 and is expected to grow by 2.9% by the end of 2027, reaching USD 1725.4 million.

On the other hand, it has been reported that CPH had pigment content, but the type of pigment compounds was not identified (Nguyen, 2015). According to Vriesmann et al. (2011), flavan-3-ols (epicatechin, catechin, and procyanidin) with small amounts of anthocyanin pigment were present in cacao and usually accumulated in the shells, husks, or skin. Therefore, it is hypothesised that pigment content in CPH extract is in the form of anthocyanin. Anthocyanins are a class of phenolic compounds

with antioxidant activity and low-to-no toxicity that are promising as natural food colourants (Khoo et al., 2017). Anthocyanin can be applied for production of purple-coloured jam, yoghurt drinks, or fruit juice as food colourant and to add antioxidant benefits. The significance of anthocyanins' value has also garnered attention to investigate the anthocyanin content in the phenolic extract from CPH.

Solvent extraction technique is an easy way to recover bioactive compounds, including phenolics and pigment compounds, from biomass. Hence, choosing an appropriate extraction solvent is an essential step to ensuring that all expected compounds dissolve while leaving undesired compounds. According to Teboukeu et al. (2018), solvent's impact on the phenolic content and antioxidant activity was the most significant factor in CPH extraction. Meanwhile, to improve solvent selectivity, the Hansen solubility parameter (HSP) calculation can suggest suitable solvents for phenolic compounds. Charles M. Hansen (1967) developed the HSP theory based on "like dissolving like" principle (Hansen, 1967). When the HSP values of two materials are similar, they are more likely to dissolve one another. Therefore, the HSP value can help choose the proper solvents for phenolic compounds before solvent extraction.

Maceration (Martínez et al., 2012; Valadez-Carmona et al., 2017), sequential conventional solvent extraction (CSE) (Yapo et al., 2013), supercritical fluid extraction (SFE) (Valadez-Carmona et al., 2018), and microwave-assisted extraction (MAE) (Nguyen et al., 2020; Wibisono et al., 2021) have been successfully applied to extract the phenolics from CPH. The basic extraction technique, maceration, yields less and requires more extraction time compared to other techniques. Sequential conventional solvent extraction (CSE) can yield more than maceration, but it involves a sequential process and needs more solvent volume. In comparison to a conventional technique, microwave-assisted extraction (MAE) was found to have a 29% higher phenolic content

(Nguyen et al., 2020). Others also reported that MAE extracted phenolic compounds in less time, at lower temperatures, and with a higher yield (Dahmoune et al., 2015; Galan et al., 2017; Gharekhani et al., 2012; Huma et al., 2018; Kaderides et al., 2019; Li et al., 2017; Pan et al., 2003). However, work still needs to be done to investigate the influence of different extraction methods to obtain maximum yield on CPH extraction.

The literature, therefore, suggests that CPH has phenolic compounds, including anthocyanins pigment, which may be of value as antioxidants and/or food colourants. CPH itself has been reported to consist of three layers: epicarp (outer), mesocarp (middle), and endocarp (inner), and it is believed that phenolic compounds are present in CPH, but these distributions in each CPH layer have never been reported. To answer this challenge, therefore, a systematic work of various extraction parameters that affects the extracts' yields (phenolic and anthocyanin contents) and functionality (antioxidant activity) is studied to comprehend the extraction of bioactive compounds from CPH. Few reports propose that MAE might enhance the phenolic yields, still, it has not been explored how other system variables can maximise the potential benefits of microwave heating in CPH extraction. In addition, to our knowledge, no study reported a direct comparison between conventional and microwave heating on phenolic extraction from CPH. Thereof, how system variables interact within conventional and microwave heating to maximise the yield is also studied.

Finally, to prevent new waste by-products from the bioactive extraction, the CPH solid residue is characterised to assess their proximate contents and pore characteristics. Thus, this study can provide basic information to select potential further processes in employing the CPH solid residue. These end-products are expected to be commercial products which are more valuable, such as bio-oil, non-condensed gases, and activated

carbon so that the CPH waste can be optimally valorised to increase its economic value and reduce its negative impact on the environment.

1.2 Aim and Objectives

This research aimed to study how processing parameters can maximise the extraction yields and consider the process efficiency in the valorisation of cacao pod husk (CPH) as a resource of phenolic-based antioxidants. The aim is developed into four research objectives, as described below. This study will help to design a flowsheet for the CPH valorisation process that would then lead to an ability to assess the viability of the process. The works would also help reduce the environmental impact of CPH and provide beneficial products in the food industry. The study's objectives are:

1. *To investigate the influence of sample pretreatment (drying and size reduction), solvent type, and different extraction methods (conventional and microwave heating) on bioactive yields: total phenolic content (TPC), total monomeric anthocyanin content (TMA), and antioxidant activity (AOA)*. The pretreatment process may affect plant material's bioactive contents; size reduction could improve the accessibility of target compounds but will have an impact on the calculation of required energy and equipment size. While solvent selection as the key factor will be firstly predicted based on Hansen solubility parameter (HSP) to improve the extraction selectivity. Hence, work needs to be done to understand a systematic preliminary study of CPH pretreatment and different extraction conditions that affect the yields and process viability whether this energy-intensive step is required in the flowsheet of CPH valorisation. The proper conditions and the methods selected from comparing four different extraction methods (maceration, reflux, CSE, MAE) are used for the following study (Objective 2).

2. *To evaluate the influence of extraction parameters: extraction time and temperature, solvent extraction, solvent-to-feed (S/F) ratio) within the microwave and conventional extraction methods to maximise the bioactive yields*. Using the knowledge obtained from Objective 1, how other system variables (time, temperature, solvent concentration and S/F ratio) interact within the extraction methods (MAE and CSE) is investigated to maximise the extraction yields and determine the process efficiency. The effect of heating methods (microwave and conventional) on the phenolic and antioxidant yields is compared under similar conditions, including heating rate, to understand the potential benefits of microwave heating over conventional method. The study for Objectives 1-2 will provide information on selecting an efficient process for designing the CPH valorisation process.
3. *To investigate the distribution of bioactive compounds across the three distinct CPH layers (epicarp, mesocarp, and endocarp) under microwave and conventional heating*. To achieve this objective, CPH is separated into three distinct layers (epicarp, mesocarp, endocarp), and bioactive compounds are extracted from each layer. The effect of heating methods (MAE and CSE) and different CPH particle sizes will also be compared under similar conditions (maximum condition obtained from previous work – Objectives 1-2). The extract yields and quality from three CPH layers are then compared to understand the distribution of phenolic compounds between the layers.
4. *To characterise the proximate contents and pore characteristics of CPH solid residue and identify potential applications based on its properties*. To accomplish this objective, the CPH solid residue from the extraction process is characterised using several analytical methods, and the obtained results

(proximate contents, lignocellulose content, BET surface area, pore characteristics, and chemical compositions) are used to consider the further processing application. This study will be part of the CPH valorisation process with order to prevent negative environmental impacts.

1.3 Structure of thesis

This thesis is structured into eight chapters, including the current introductory chapter (Chapter 1), literature review (Chapter 2), and conclusion and future recommendations (Chapter 8).

Chapter 1 gives a general introduction to the main research questions, research aim and objectives.

Chapter 2 provides a literature review behind this research, including a description of cacao pod husk (CPH) and its bioactive compounds, extraction method, extraction parameters, and solubility parameters.

Chapter 3 focuses on the details of experimental methodologies, including material, sample preparation, experimental design, and analytical measurements.

The result and discussion are divided into four chapters to answer the four study objectives described in Section 1.2. Chapter 4 presents the preliminary study of the extraction of bioactive compounds from CPH to achieve Objective 1, including how to sample pretreatment (drying and size reduction), the appropriate solvent and extraction method would influence the bioactive yields (phenolic and anthocyanin contents, and their antioxidant activities). Chapter 5 demonstrates the effect of various extraction parameters: extraction time and temperature, solvent concentration, and solvent-to-feed (S/F) ratio on maximising the bioactive yields, including comparing the MAE and CSE performances; this chapter will meet Objective 2. At the same time, the comparison of bioactive compounds from each layer of CPH (epicarp, mesocarp, endocarp) is

presented in Chapter 6 to answer Objective 3. While addressing Objective 4, Chapter 7 discusses the characterisation of CPH solid before and after extraction.

Chapter 8, finally, explains the general conclusions of this work and recommendations for future study.

CHAPTER 2. LITERATURE REVIEW

Chapter 2 is divided into two major parts, which provide information about cacao pod husk (CPH) and its bioactive compounds (Part I) and extraction of bioactive compounds from CPH (Part II). The first section explains an overview of cacao and CPH, bioactive compounds in CPH and their potential functionality. This study evaluated the extraction of bioactive compounds (phenolic and anthocyanin compounds) from CPH by conventional and microwave techniques. The mechanism of both methods and the influences of extraction parameters on extract yields are discussed in the second section.

Part I: Cacao pod husk (CPH) and its bioactive compounds

Cacao pod husk (CPH) is a major by-product of the cacao industry, containing natural antioxidants derived from phenolic compounds. CPH has been reported to have a high potential to be valorised due to its bioactive compound. The potential of bioactive compounds and their functionality were discussed in this section.

2.1 Cacao

The cacao (*Theobroma cacao* L.) tree originated from Central and South America, and the seeds of its fruit were consumed by Preclassic Mayan in early 600 BC. The discovery of America caused a migration of cacao to other countries. In the mid-1500s, the Dutch brought it to Indonesia, while Spain introduced it to the Philippines and West India in the early 1600s (Vega and Kwik-Uribe, 2012). Cacao, often known as cocoa, is a highly traded commodity in some world's regions. Truth be told, cacao is different from cocoa, and there are lots of misconceptions surrounding the subject. Cacao refers to the raw, untreated beans that are unroasted into powder or nibs, whereas cocoa refers to raw cacao that has been roasted up to 150 °C (Gibson and

Newsham, 2018). To date, almost 94% of the world's total cocoa production is being contributed by the top ten countries (Table 2.1), with Indonesia contributing 12.7% (https://worldpopulationreview.com/country-rankings/cocoa-producing-countries). Indonesia has produced 683-kilo tonnes of cacao per year from 1,658,421 hectares' areas (Ministry of Agriculture, 2021).

Table 2. 1 Production of cacao in 2020 for the top ten countries

Country	Production (tonnes)	% Total
Cote D'Ivoire	2,034,000	39.11
Ghana	883,652	16.99
Indonesia	659,776	12.69
Cameroon	328,263	6.31
Nigeria	295,028	4.53
Brazil	235,809	3.96
Ecuador	205,995	2.34
Peru	121,825	1.67
Dominican Republic	86,599	1.09
Colombia	56,808	1.26

Source: https://worldpopulationreview.com/country-rankings/cocoa-producing-countries

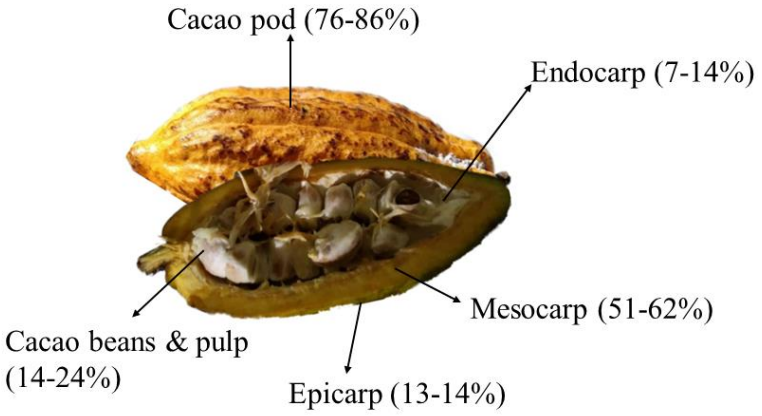


Figure 2. 1 Cacao fruit part (Experimental data from this thesis)

Cacao fruit (Figure 2.1) consists of cacao pod and cacao beans, with only the beans used as raw material in the chocolate industry. Cacao is usually exported as processed products, such as fermented cacao beans, cocoa butter, cocoa powder, cocoa cake, cocoa paste, chocolate and other cocoa-containing food preparations. Cacao beans are commonly processed into some products, such as cocoa powder and chocolate bars. Not only has it many benefits for health because of its bioactive compounds, but cacao

has also been reported to contain phenolic compounds, including flavonoid (epicatechin, catechin, procyanidins, anthocyanins, quercetin, narigenin), non-flavonoid (chlorogenic acid, vanilic acid, coumaric acid, phloretic acid, caffeic acid, ferulic acid, phenylacetic acid, syringic acid, resveratrol, piceid, clovamide), theobromine and caffeine that have strong antioxidant activities (Hernández-Hernández et al., 2018; Kim et al., 2014).

2.2 Cacao pod husk (CPH) and its potential

Cacao pods husk (CPH) (Figure 2.1) is discarded during cacao processing. Every year, approximately approximately 505-kilo tonnes of CPH are discarded in Indonesia, accounting for 74% of fresh pod weight (L. C. Vriesmann et al., 2011). Only a small portion of this waste is utilised either directly as fertiliser (34%) and animal feed (8%) (Picchioni et al., 2020) or after being processed into products such as soap and biogas. The used of CPH as fertiliser gave important roles in carbon (15.6%) and potassium (32.6%) balances in maintaining soil quality, but it only played minor role in maintenance of soil phosphorus (1.1%), calcium (1.5%), and magnesium (2.7%) balances; CPH on the farms will decay and recycle the nutrients into the soil (Picchioni et al., 2020). On the other hand, the remaining CPH waste that left on the cacao plantation will cause environmental problems, such as foul odours and black pod rot, reducing cacao production (Campos-Vega et al., 2018). Untreated CPH left on the farms may cause black pod rot disease due to the presence of *Phytophthora sp*, which can reduce the annually cacao production from 30 to 90% (Lu et al., 2018). This condition will lead to serious issues which need to be addressed. Instead of being wasted, the unexploited CPH has a high potential to be valorised as it contains soluble carbohydrate, protein, lipid, phenolic compounds, and fibre including pectin and lignocellulosic compounds, as reported in Tables 2.2 and 2.3. Total dietary fibre refers

to non-starchy polysaccharides, resistant starch, fructan oligosaccharides, and lignin, that are resistant to hydrolysis by human alimentary enzymes (Yapo et al., 2013).

Table 2. 2 Chemical composition of CPH

Composition	**[1]**	**[2]**	**[3]**	**[4]**	**[5]**
Moisture (%)	8.50 ± 0.06	6.72 ± 0.17	8.45 ± 0.31	5.40 ± 0.31	NR
Total ash (%)	6.70 ± 0.02	8.32 ± 0.07	7.92 ± 0.39	8.40 ± 0.06	7.6 ± 0.30
Carbohydrate (%)	32.30 ± 1.80	28.73 ± 0.11	NR	46.02 ± 0.11	58.6 ± 2.80
Protein (%)	8.60 ± 0.09	4.22 ± 0.07	8.91 ± 0.29	3.21 ± 0.03	8.60 ± 0.98
Fat (%)	1.50 ± 0.13	2.24 ± 0.10	2.27 ± 0.25	7.72 ± 0.01	NR
Total dietary fibre (%)	36.60 ± 0.01	55.99 ± 0.22	59.02 ± 0.54	27.25 ± 0.01	82.1 ± 0.20
Pectin (%)	12.60 ± 0.60	NR	NR	NR	8.30 ± 0.62
Soluble phenolics (%)	4.60 ± 0.04	NR	6.89 ± 0.56	NR	6.20 ± 0.15

NR: not reported. [1] (L. C. Vriesmann et al., 2011); [2] (Martínez et al., 2012); [3](Yapo et al., 2013); [4] (Shodehinde and Abike, 2017); [5] (Muñoz-Almagro et al., 2019)

Table 2. 3 Lignocellulosic contents in CPH

References	**Cellulose (%)**	**Hemicellulose (%)**	**Lignin (%)**
(Daud et al., 2013)	35.4 ± 0.3	37.0 ± 0.5	14.7 ± 0.4
(Laconi and Jayanegara, 2015)	35.3	6.0	38.3
(Marsiglia et al., 2016)	18.4	10.0	12.1
(Nazir et al., 2016)	44.7	11.2	34.8
(Shet et al., 2018)	16.9	4.0	69.0

CPH consist of three layers: *epicarp (outer), mesocarp (middle), and endocarp (inner)* (Figure 2.1). The endocarp is a soft tissue protecting cacao beans in a well-lubricated inner chamber containing 60% of pectic compounds; the mesocarp is a hard-composite structure that covers cacao beans in place, having ±50% bulk crude fibre and cellulose; and the epicarp is the outermost layer with yellow colour (when ripe) indicating the presence of pigment (Campos-Vega et al., 2018). The epicarp is enriched with lignin and contains 30% of pectic substances (Sobamiwa and Longe, 1994) and pigment compounds. The epicarp accumulated high levels of soluble and insoluble proanthocyanidins, about 170 and 8 mg/g dw, respectively (Campos-Vega et al., 2018). The composition of each layer of CPH can be seen in Table 2.4.

Table 2. 4 Composition of pericarp fractions of CPH (g/100 g) (Sobamiwa and Longe, 1994)

Composition	Whole CPH	Epicarp	Mesocarp	Endocarp
Moisture (%)	80.2 ± 1.1	82.8 ± 1.0	64.0 ± 0.9	87.1 ± 0.2
Total ash (%)	9.1 ± 0.3	10.1 ± 0.2	4.6 ± 0.1	6.7 ± 0.2
Protein (%)	5.9 ± 0.4	5.0 ± 0.3	1.9 ± 0.1	6.9 ± 0.2
Crude fat (ether extract) (%)	1.2 ± 0.1	0.8 ± 0.1	0.3 ± 0.0	1.1 ± 0.1
Nitrogen-free (%)	62.2 ± 0.7	66.8 ± 0.7	63.7 ± 0.5	70.0 ± 0.9
Crude fibre (%)	22.6 ± 0.7	17.3 ± 0.8	29.5 ± 0.2	15.3 ± 0.6
Neutral detergent fibre (%)	61.0 ± 0.2	62.0 ± 0.4	80.0 ± 0.5	41.0 ± 0.7
Acid detergent fibre (%)	50.0 ± 0.4	45.0 ± 0.4	70.0 ± 0.3	34.0 ± 0.4
Cellulose (%)	35.0 ± 0.2	30.0 ± 0.2	57.5 ± 0.4	20.8 ± 0.3
Hemicellulose (%)	11.0 ± 0.1	17.0 ± 0.1	10.0 ± 0.1	0.7 ± 0.1
Lignin (%)	14.6 ± 0.2	15.0 ± 0.2	12.0 ± 0.1	13.2 ± 0.1
Pectin (%)	6.1 ± 0.1	5.1 ± 0.1	2.1 ± 0.1	10.5 ± 0.2
Ca (%)	0.32 ± 0.0	0.58 ± 0.0	0.19 ± 0.0	0.13 ± 0.0
K (%)	3.19 ± 0.3	4.61 ± 0.3	1.56 ± 0.2	2.66 ± 0.2
P (%)	0.15 ± 0.0	0.16 ± 0.0	0.06 ±0.0	0.09 ± 0.0
Mg (%)	0.22 ± 0.0	0.39 ± 0.0	0.1 ± 0.0	0.15 ± 0.0
Na (mg/100 g)	0.31 ± 0.03	0.91 ± 0.02	0.6 ± 0.02	0.72 ± 0.02
Zn (mg/100 g)	4.04 ± 0.04	6.49 ± 0.05	2.35 ± 0.03	3.08 ± 0.03
Fe (mg/100 g)	9.01 ± 0.04	19.71 ± 0.06	10.63 ± 0.06	11.24 ± 0.04
Cu (mg/100 g)	0.72 ± 0.05	1.32 ± 0.05	0.56 ± 0.03	0.71 ± 0.03
Mn (mg/100 g)	3.36 ± 0.14	10.32 ± 0.15	2.13 ± 0.12	3.19 ± 0.09

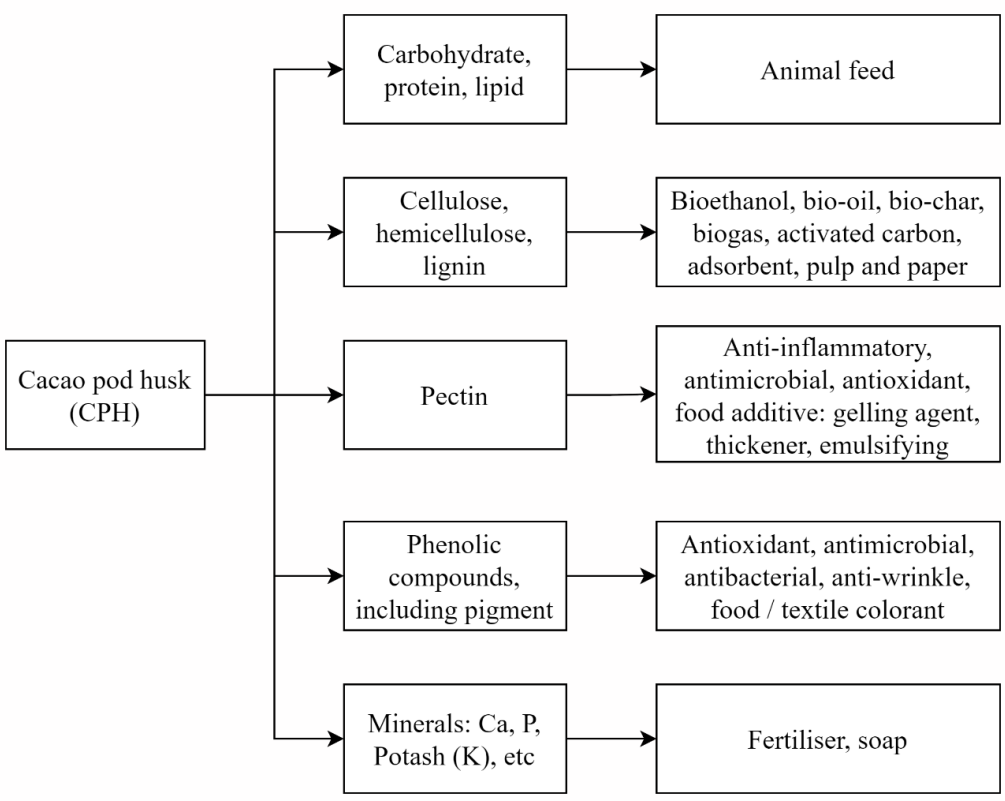


Figure 2. 2 Schematic of potential valorisation of CPH

As regards to those promising CPH compositions, several studies have been reported on the potential valorisation of CPH into some products, as seen in Figure 2.2. CPH has a high potential for use as animal feed due to its carbohydrate and protein contents (Laconi and Jayanegara, 2015; Shodehinde and Abike, 2017; Sobamiwa and Longe, 1994), whereas the mineral compounds have a high possibility for use as fertiliser (Agbeniyi et al., 2011). CPH containing lignocellulosic compounds (cellulose, hemicellulose and lignin) could be pyrolysed to produce bio-oil, bio-char, and non-condensable gas (Adjin-Tetteh et al., 2018). Studies on converting the CPH into activated carbon have been reported by Cruz et al. (2012); Tsai et al. (2020); and Tsai and Huang (2018), and their application as an adsorbent for methylene blue has been investigated (Pua et al., 2013). CPH, on the other hand, indicated a promising as alternative fibre source for pulp and paper making based on its holocellulose (74%) and lignin (7.5-14.7%) contents (Daud et al., 2013).

In the energy sector, Syamsiro et al. (2012) reported CPH as renewable energy by carbonisation at 400 °C for 2 hours resulting in energy with a high heating value of 17.0 MJ/kg. Whilst, Shet et al. (2018) converted CPH into bioethanol and achieved 2 g/L at 2% (v/v) inoculum concentration after 72 hours of fermentation. Another study by Kilama et al. (2019) showed that 9,092 tonnes of CPH had a calorific value of 17.5 MJ/kg dry sample, comparable to wood (18.6 kJ/kg dry sample) and bagasse. CPH was high-potential energy that might generate 41.7 GJ of energy per year in Uganda. Meanwhile, in the food industry, CPH has potential to be applied for food additives such as dietary fibre, colourant pigment, pectin-emulsifying and gelling agents (Nguyen, 2015; Vriesmann and Petkowicz, 2013, 2017; Yapo et al., 2013; Yapo and Koffi, 2013), antibacterial (Wibisono et al., 2021) and antioxidant agents (Karim et al.,

2014a; Lu et al., 2018; Martínez et al., 2012; Nguyen et al., 2021; Teboukeu et al., 2018).

2.3 Bioactive compounds in CPH

The plant contains a wide variety of bioactive compounds, particularly terpenes and phenolics; phenolics are a major group of compounds that contain at least one aromatic ring and one or more hydroxyl groups. Based on their chemical structure, phenolic compounds can be classified into phenolic acids and polyphenols. Phenolic acids have only one phenol subunit, resulting in low molecular weight compounds, while polyphenols are made up of two or more phenol subunits. Phenolic acids are mainly located in plant cell walls (Cavalcanti et al., 2013). The classification of phenolic compounds can be seen in Figure 2.3.

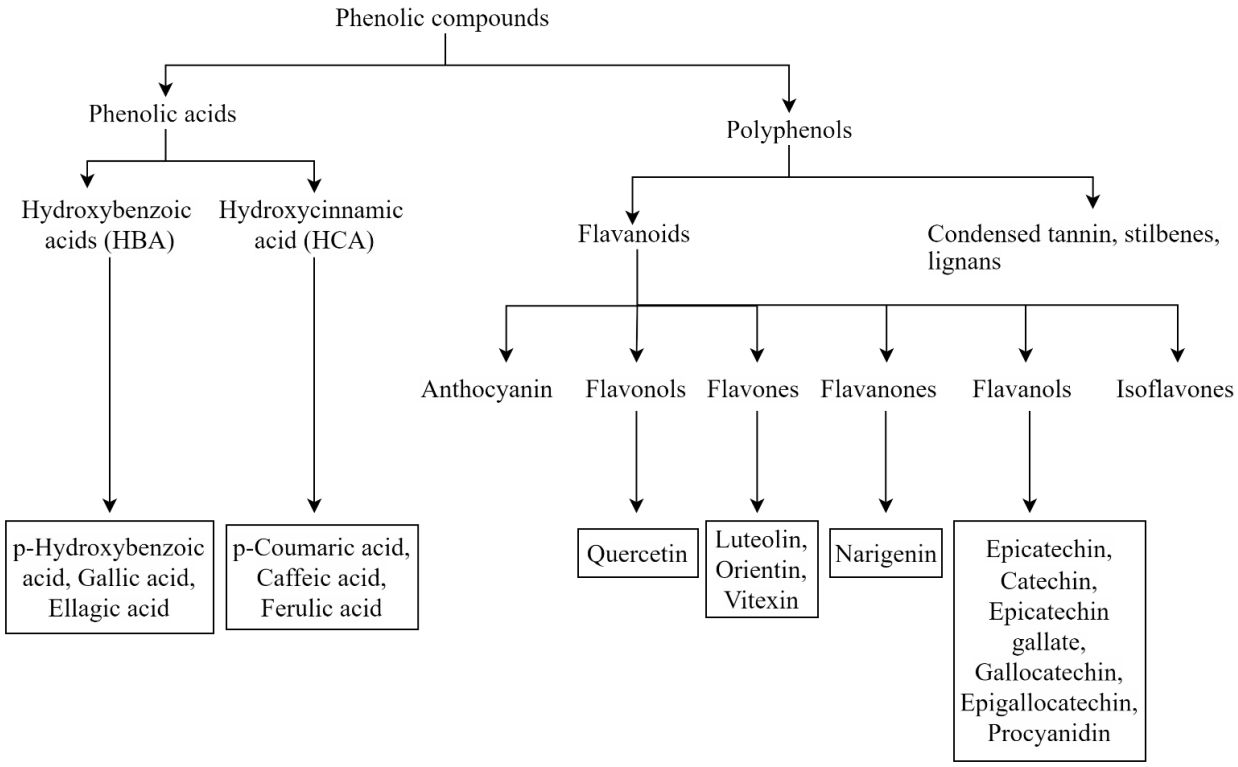


Figure 2. 3 Schematic of phenolic compounds classification (Cavalcanti et al., 2013; Kim et al., 2014)

CPH is a source of pectin, fibre and phenolic compounds (L. C. Vriesmann et al., 2011) that proposes high potential in food application. Pectin can be used as a food additive or functional food, gelling agent, and emulsifier. Pectin from CPH was found to be an effective oil-water emulsifier and emulsion stabiliser without the need for depolymerisation, as well as a moderately effective gelling agent in sugar-acid gels containing 65-75% sucrose (Yapo and Koffi, 2013). CPH-pectin has been proposed by Vriesmann and Petkowicz (2013) as gelling and thickening additive. Additionally, CPH-pectin has been shown to have antimicrobial and antioxidant properties; it had moderate activity against both gram-positive and negative microorganisms: was ineffective against *Listeria spp*. and *Aspergillus niger* but worked best against *E. coli* and *S. aureus* (Adi-Dako et al., 2016). Amorim et al. (2016) also investigated the modified pectin from CPH as an antitumor agent, which may decrease susceptibility to microbial infection.

Both cacao pods and cacao bean shells contained a promising amount of phenolic content with antioxidant activity, whereby antioxidant activity in pods was higher than in shells. This high antioxidant activity in cacao pod might not only be caused by phenolic compounds but also due to the extracted pigment (Karim et al., 2014a). The phenolic compounds in CPH extract were gallic acid, catechin, (-)-epicatechin, quercetin, p-coumaric acid, and protocatechuic acid (Valadez-Carmona et al., 2017), as shown in Table 2.5. Meanwhile, Nguyen et al. (2021) identified seven phytochemical compounds in CPH extracts, including gallic acid, theobromine, theophylline, (+)-catechin, (-)-epicatechin, (-)-epigallocatechin, and (+)-epigallocatechin gallate. The chemical structure of the phenolic compounds in CPH is shown in Figure 2.4.

Table 2. 5 Phenolics profile in CPH extract by HPLC (Valadez-Carmona et al., 2017)

Compound name	Phenolic content(μg/g)	
	Fresh CPH	**Dry CPH (hot air drying)**
Gallic acid	324.7 ± 19.6	973.4 ± 34
Catechin	1024.7 ± 10.6	867.9 ± 28.7
(-)-epicatechin	594.4 ± 55.3	1589.5 ± 54.4
Coumaric acid	186.9 ± 19.6	504.9 ± 0.8
Protocatechuic acid	129.7 ± 0.60	120.8 ± 1.8
Quercetin	601.8 ± 11.2	190.3 ± 1.2
Total	2862.21	4246.8

Nguyen (2015) reported that cacao pod shells also contained pigment compounds, but there was no data on those compounds or contents. On the other hand, Vriesmann et al. (2011) mentioned that cacao had phenolic compounds with small amounts of anthocyanin pigment and flavonol that usually accumulated in the outermost layer (such as shells, husks, and skin) and may be bound to pectin. Another study also stated that catechins or flavan-3-ols (37%), proanthocyanidins (58%), and anthocyanins (4%) in cocoa had good antioxidant activity (Belščak et al., 2009). Thus, it can be expected that the pigment compound in CPH is anthocyanin.

Anthocyanins are a class of phenolic compounds promising as natural food colourants with antioxidant activity and low toxicity (Khoo et al., 2017). Anthocyanins are anthocyanidins with sugar groups, most of which are 3-glucoside. The sugar groups are typically attached to anthocyanidins via 3- or 5-hydroxyl positions (Cavalcanti et al., 2013), and so there are six common anthocyanidins: pelargonidin, cyanidin, peonidin, delphinidin, and malvidin, with cyanidin-3-glucoside being the most common in nature and used to calculate total monomeric anthocyanin content (Lee et al., 2005). The basic structure of anthocyanin pigment can be seen in Figure 2.5. Anthocyanin can be applied as a food colourant in producing purple-coloured jam, beverages, yoghurt drinks or mixed fruit juice. According to those previous studies, thus, the extraction of

the phenolics-antioxidant, including anthocyanin, could provide a viable route for valorisation of cacao pod waste.

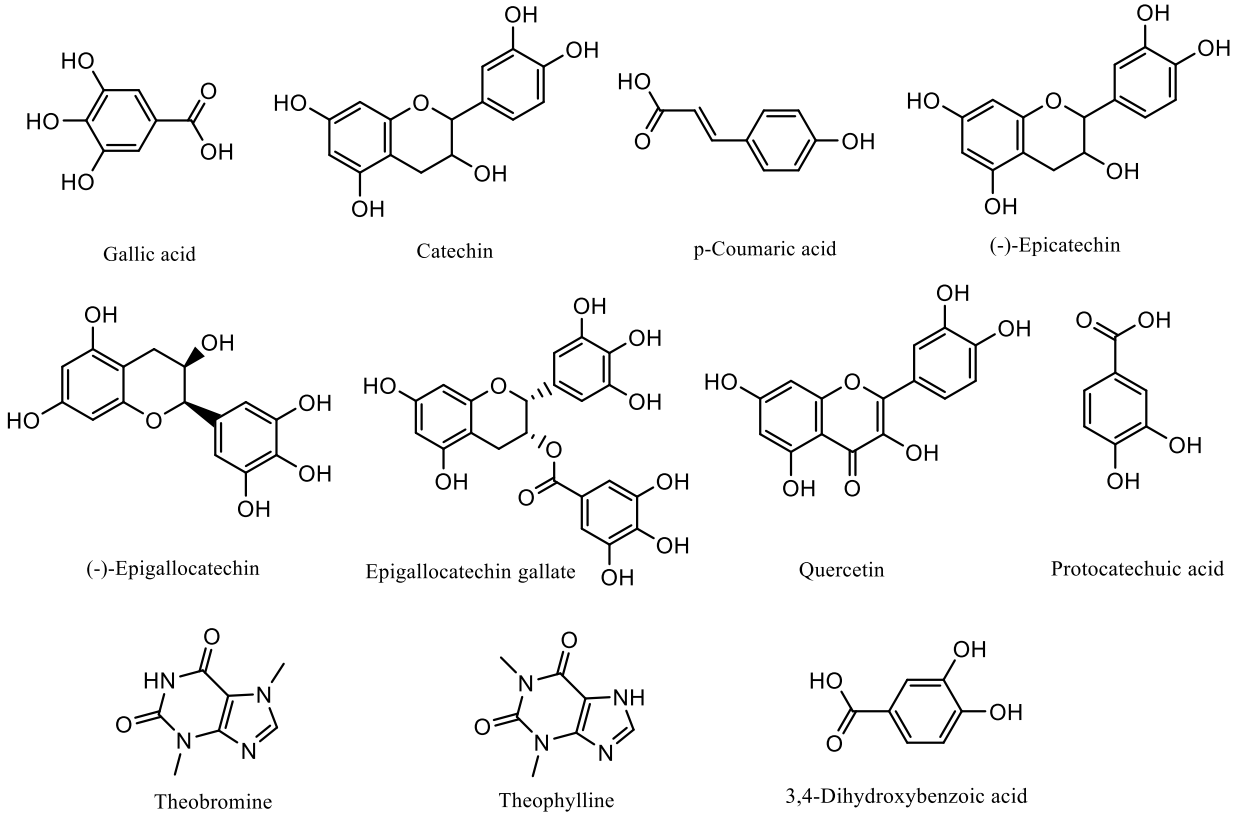
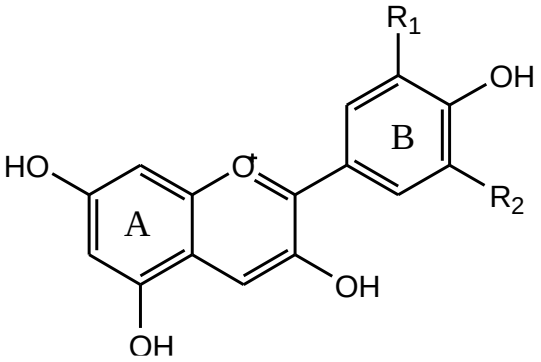


Figure 2. 4 Chemical structure of phenolic compounds in CPH



	R_1	R_2
Pelargonidin	H	H
Cyanidin	OH	H
Peonidin	OCH_3	H
Delphinidin	OH	OH
Petunidin	OCH_3	OH
Malvidin	OCH_3	OCH_3

Figure 2. 5 Basic structure of anthocyanin pigment

2.4 Antioxidant activity of bioactive compounds and their potential application

Antioxidants act as free radical scavengers, donating a proton to neutralise harmful free radicals. Free radicals are atoms or molecules having unpaired electrons, such as hydroxyl radical (˙OH), superoxide radical ($O_2^{\cdot-}$) and hydroperoxyl radical ($HO^{\cdot}_2$). Free radicals can cause cell stress which initiates cancer by oxidising the DNA of cellular macromolecules in our bodies (Hangun-balkir and Mckenney, 2012; Yapo et al., 2013), or they cause lipid oxidation to produce harmful peroxide in food products (Teboukeu et al., 2018). Phenolic compounds have been reported to have antioxidant activity because of protonated carboxyl (-COOH) groups and secondary alcohol (-OH). These groups have the potential to neutralise oxidants or free radicals by acting as proton (hydrogen) donors (Yapo et al., 2013). The reaction between the phenolic compounds and antioxidants is presented in Figure 2.6.

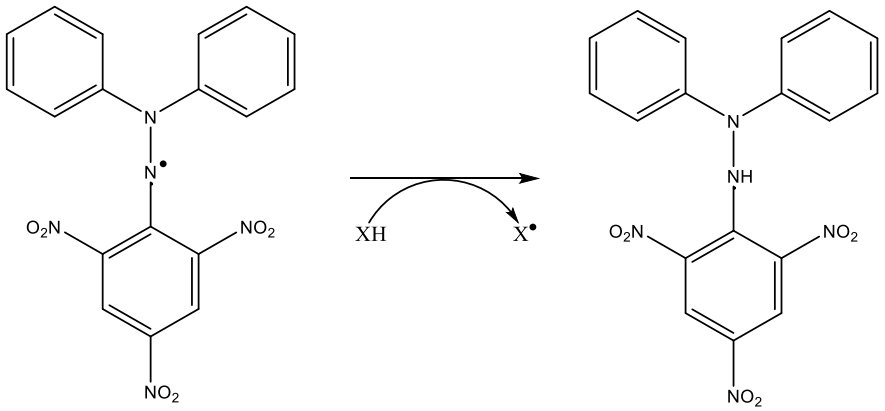


Figure 2. 6 Reaction of DPPH and antioxidant (oxidant scavenging)

Some studies reported the application of phenolic compounds as an antioxidant in the food industry. The application of phenolic compounds from various biomass to prevent lipid oxidation and extend the shelf life of products has been reported; for instance, the use 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 phenolic compounds from peanut skin extract in soybean oil (Franco et al., 2018), CPH extract to palm olein (Teboukeu et al., 2018) or rosemary extract to hemp seed oil (Moczkowska et al., 2020). Mango pulp and yerba extract were added into a cassava starch to produce antioxidants bio-based film for palm oil packaging; the palm oil packaged (stored for 90 days) demonstrated a decreased oxidative rate, which films containing a high concentration of mango pulp and yerba mate extract enhanced palm oil stability (Reis et al., 2015). In another case, the phenolic extract from CPH was efficient in delaying palm olein oxidation during heating at 180 °C for 1-7 days (3 hours of heating per day) of treatment (Teboukeu et al., 2018).

Some studies reported that CPH extracts have a high potential for phenolic compounds, which act as an antioxidant (Karim et al., 2014a; Martínez et al., 2012; Teboukeu et al., 2018; Valadez-Carmona et al., 2017; Yapo et al., 2013). Antioxidants in CPH extract can prevent lipid oxidation in oil products (Teboukeu et al., 2018) and neutralise harmful free radicals by donating a proton to its free radical (Yapo et al., 2013). Martínez et al. (2012) found that CPH extract contained ~3.7 mg GAE/g sample that had the ability to act as antioxidant-dietary fibre with the activity of 33.9 µM TE/g, whereas Yapo et al. (2013) reported the phenolic content in CPH extract was 69 mg GAE/g with antioxidant capacity of 85 % scavenging.

The amount of phenolic and flavonoid content in CPH was higher antioxidant activity compared to cocoa shells (Karim et al., 2014a), which was promising as raw material for cosmeceutical applications (Karim et al., 2014b). CPH extract contained carboxylic acid (citric acid and malic acid), phenolic acid, fatty acid, kaempferol, flavones, resveratrol, and terpenoid, kaempferol and resveratrol were suggested to inhibit elastase and collagenase in reducing wrinkles. In addition, the flavones

derivatives in CPH extract showed UVB sunscreen effect, which was better than commercially UV-protection agents such as avobenzone and octyl methoxycinnamate (Karim et al., 2014b). The use of CPH extract as antioxidant activity was reported for skin treatments such as anti-wrinkle, skin whitening, and soap (Abdul Karim et al., 2016; Gyedu-Akoto et al., 2015; Karim et al., 2014b). Therefore, it is fascinating to recover the phenolic compounds from CPH due to their antioxidant properties, which are very promising for industrial applications.

Part II: Extraction of plant materials

The term "extraction" literally means pulling something out (ex: out, traction: the action of pulling). However, extraction is defined as a separation process based on solubility differences. A solvent is used to dissolve and separate a solute (target compound) from other materials that are less soluble in the solvent (Berk, 2009). In extraction, there are two common procedures: 1) Solid-liquid extraction, which is an extraction of a solute or analyte from a solid material using a solvent, and 2) Liquid-liquid extraction, which involves separating a solute or analyte from a solution in a certain solvent with another immiscible solvent.

2.5 Fundamental extraction of the bioactive compounds from plant material /biomass

Solid-liquid extraction, also known as solvent extraction, is a method to get bioactive compounds from plants. Solid-liquid extraction is a separation process in which a liquid solvent dissolves target compounds from a solid matrix, forming a solution (Chanioti et al., 2014). A stage of solid-liquid extraction can be seen in Figure 2.7.

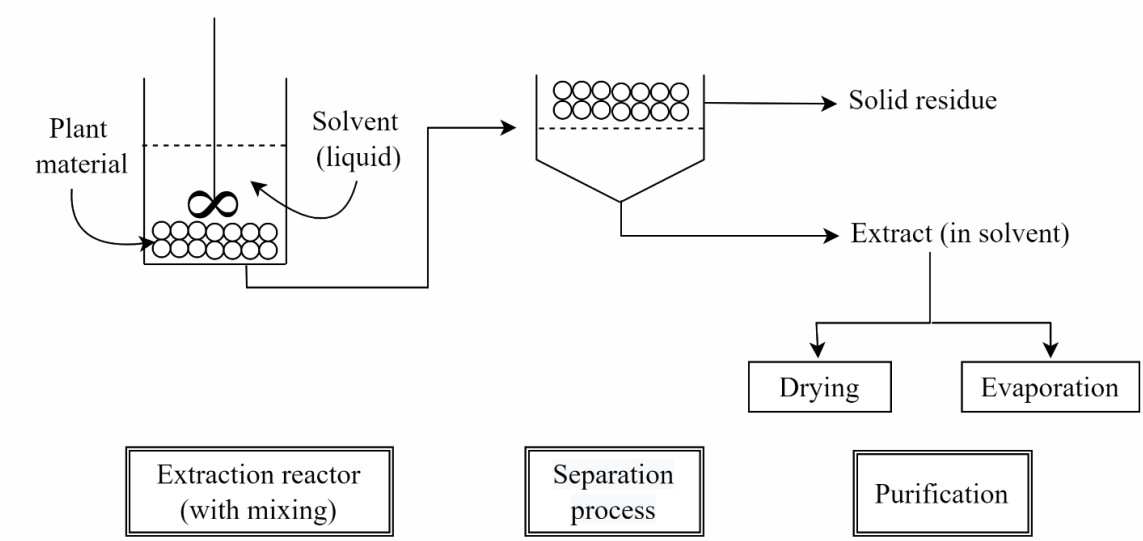


Figure 2. 7 Solid-liquid extraction process

The solvent extraction process involves a series of steps: (1) solvent pervades the solid surface after being transferred from the fluid phase; (2) penetration of the solvent into the solid matrix (plant) by molecular diffusion; (3) breakdown of the chemical bonds of extractive compounds from solid matrix and solubilisation of the soluble compounds (solute); (4) transport of solution containing solute out of the solid matrix (plant) by molecular diffusion; (5) migration of extracted compounds from the external surface of solid into the bulk solution due to natural or forced convection (Palma et al., 2013). The solvent used should be able to dissolve (appropriate properties) the target compounds from insoluble permeable solid and transfer the soluble compounds (solute) to solvent; thus, solvent selection is the critical point to maximise the extraction yield.

The main objective of the extraction process is to achieve high extraction yield, high extract purity, and high extract sensitivity that allows extract to be analysed using instruments with low detection limits. These properties are important to consider in lab-scale experiments, whereas on an industrial scale, the emphasis is on extraction yield, extract purity, process efficiency, and sustainability (Palma et al., 2013). Therefore, several extraction factors that influence them must be controlled to enhance the process efficiency, as explained in Section 2.12.

2.6 Conventional solvent extraction (CSE)

In conventional solvent extraction (CSE), heat is transferred into materials by convection, conduction, and radiation via the external surface of the material due to the thermal gradients (Veggi et al., 2013). Various extraction methods have been studied for obtaining bioactive compounds. There is no standard extraction method as each one provides benefits and drawbacks, but the most widely used conventional techniques are maceration, reflux extraction, Soxhlet, and conventional solvent heating. The extraction method is usually selected based on the raw material properties (such as thermolabile) or process conditions such as temperature, pressure, or solvent type; the use of heat and stirring may enhance the extraction kinetics and increase the extraction yield. Agitation facilitates the dispersion of the solid particles in the solvent, making them easier to interact with; it accelerates the diffusion of the extracted compounds and avoids supersaturation near the solid surface (Palma et al., 2013; Selvamuthukumaran and Shi, 2017).

Maceration is a simple procedure that involves soaking samples in the appropriate solvent with constant agitation at room temperature for several hours or even days, followed by separation methods such as filtration, decantation or clarification to separate extract from solid material (Alara et al., 2021). Extraction by maceration is easy and simple because it only requires simple equipment, such as Erlenmeyer or bottle vial and magnetic stirrer, as shown in Figure 2.8.A. Maceration is low capital investment, low temperature and easy-to-operate equipment compared to other conventional extraction techniques; thus, it is widely used to extract bioactive compounds from plants (Garcia-Vaquero et al., 2020).

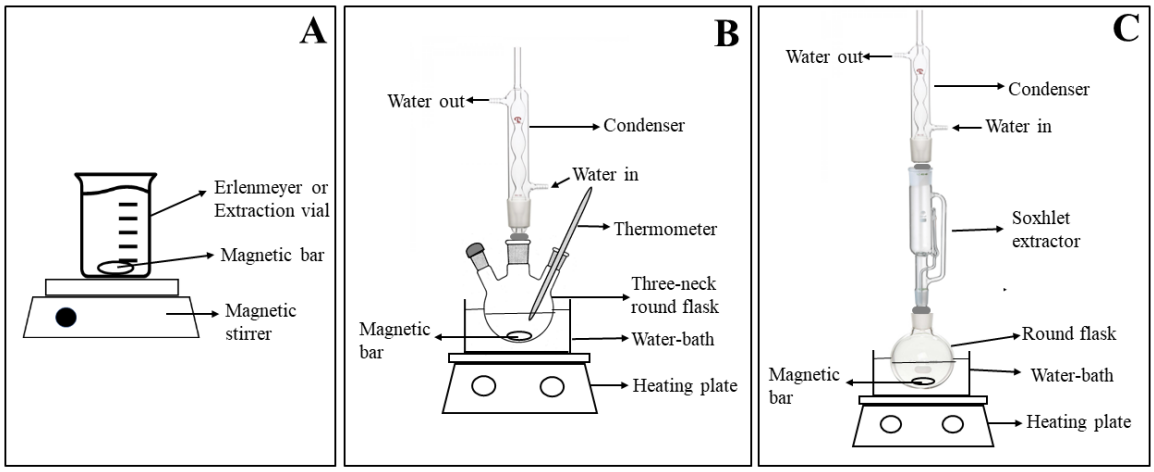


Figure 2. 8 Pictorial representation of conventional extraction techniques: (A) Maceration; (B) Reflux; (C) Soxhlet

Some reports showed the application of maceration to extract the phenolic compounds from *Eucalyptus camaldulensis* Dehn leaves (Gharekhani et al., 2012), *Gordonia axillaris* fruit (Li et al., 2017), purple corn cob (Lao and Giusti, 2018), and propolis (Oroian et al., 2020). They showed that maceration successfully extracted phenolics from plants. Still, it presented several drawbacks compared with other methods, such as a required long extraction time (20-24 hours) and a lower yield (Li et al., 2017; Oroian et al., 2020). In addition, maceration has also been reported to have disadvantages: 1) requires high solvent loading; 2) low mass transfer rate, which causes time-consuming (Garcia-Vaquero et al., 2020; Palma et al., 2013), which makes inefficient extraction process to produce higher yield (Daso and Okonkwo, 2015). However, maceration was good for recovering thermolabile compounds which are not resistant to heating, such as anthocyanin (Lao and Giusti, 2018). A summary of the maceration technique to extract the bioactive compounds from plants can be found in Table 2.6.

Reflux 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). The reflux system consists of a borosilicate round flask that connects to a condenser. A mixture of sample and solvent is put in the round flask and heated at the solvent's boiling point (Alonso-Carrillo et al., 2017). When the solvent evaporates and rises to the top, it will meet the condenser and re-condense, so the solvent is always in the flask. Agitation can be applied in reflux extraction to enhance the interaction between plant material and solvent. The reflux design is presented in Figure 2.8.B.

Some studies reported the use of reflux in phenolic extraction. Wong-Paz et al. (2015) studied the significant effect of extraction time and ethanol concentration on extracting the phenolics and antioxidants from the semiarid Mexican region plants (*J. dioica, F. cernua, E. camaldulensis, T. diffusa*) by using heat-reflux system; the use of 35% aqueous ethanol showed a maximum TPC yield compared to water and 80% aqueous ethanol. While optimisation of reflux condition was reported on Pandan (Ghasemzadeh and Jaafar, 2014) and *Pleioblastus amarus (Keng*) Shell (Ma et al., 2022) extractions to enhance the phenolics and antioxidant capacity. However, extraction of bioactive compounds using reflux seems to require a longer extraction time, which was about 1-2 hours. Thus, a combination of reflux extraction with other extraction methods can be a solution to speed up the processing time and may enhance the extraction yield. The combination of reflux with microwave-assisted extraction (MAHE) could increase the extraction yield twofold in a shorter time (30 min), as reported by Alara and Abdurahman (2019) in *Vernonia cinerea* leaf extraction. Detailed extraction conditions and yields of that literature are shown in Table 2.6.

In 1879, the Soxhlet apparatus was designed by Franz von Soxhlet, a German scientist; it is used as a reference method for evaluating the performance of novel solvent extraction methods due to its simplicity and low cost (Palma et al., 2013). Soxhlet uses the principle of reflux and siphoning to continuously extract the plant

material using fresh solvent (Zhang et al., 2018). Soxhlet extraction is based on transferring the target compounds from solid matrix to an appropriate solvent with continuous contact between solvent and sample during the heating process (Daso and Okonkwo, 2015). The Soxhlet apparatus generally consists of a round boiling flask, thimble (sample holder), glass extraction chamber (siphon), and condenser (Figure 2.8.C). Plant material should be put in the thimble, which is placed in the glass extraction chamber. This chamber is connected with a round flask containing solvent on the bottom and a condenser on top. By heating, the solvent will evaporate and goes to the condenser. The condensate dripped into a reservoir containing a thimble with the solid sample. When the solvent level reached the siphon, it was refluxed back into the round flask. The process repeatedly runs for desired time (Alara and Abdurahman, 2019). During Soxhlet extraction, fresh solvent can flow continuously, and the heat effect can be retained on the sample, so there is no need for a filtration process to separate the solid residue.

The application of Soxhlet extraction in recovering bioactive compounds was reported by Alara et al. (2018); Alara and Abdurahman (2019). They successfully extracted the phenolic and flavanoids from *V. cinerea* leaves by the Soxhlet method and showed that Soxhlet was significantly affected by the extraction parameters: extraction time, solvent-to-feed ratio and ethanol concentration. According to the reference, extraction using Soxhlet required a long extraction time (2 hours) and still produced a lower extraction yield than the MAHE technique. Garcia-Vaquero et al. (2020) also gave the statement that the Soxhlet method is time-consuming, needs a large number of solvents, cannot be accelerated by using agitation, and thermolabile extracted compounds are easy to degrade. However, this method achieves high

extraction efficiency with less time and solvent consumption compared to maceration (Zhang et al., 2018).

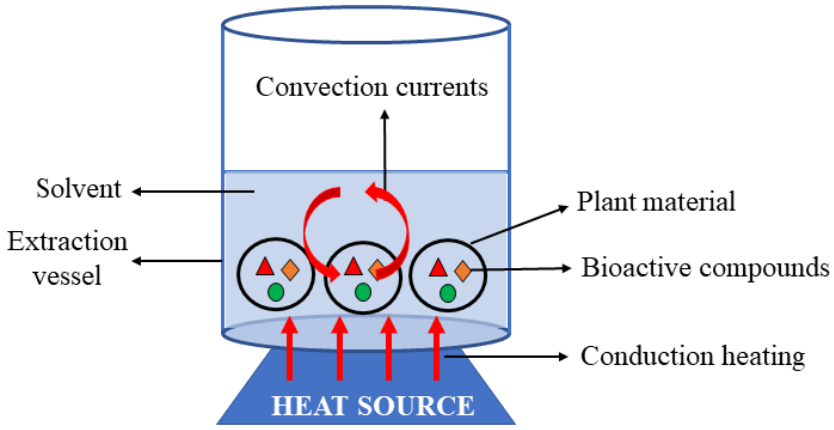


Figure 2. 9 Conventional solvent extraction

Conventional solvent extraction, in principle, is an extraction from plant materials using an appropriate solvent under conventional heating. During conventional heating, the heat is transferred via conduction from the heat source (such as a hotplate) to the heating medium (e.g. water bath) and dissipated by convection to heat the sample mixture inside the vessel (Veggi et al., 2013); the heating process occurs from the outside to the inside because of the thermal gradient. As illustrated in Figure 2.9, heat is transferred from high to low temperatures. In most studies, conventional solvent extraction (CSE) was applied using a water bath as medium heating. Chew et al. (2011a) extracted 20 mg GAE/g of phenolic compounds from *Orthosiphon stamineus* using a water bath shaker at 65 °C for 2 hours. While extraction of phenolic compounds from dried blackcurrant skins and pomace using a shaking water bath required 2 and 6 hours, respectively, to obtain the highest level of free phenolics content (Azman et al., 2022, 2020). When compared to maceration (27 mg GAE/g in 60 min), CSE required a shorter extraction time (15 min) with a higher yield (30 mg GAE/g) in phenolic extraction from *Thymus serpyllum* L. herb (Jovanović et al., 2017). In contrast, CSE needed a substantially longer extraction time (120 min) than ultrasound- (15 min) and

microwave-assisted extraction (1 min) to extract the polyphenols from *Myrtus communis* L. leaves, which only produced a lower yield (Dahmoune et al., 2015). However, the CSE experiment could be designed to have a similar extraction time as microwave-assisted extraction (MAE) by using an ethylene glycol bath to reach the temperature setting (heating phase) before extraction with a water bath, as reported by Galan et al. (2017). Some examples of bioactive extraction with the CSE method have been summarised in Table 2.6.

Table 2. 6 A summary of extraction techniques used to recover bioactive compounds from plant materials

Sample	Target compounds	Extraction method	Optimised extraction conditions					Yield	Remarks	Ref
			Solvent	Temp (°C)	Time (min)	S/F ratio (mL/g)	Other			
Green tea leaves	Polyphenols, caffeine	Maceration	50% ethanol	20	1200	20: 1		28% of phenolics, 3.6% of caffeine	Compared to conventional methods, MAE provided high extraction, high selectivity and required a short time	(Pan et al., 2003)
		Heat Reflux	50% ethanol	85	45	20: 1		28% of phenolics, 3.6% of caffeine		
		UAE (Ultrasound-assisted extraction)	50% ethanol	20-40	90	20: 1		28% of phenolics, 3.6% of caffeine		
		MAE (after pre-leaching for 90 min at 20 °C): 45 s power on, 10 s power off, 3 s for heating, 10 s for cooling	50% ethanol	85-90	4	20:1	Power: 700 W	30% of phenolics, 4% of caffeine		
Orthosiphon stamineus	Phenolic compounds	CSE (Conventional solvent extraction - water bath shaker)	40% ethanol	65	120	10:1		TPC: 20 mg GAE/g; TFC: 16.1 mg CE/g dw; AOA: 21.9 µmol TEAC/g dw (DPPH)	All extraction parameters: ethanol concentration, extraction time, and temperature showed a significant effect on phenolics yield	(Chew et al., 2011a)

Sample	Target compounds	Extraction method	Optimised extraction conditions					Yield	Remarks	Ref
			Solvent	Temp (°C)	Time (min)	S/F ratio (mL/g)	Other			
Eucalyptus camaldulensis Dehn leaves	Phenolic and flavonoid	Maceration	50% ethanol	25	1440	20:1		TPC: 82 mg GAE/g; TFC: 5.5 mg QE/g	Compared with traditional extraction and UAE, MAE provided higher extraction yield and selectivity and required relatively shorter times and less intensive treatment	(Gharekhani et al., 2012)
		UAE	50% ethanol	30-40	60	20:1		TPC: 81.8 mg GAE/g; TFC: 5.5 mg QE/g		
		MAE (10 s power on, 15 s power off for three times, then 3 s power on for heating, 10 s for cooling	50% ethanol	75-85	5	20:1	Power: 600 W	TPC: 76.6 mg GAE/g; TFC: 5.8 mg QE/g		
Chilean Berries: chequen, murta, arrayan, blueberries, meli, calafate	Phenolic and anthocyanin compounds	UAE (ultrasonic bath)	Methanol with 0.1% HCl	Room temp (RT)	60	10:1		Maximum in calafate with TPC of 65.3 mg GAE/g dw, TFC of 45.7 mg QE/g dw, and TMA of 51.6 mg Cy_3GE/g dw	Thirty-one anthocyanins, 3 phenolic acids, and 6 flavonols were found in 6 edible berries from the Chile region.	(Brito et al., 2014)
Pandan (*Pandanus amaryllifolius*)	Phenolic and flavonoid compounds	Reflux	75-79% methanol	70	120	32:1		TPC: 6.6 mg/g dw TFC: 1.8 mg/g dw AOA: 87.4 %	Reflux was successfully optimised using RSM. Methanol concentration, temperature and solvent-to-feed ratio had a significant effect on increasing the yields	(Ghasemzadeh and Jaafar, 2014)

Sample	Target compounds	Extraction method	Optimised extraction conditions					Yield	Remarks	Ref
			Solvent	Temp (°C)	Time (min)	S/F ratio (mL/g)	Other			
Semiarid Mexican region plants: *Jatropha dioica Flourensia cernua, Eucalyptus camaldulensis, Turnera diffusa*	Phenolic compounds	Heat-reflux	70% ethanol	60	120	4:1		*J. dioica:* 2.1 mg GAE/g dw *F. cernua:* 7.9 mg GAE/g dw *E. camaldulensis:* 12.8 mg GAE/g dw *T. diffusa:* 2.5 mg GAE/g dw	The three plant extracts (*F. cernua, E. camaldulensis, T. diffusa* showed similar strong antioxidant activities in scavenging DPPH and lipid oxidation inhibition.	(Wong-Paz et al., 2015)
Myrtus communis L. leaves	Polyphenols, flavonoids, condensed tannin	MAE	42% ethanol		1.04	32	Power: 500 W	TPC: 162.5 mg GAE/g; TFC: 5.0 mg QE/g; Condensed (CT) tannin: 32.7 mg/g	Extraction time for MAE was about 14 and 15 faster than UAE and CSE, respectively	(Dahmoune et al., 2015)
		UAE	50% ethanol	(27±2)	15	50	Frequency: 20 kHz	TPC: 144.8 mg GAE/g; TFC: 3.9 mg QE/g; CT: 23.3 mg/g		
		CSE (thermostatic water bath)	50% ethanol	60	120	50		TPC: 128.0 mg GAE/g dw; TFC: 4.2 mg QE/g; CT: 17.2 mg/g		
Chinese bayberry	Anthocyanin	MAE	95% ethanol acidified with 1% HCl	80	15	50:1	Power: 800 W	TMA: 2.95 mg/g; AOA: 279.96 μmol TE/g dw	MAE could reduce the processing time. However, microwave irradiation could hydrolyse cyanidin-3-O-glucoside to cyanidin without changing its antioxidant activity	(Duan et al., 2015)

Sample	Target compounds	Extraction method	Optimised extraction conditions					Yield	Remarks	Ref
			Solvent	Temp (°C)	Time (min)	S/F ratio (mL/g)	Other			
Jamun fruit pulp	Anthocyanin	CSE (water bath)	Distilled water	44	93	15:1		TMA: 10.6 mg/100 g; TCS: 10618.3 mg/L	The extraction temperature, time, and S/F ratio significantly affected the yield.	(Maran et al., 2015)
Peach (*Prunus persica* L.) fruit	Phenolic antioxidant	CSE (water bath shaker)	60% acetone	RT	180	30:1		TPC: 3.6 mg GAE/g; 48% DPPH RSA; 3.17 mg AAE/g (FRP)	TPC, TFC, DPPH-RSA and FRP were significantly affected by solvent extraction (type, concentration, acidity), extraction time and temperature	(Mokrani and Madani, 2016)
Thymus serpyllum L. herb	Polyphenols	Maceration	50% ethanol	RT	60	30:1		26.6 mg GAE/L	According to phenolic content, the efficiency of the extraction method was ranked in the following order: UAE > HAE > maceration. UAE produced a higher yield than others	(Jovanović et al., 2017)
		Heat-assisted extraction (HAE)	50% ethanol	80	15	30:1		29.8 mg GAE/L		
		UAE	50% ethanol	25	15	30:1	750 W output; 20 kHz	32.7 mg GAE/L		
Blackthorn flower	Phenolic compounds: Phenolic, flavanoid, flavonols, hydroxycinnamic acids	MAE	70% ethanol	60	5	40:1	300 W	61.6 mg/g	The aqueous ethanol was a more appropriate solvent for phenolics: phenolics were higher in 70% ethanol, whereas the antioxidant capacity was rich in 50% methanol	(Lovrić et al., 2017)

Sample	Target compounds	Extraction method	Optimised extraction conditions					Yield	Remarks	Ref
			Solvent	Temp (°C)	Time (min)	S/F ratio (mL/g)	Other			
Sea buckthorn leaves	Phenolic compounds	CSE (ethylene glycol bath for reaching temperature setting and water bath for extraction)	50% ethanol	80	7.5	20:1		162 mg GAE/g dw	A direct comparison of MAE and CSE under the same experimental conditions (including heating rate) showed that MAE produced a 8% higher yield due to the selective heating effect.	(Galan et al., 2017)
		MAE	50% ethanol	80	7.5	20:1		150 mg GAE/g dw		
Gordonia axillaris fruit	Phenolic compounds	MAE	37% ethanol	40	71	20:1		17.7 mg GAE/g dw	Ethanol concentration, solvent/material ratio and extraction time influenced extraction efficacy. The antioxidant capacity of the extract by MAE was stronger than that by maceration or Soxhlet.	(Li et al., 2017)
		Soxhlet extraction	37% ethanol	85	240	20:1		9.6 mg GAE/g dw		
		Maceration	37% ethanol	25	1440	20:1		13.7 mg GAE/g dw		
Satureja macrostema	Phenolic compounds	Reflux	100% ethanol	bp	60	30:1		TPC: 141.6 mg GAE/g dw; TFC: 100.5 mg CE/g dw	MUAE technique showed advantages over reflux extraction: higher yield and shorter extraction time	(Alonso-Carrillo et al., 2017)
		Microwave-ultrasound assisted extraction (MUAE)	75% ethanol	40	30	30:1	500 W microwave power; 50 W ultrasound power at a frequency of 40 kHz	TPC: 166.1 mg GAE/g dw; TFC: 99.2 mg CE/g dw		

Sample	Target compounds	Extraction method	Optimised extraction conditions					Yield	Remarks	Ref
			Solvent	Temp (°C)	Time (min)	S/F ratio (mL/g)	Other			
Carob (*Ceratonia siliqua*) kibbles	Phenolic compounds	MAE	45% ethanol		4.5	30:1	340 W	TPC: 70.1 mg GAE/g dw; Tannins: 4.1 mg CE/g dw	Microwave power and ethanol concentration affected the TPC and condensed tannin yields. In shorter extraction time, the extraction yields by MAE were comparable to UAE.	(Huma et al., 2018)
		UAE	45% ethanol	25	30	30:1	Power: 100 W; frequency: 20 kHz	TPC: 68.8 mg GAE/g dw; Tannins: 3.9 mg CE/g dw		
		CSE (water bath)	45% ethanol	50	120	30:1		TPC: 69.9 mg GAE/ g dw; Tannins: 4.2 mg GAE/g dw		
Basil (*Ocium basilicum* L.) leaves waste	Phenolic compounds: rosmarinic acid (RA) and caffeic acid (CA)	PLE	RA: 75% ethanol CA: 100% ethanol	50	20	NR	10 Mpa	RA: 23.9 mg/g extract (efficiency: 75.9%) CA: 2.4 mg/g extract (efficiency: 13.9%)	Theoretical modelling of Hansen solubility parameters was used to select a list of selective solvents. PLE has high selectivity to enrich the extract yield	(Pagano et al., 2018)
Purple corn (*Zea mays* L.) cob	Phenolics and anthocyanin compounds	Maceration	0.01% (v/v) 6N HCl acidified 50% aqueous ethanol	20-25	45	50:1		TMA: 14.3 mg/g; Polymeric anthocyanin: 20.4%; TPC: 49.8 mg/g; Acylated anthocyanin: 32.4%	Solvent selection and acidity affected the anthocyanins and phenolics extraction	(Lao and Giusti, 2018)
Vernonia cinerea leaves	Phenolic compounds	Soxhlet	60% ethanol	bp	120	20:1		TPC: 54 mg GAE/g dw; TFC: 30.1 mg QE/g dw	Soxhlet has succeeded in phenolic extraction, and *V. cinerea* extract possessed potent antioxidant activity with reference to ascorbic acid.	(Alara et al., 2018)

Sample	Target compounds	Extraction method	Optimised extraction conditions					Yield	Remarks	Ref
			Solvent	Temp (°C)	Time (min)	S/F ratio (mL/g)	Other			
Vernonia cinerea leaf	Bioactive compounds	Soxhlet	Distilled water	bp	120	10:1	450 W; relative electric consumption: 0.89 kWh/g; relative CO_2 emission: 0.71 kg/g	1.38%	MAHE could extract a higher yield of bioactive compounds in a shorter time and reduce solvent consumption compared with Soxhlet extraction. MAHE also showed lower consumption of electricity and reduced emission of CO_2.	(Alara and Abdurahman, 2019)
		Microwave-assisted hydrodistillation extraction (MAHE)	Distilled water		30	10:1	500 W; relative electric consumption: 0.12 kWh/g; relative CO_2 emission: 0.096 kg/g	2.92%		
Momordica Charantia L. Leaves	Phenolic compounds	Maceration	70% methanol	20	90	NR		TPC: 17.3 mg GAE/g dw; AOA: 91.1 mg TE/g (CUPRAC) and 55.1 mg TE/g (FRAP)	The TPC was highly influenced by the linear term of solvent concentration and temperature as well as by the cross product of temperature/time and temperature/ solvent concentration	(Uysal et al., 2019)
Pomegranate peels	Phenolic compounds	MAE	50% ethanol		4	60:1	Power: 600 W	TPC: 199.4 mg GAE/g dw; AOA: 94.91% RSA	MAE was found to increase the yield but mainly to shorten the extraction time by over 60 times compared to conventional. MAE had 1.7 times higher yield in a shorter process (4 min) in comparison with UAE (10 min)	(Kaderides et al., 2019)
		UAE	Water	35	10	32.2	Amplitude 52 W	TPC: 119.8 mg GAE.g dw; AOA: 94.8 % RSA		

Sample	Target compounds	Extraction method	Optimised extraction conditions					Yield	Remarks	Ref
			Solvent	Temp (°C)	Time (min)	S/F ratio (mL/g)	Other			
Butterfly pea (*Clitoria ternatea* L. flower)	Anthocyanin	Reflux	50% ethanol	60	30	25:1		143.49 mg/L monomeric anthocyanin	The extraction yield was affected by temperature and raised with increasing solvent volume.	(Pham et al., 2019)
Cacao pod husk	Phenolic compounds	MAE	Water		5 s irradiation, 30 min extraction	50:1	Power: 600 W	TPC: 10.97 mg GAE/g dw; Extraction efficiency: 76.8%; AOA: 121.5 mg DPPH/g dw	TPC yield was 29% higher than the previous study using conventional (~8.5 mg GAE/g dw). Microwave power, irradiation time, solvent/sample ratio, and extraction time were important to optimise extraction yield as well as reduce production costs.	(Nguyen et al., 2020)
Dried blackcurrant skins	Phenolic compounds (anthocyanin, hydroxycinnamic acids, flavonols)	CSE (shaking water bath)	Acetic acid buffer pH 1.5	50	120	10:1	200 rpm shaking speed	37.02 mg GAE/g of free phenolics; 17.12 mg/g of free anthocyanin with antioxidant activity of 61% DPPH inhibition	The extraction yields were mainly affected by solvent and temperature. Using an acidic solvent (pH 1.5) produced higher yields and antioxidant activity (AOA) than water or methanol.	(Azman et al., 2020)

Sample	Target compounds	Extraction method	Optimised extraction conditions					Yield	Remarks	Ref
			Solvent	Temp (°C)	Time (min)	S/F ratio (mL/g)	Other			
Potato peels	Phenolic compounds	DUAE	50% methanol	25	1	20:1	40 kHz	7.6 mg GAE/g dw	DUAE was more effective compared to IUAE and CSE; its AOA was comparable to commercial synthesis antioxidants.	(Wang et al., 2020)
		IUAE	50% methanol	25	5	20:1	40 kHz	7.6 mg GAE/g dw		
		CSE (incubator shaker)	50% methanol	25	60	20:1	150 rpm	6.0 mg GAE/g dw		
Blueberry	Anthocyanin	Freeze-ultrasonic thawing technology (FUTE)	60% ethanol	41.64	Freezing time: 5.43 min; ultrasonic thawing time: 23.56 min	24.07:1	40 kHz frequency, 600W power	2.53 mg/g	FUTE extract showed a higher yield and greater ability to scavenge DPPH and inhibit lipid peroxidation than UAE and FTE extract. FUTE could rapidly and effective extraction compared to conventional freezing and ultrasonic in terms of yield and biological activity.	(Yuan et al., 2020)
		UAE	60% ethanol	55	40	20:1		1.25 mg/g		
		Freeze-thawing extraction (FTE)	60% ethanol		5	20:1		1.01 mg/g		
Potato peels	Phenolic compounds	SFE	CO_2 and co-solvent of 20% methanol	80	60		Pressure: 350 bar; flow rate of 18 g/min	TPC of 37%, 82% caffeic acid recovery, and AOA of 73% scavenging capacity	Co-solvent concentration and CO_2 flow rate significantly impacted the final response. The use of organic solvent and water mixture as co-solvent gave better dissolution of chlorogenic acid	(Lima et al., 2021)

Sample	Target compounds	Extraction method	Optimised extraction conditions					Yield	Remarks	Ref
			Solvent	Temp (°C)	Time (min)	S/F ratio (mL/g)	Other			
Dried blackcurrant pomace	Phenolic compounds (anthocyanin, hydroxycinnamic acids, flavonols)	CSE (shaking water bath)	Acidified water (pH 1.5)	30	360	10:1		TPC: 20.94 mg GAE/g; TFC: 0.47 mg/g of flavonols	TPC and AOA increased by the increasing extraction time and decreasing pH. Anthocyanins were the primary contributor to the antioxidant activity of the extract.	(Azman et al., 2022)

TPC: total phenolic content; TFC: total flavonoid content; TMA: total monomeric anthocyanin; CT: condensed tannin; AOA: antioxidant activity; DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRP: ferric reducing power ; FRAP: ferric reducing antioxidant power; CUPRAC: cupric ion reducing antioxidant capacity; RSA: radical scavenging activity; GAE: gallic acid equivalent; QE: quercetin equivalent; CE: catechin equivalent; AAE: ascorbic acid equivalent; TE: Trolox equivalent; TEAC: Trolox equivalent antioxidant capacity; RT: room temperature; bp: boiling point

2.7 Supercritical fluid extraction (SFE)

SFE is an extraction process using supercritical fluid as a solvent. A supercritical fluid is fluid at supercritical condition, i.e. conditions beyond its critical point in which no liquid-gas phase boundary exist (Figure 2.10); it has the same solubility as liquid and similar diffusivity to gas (Zhang et al., 2018). The critical point is defined as critical temperature and pressure where a substance can be liquefied, while supercritical fluid (beyond its critical point) cannot be liquefied by modifying pressure and temperature; this fluid has gas-like properties of diffusion, viscosity, surface tension, and liquid-like density and solvation power (Selvamuthukumaran and Shi, 2017).

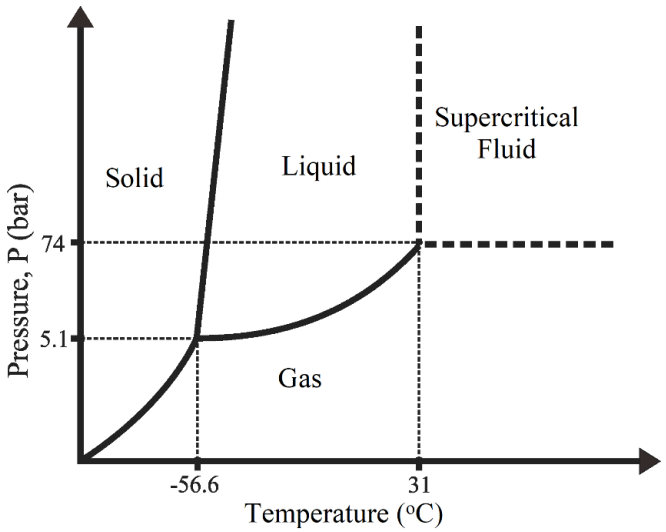


Figure 2. 10 Phase diagram of carbon dioxide

The most common solvent used in SFE is carbon dioxide (CO_2) because it is low cost, non-toxicity, inert, selective, has a low critical temperature (31 °C) and low critical pressure (74 bars) (Selvamuthukumaran and Shi, 2017; Zhang et al., 2018). Thus, at supercritical conditions (31°C, 74 bars) (Figure 2.10), carbon dioxide fluid can diffuse into solid matrix/plant material like a gas and dissolve the target compounds like a liquid. In SFE, the appropriate solvent is selected based on its safety, hazard, energy requirement, and operability. Increasing the solvent temperature above a critical point

needs lots of energy, so the solvent with a low critical temperature, such as carbon dioxide, is preferable. Moreover, a higher temperature could degrade bioactive compounds and reduce selectivity (Panja, 2017).

The SFE apparatus generally consists of a CO_2 tank comprising a pump and a cooler, an extraction chamber and a collector or trapping vessel (Caballero et al., 2020). However, low-polarity CO_2 is preferable for extracting non-polar compounds. Hence, to extract polar compounds, organic solvents, including ethanol, can be added as co-solvent to increase solvent polarity (Alara et al., 2021). The SFE apparatus for antioxidants extraction consists of a tank including CO_2 solvent, a pump to pressurise gas, a co-solvent vessel and pump, an oven containing extractor, a controller to maintain the operating system, a flow meter, and a trapping vessel (Selvamuthukumaran and Shi, 2017). A systematic diagram of SFE-CO_2 is given in Figure 2.11. Both CO_2 from cylinder (1) and co-solvent from a reservoir (2) are pressurised by a pneumatic pump (3) to reach the pressure setting. Both are mixed in a mixer (5) and then preheated inside a tube in oven (6) and put in contact with the sample in an extraction chamber (made of stainless steel rated at 10,000 psi). CO_2 pressure, flow rate, and co-solvent flow are controlled by the micro metering valve and back pressure regulator. The obtained extract is collected in a collection vessel by depressurisation of the system. Due to the decrease in pressure, CO_2 returns to a gaseous state when the extract flows into the collector. The residual co-solvent is evaporated under a vacuum to calculate the extraction yield (Caballero et al., 2020; Fabrowska et al., 2016). To optimise the process, extraction parameters such as temperature, pressure, and extraction time must be considered (Panja, 2017).

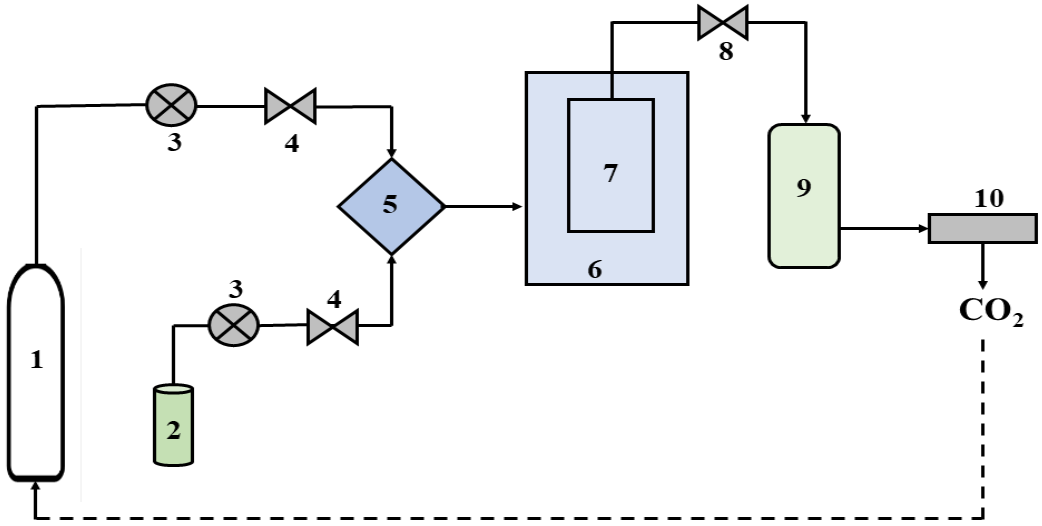


Figure 2. 11 Systematic diagrams of SFE: 1. CO_2 cylinder; 2. Co-solvent reservoir; 3. Pump; 4. Micro metering valve; 5. Mixer; 6. Oven; 7. Extraction chamber; 8. Back pressure regulator; 9. Collection vessel; 10. Wet-dry-gas meter, redrawn with modification from Fabrowska et al. (2016); and Selvamuthukumaran and Shi, (2017)

The benefits of using SFE for extracting bioactive compounds are: 1) extraction time can be reduced because the supercritical fluid has a higher diffusion coefficient, lower viscosity, and lower surface tension compared to liquid solvent, allowing more solvent to penetrate the sample; 2) repeatedly extraction cycle; 3) high selectivity; 4) extract can be easily separated by depressurisation; 5) prevent degradation of thermolabile compounds since operates at low temperature; 6) need a small amount of sample/solid matrix; 7) low solvent consumption: 8) environmentally friendly; 8) solvent can be reused so minimising the waste; 9) possible to scale-up for industrial scale; 10) better reproducibility (Selvamuthukumaran and Shi, 2017; Valadez-Carmona et al., 2018).

The use of SFE using CO_2 solvent was presented by Caballero et al. (2020) to extract polyphenolic compounds from olive waste extracts. The 60% ethanol was used as a co-solvent to improve the solubility of phenolic compounds. They showed that SFE at 300 bar produced higher total phenolic and antioxidant activity in less time and lower solvent consumption compared to the maceration process. In another study, recovery

of phenolics from potato peels using SFE at 80 °C, 350 bar, a flow rate of 18 g/min and 20% methanol as co-solvent has successfully recovered 37% of phenolics, 82% caffeic acid with 73% of scavenging activity (Lima et al., 2021). SFE was also applied to extract the phenolic pigment from cacao hulls (Arlorio et al., 2005) and phenolic compounds from cacao pod husks (Valadez-Carmona et al., 2018).

SFE method indicated a very high selectivity to antioxidant compounds, and using 13.7% ethanol as a co-solvent increased the extractability of target compounds. Applying SFE at 60°C and 299 bar for extracting CPH yielded 13 mg GAE/g extract of TPC with 0.213 mmol TE/g extract of antioxidant activity. The extraction pressure and co-solvent concentration were the main factors influencing the extraction yields (Valadez-Carmona et al., 2018). The possibility of scaling up SFE with the presence of co-solvent was reported by Hassim et al. (2021); the validation of scale-up of the solvent-to-feed ratio extraction on *P. niruri* successfully obtained a similar overall extraction curve (OEC) to laboratory-scale. Moreover, the results found a low co-solvent residue in the dry extract that was safe to consume.

2.8 Ultrasound-assisted extraction (UAE)

Ultrasound is a specific type of sound wave with frequency ranging from 20 kHz to 2 MHz, producing cavitation effects which lead to the production, growth, and collapse of bubbles; sound waves work on an elastic medium such as soft tissue of plant parts, liquid solvents or liquid containing solid material (Panja, 2017). During sonication, longitudinal waves are created when a sonic wave passes the liquid materials that will form regions of alternating compression and rarefaction (expansion) waves; this expansion process produces cavitation bubbles in a liquid. The forming and collapsing bubbles are established at constant ultrasound intensity (Figure 2.12.A).

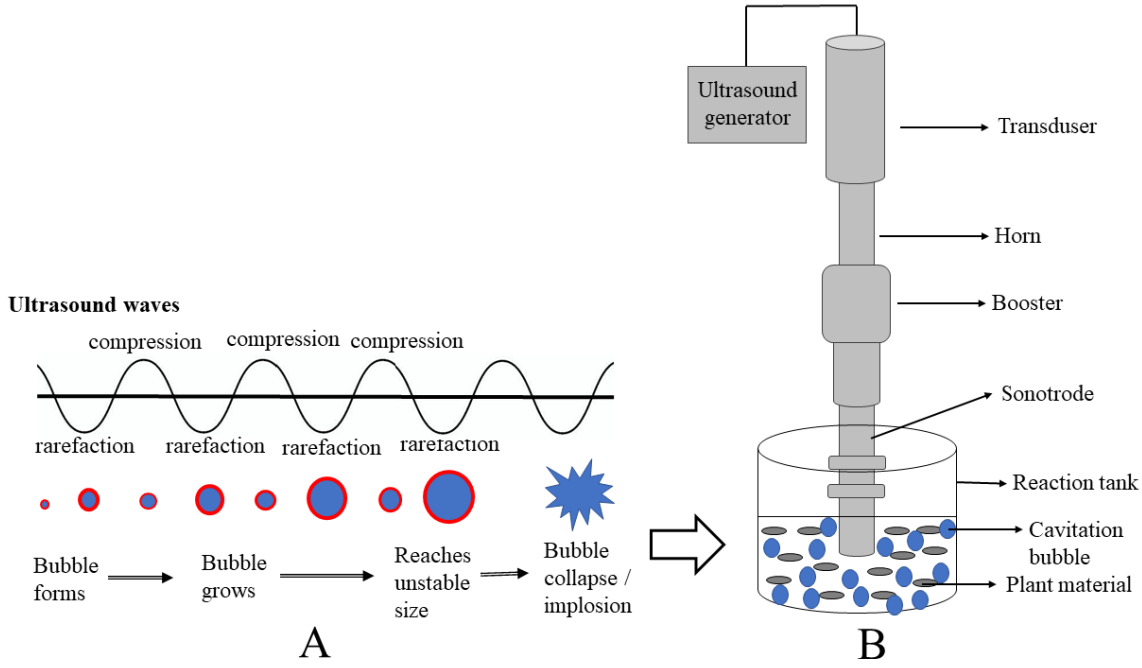


Figure 2. 12 (A) Cavitation effect; and (B) Ultrasound-assisted extraction design, redrawn with modification from Mason (2003); and Selvamuthukumaran and Shi (2017)

When cavitation bubbles collapse near cell walls, the high temperature and pressure create microjets and shock waves on a solid surface, increasing solvent penetration into cells and their mass transfer (Chanioti et al., 2014). The mechanism of the UAE process is described in four steps: 1) cavitation bubbles are generated near the surface of the plant matrix; 2) bubbles are collapsed, releasing a microjet with pressure and temperature toward the surface; 3) matrix surface is ruptured and direct contact is established between active compounds inside the cell and outside solvent; 4) active compounds are released and transported to the solvent (Panja, 2017).

UAE apparatus (Figure 2.12.B) consists of a sonotrode immersed in the glass reaction tank and a transducer connected to the horn with a booster; double-layered mantle reactor with a cooling system can be designed to control the temperature during the experiment (Selvamuthukumaran and Shi, 2017). The use of UAE could reduce the extraction time, energy, solvent loading, temperature, equipment size, and cost, as well as increase the production yield (Selvamuthukumaran and Shi, 2017; Zhang et al.,

2018); UAE also allows effective agitation, reduce the thermal gradient, which start-up, and faster response to process control transfer. However, to optimise the UAE process, the appropriate frequency, ultrasonic power, intensity, wavelength and amplitude of wave need to be designed; moreover, temperature, pressure, sonication time, solvent type, and particle size should be considered during the process (Chanioti et al., 2014).

Applications of UAE were reported to extract phenolic compounds and anthocyanin from plant material. UAE has successfully extracted polyphenols from green tea leaves (Pan et al., 2003), *Eucalyptus camaldulensis Dehn* leaves (Gharekhani et al., 2012), *Myrtus communis* L. leaves (Dahmoune et al., 2015), and *Thymus serpyllum* L. herb (Jovanović et al., 2017) in shorter extraction time than conventional extraction (Table 2.6). Meanwhile, Wang et al. (2020) compared direct ultrasound-assisted extraction (DUAE) and indirect ultrasound-assisted extraction (IUAE) to conventional shaking (CSE) for extracting phenolic compounds from potato peels. In DUAE, the sample mixture was put in a water bath then an ultrasound probe was immersed in the sample mixture during extraction. At the same time, IUAE was conducted using an ultrasound water bath. The results showed that DUAE was more effective in extracting the phenolics from potato peels which performed a higher total phenolic and faster extraction rate in a shorter extraction time. The direct ultrasound probe (DUAE) could promote higher mass transfer because it transferred higher ultrasonic energy to the samples. In another study, combining the ultrasound method with microwave extraction (microwave-ultrasound assisted extract, MUAE) could produce a slightly higher phenolic and flavonoid than those of reflux extraction of *Satureja macrostema*. Combining the two methods: microwave and ultrasound, can work in synergy; microwaves work to reduce the inner mass transfer limitation, while

ultrasound can reduce the external transport restrictions and break cell membranes, thereby reducing control of inner mass transport (Alonso-Carrillo et al., 2017).

2.9 Pressurised liquid extraction (PLE)

PLE is the application of high pressure to maintain the solvent liquid at its normal boiling point resulting in a high solubility and diffusion rate of solute as well as high penetration solvent in plant material (Zhang et al., 2018). The combination of high pressure and temperature will increase solubility and mass transfer rate, thereby accelerating the extraction process even using a small amount of solvent. In PLE, the solvent is pumped into the extraction cell containing a sintered metal filter at the bottom and the top, which is placed in an electrical heating jacket; during extraction, the system is pressurised, and the extract rapidly cooled (Selvamuthukumaran and Shi, 2017). A systematic diagram of the PLE experiment can be seen in Figure 2.13.

PLE may not be possible to extract thermolabile compounds as it uses high temperature and pressure. Still, if the extraction is conducted in a short time, it could prevent the degradation of thermolabile compounds. Polyphenol extraction using the PLE method shows several advantages: 1) higher yield at elevated temperature. Temperature PLE ranges from 50 to 200 °C, but the maximum temperature depends on the polyphenol and solvent used; 2) energy saving because less heat is required to increase the temperature than to generate vapour. The sensible heat of liquid is less than the heat of vaporisation; 3) use low-cost, non-toxic, and environmentally friendly solvents, such as aqueous alcohol and mainly water; 4) the equipment set is simple. However, the solvent selection is limited because the solvent used should have high auto-ignition temperatures and reduced metal corrosiveness at high temperatures and pressures. Typical solvents used for PLE include methanol, ethanol, aqueous methanol, aqueous ethanol, or aqueous 1-propanol (Panja, 2017).

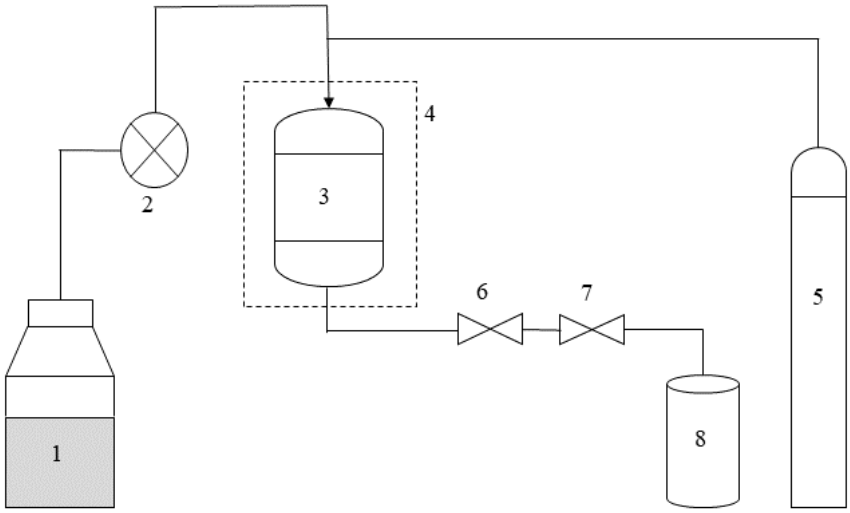


Figure 2. 13 Schematic diagrams of pressurised liquid extraction (PLE): 1. Solvent; 2. Pump; 3. Extraction cell; 4. Oven; 5. Gas cylinder; 6. Blocking valve; 7. Micrometric valve; 8. Collection vial, redrawn with modification from Selvamuthukumaran and Shi (2017).

The study of PLE application was reported on phenolic extraction from *Anatolia* propolis; PLE could recover the polyphenol compounds up to 99.7% at low temperature (40 °C) using 1500 psi of pressure. Static pressure is one of the essential parameters on the PLE process; high pressure forces the solvent into plant matrix pores and keeps the solvent in a liquid phase, thus increasing the extraction efficiency (Erdogan et al., 2011). However, the study of comparison of PLE and solid-liquid extraction (SLE) on the extraction of Irish microalgae showed that the high temperatures (up to 100 °C) and pressures (1000 psi) in PLE did not increase the antioxidant activity; the SLE extracts were much higher antioxidant activity than PLE (Heffernan et al., 2014). They found high temperature and pressure used in PLE are undesirable for food-friendly phenolics production because they could degrade the thermolabile target compounds. In addition, PLE required a significant capital cost investment.

2.10 Microwave-assisted extraction (MAE)

MAE is the application of microwave heating to extract bioactive compounds from plant material. In the extraction process, microwaves heat moisture inside the

plant cells, resulting in evaporation and generating tremendous pressure on the cell wall, which will rupture the cell wall (Tatke and Jaiswal, 2011). MAE process differs from conventional techniques in that heat and mass gradients work in the same direction from inside to outside, and heat is dissipated volumetrically. In contrast, mass and heat transfers in conventional heating work in opposite directions; heat is transferred from outside to inside the system, while mass transfer occurs from inside to outside (Figure 2.14) (Veggi et al., 2013). In extraction processes, the advantages of microwave heating are reported in terms of volumetric and selective heating, which allow the rapid and uniform heating process in minutes (Flórez et al., 2015).

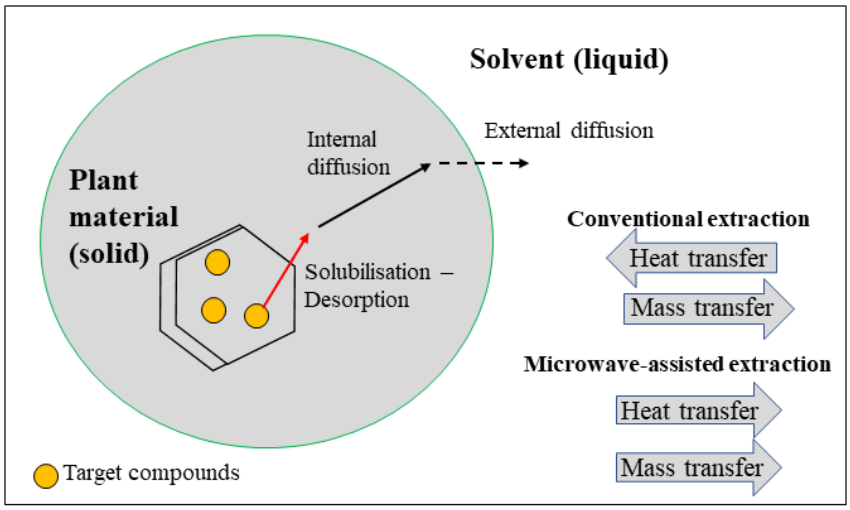


Figure 2. 14 Mechanism of heat and mass transfer in conventional and microwave extraction, redrawn with modification from Flórez et al. (2015); and Veggi et al. (2013)

Microwave apparatus consists of four components: 1) magnetron tube or microwave generator which generates the microwave energy; 2) waveguide to transmit the microwave from source to microwave cavity; 3) applicator for sample holder; and 4) circulator which allows microwaves to pass only in a forward direction (Chanioti et al., 2014). A schematic of microwave extraction is given in Figure 2.15.

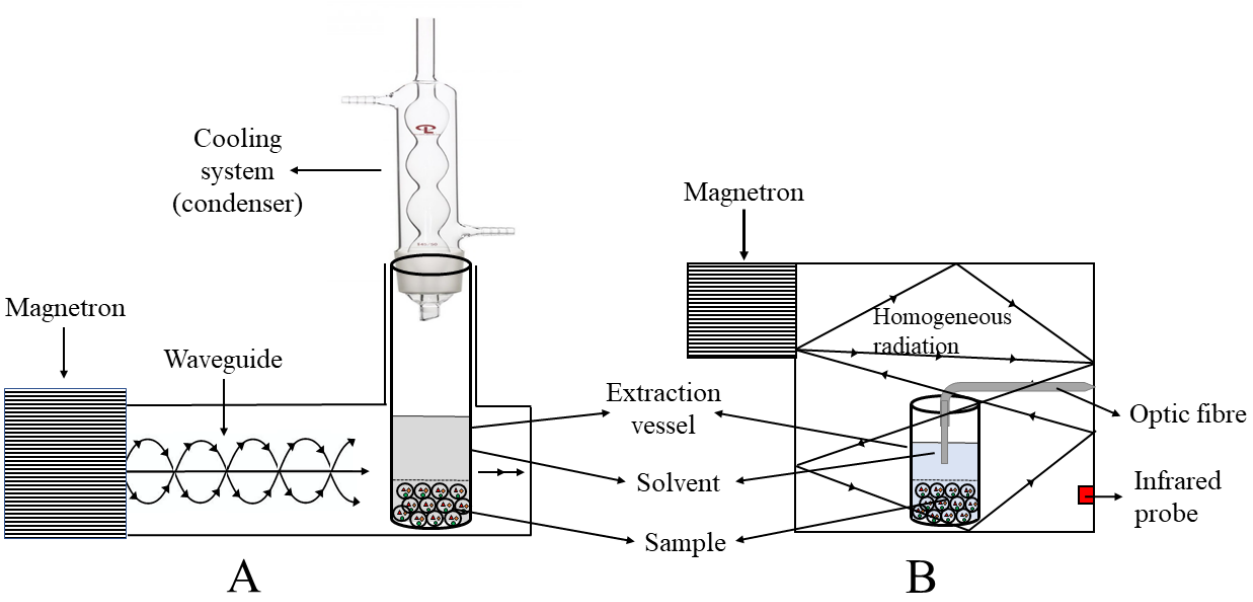


Figure 2. 15 Schematic of microwave system: (A) Open-vessel in monomode cavity; (B) Closed-vessel in multimode cavity, redrawn with modification from Chanioti et al. (2014 and Destandau et al. (2013)

There are two microwave systems: multimode and single mode (focused system). The multimode cavity allows the microwave radiation to be dispersed randomly; thus, every cavity region, including sample, is irradiated; whereas the monomode cavity can produce a frequency that only excites one resonance mode, so microwave radiation focuses on a restricted region exposed to a greater electromagnetic field. The multimode system is a closed-vessel in which temperature and pressure are controlled, while the monomode system is an open-vessel system that works under atmospheric pressure (Chanioti et al., 2014; Destandau et al., 2013).

2.10.1 Microwave heating

Microwaves are electromagnetic waves generated by two oscillating perpendicular fields: electrical field and magnetic field. Microwave energy is non-ionising electromagnetic energy at frequencies from 300 MHz to 300 GHz; it is transmitted as waves into materials and interacts with polar molecules inside the materials to generate heat. The application of microwave heating in food industries is performed at frequencies of 915 and 2450 MHz (Ibrahim and Zaini, 2018). The

frequency and wavelength range for the electromagnetic spectrum are displayed in Figure 2.16.

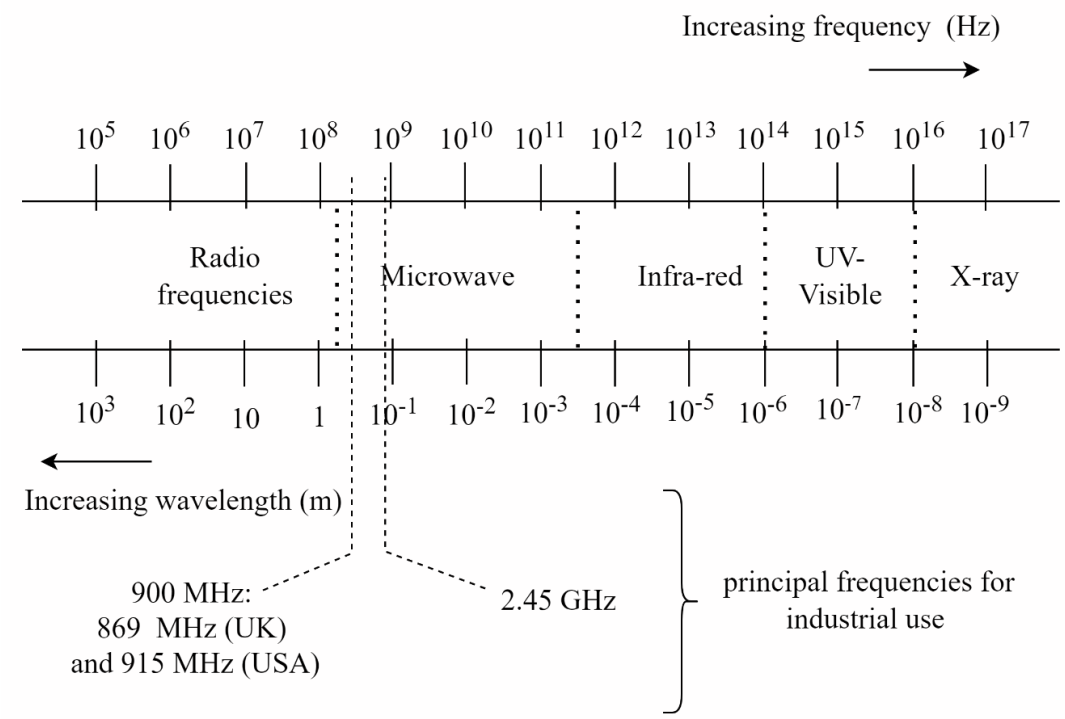


Figure 2. 16 Electromagnetic spectrum, adapted from Metaxas and Meredith (1983)

Electromagnetic energy is converted to heat following ionic conduction and dipole rotation mechanisms. When electromagnetic field is applied, ionic conduction works by electrophoretic migration of ions and the solution's resistance results in friction to heat the solution. While dipole rotation is due to the rearrangement of dipoles (Chanioti et al., 2014). Dipole rotation refers to the interaction of the electric field with the plant matrix. When microwave heating is exposed to the mixture of plant matrix and solvent, an oscillating electromagnetic field causes dipole rotation of polar molecules to align within the applied electric field. The dipole reorients constantly follow the movement that causes them to produce and dissipate energy in the form of heat to the surrounding environment through molecular friction and dielectric loss (dipolar polarisation or dipole rotation mechanism). The matrix's ability to realign itself with the applied field is directly proportional to the amount of heat released (Kappe and

Stadler, 2005). The dipole rotation of molecules promotes the breaking of weak hydrogen bonds during microwave heating (Selvamuthukumaran and Shi, 2017). These two mechanisms could occur simultaneously or individually. Mao et al. (2021) reported the pectin extraction of mango peel above 60 °C, and the ionic conduction significantly contributed to accelerating the volumetric heating by increasing the dielectric loss; while the dipole rotation (dipole polarisation) appeared at below 60 °C.

The basic concepts of microwave heating in MAE differ from conventional solvent extraction (CSE) due to the electromagnetic waves involved in MAE, as opposed to the conventional heat transfer mechanisms of conduction and convection during CSE. Microwave energy works volumetrically and selectively in the heating process. Through direct molecular interactions with the electromagnetic field, microwave energy is directly and volumetrically dissipated into materials and converted into heat, generating efficient internal heating (in core volumetric heating). In contrast, heat is transferred from the heating medium to inside the sample during conventional heating (Kappe and Stadler, 2005; Veggi et al., 2013). The difference between heating mechanism in microwave and conventional techniques can be seen in Figure 2.17. During microwave selective heating, the plant material is hotter than the surrounding solvent phase, and this temperature difference will cause the potential chemical gradient; thus, water and solute are easy to dissolve into the solvent. Consequently, MAE can require lower extraction time, solvent and energy consumption as well as increase the yield and quality of extract (Galan et al., 2017; Lee et al., 2016). The main characteristics between microwave and conventional heating are given in Table 2.7.

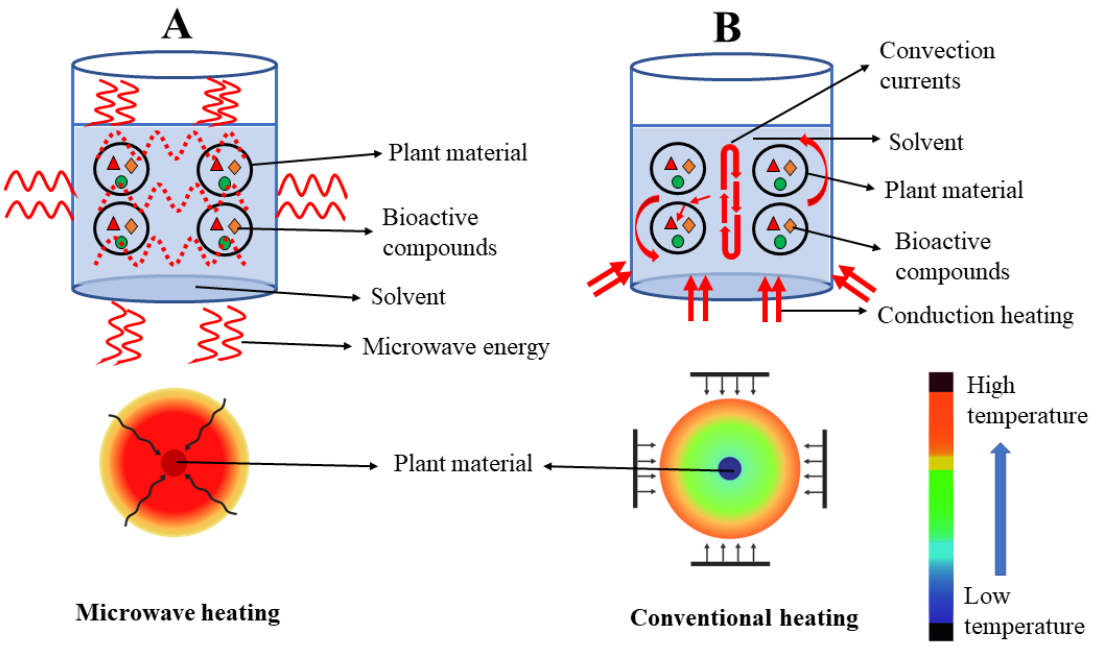


Figure 2. 17 Comparison of heating mechanisms: (A) Microwave heating; and (B) Conventional heating, adapted with modification from Nizamuddin et al. (2018)

Table 2. 7 Characteristics of microwave and conventional heating (Mishra et al., 2016)

Microwave heating	**Conventional heating**
Energetic coupling	Conduction/convection
Coupling at molecular level	Superficial heating
Rapid	Slow
Volumetric	Superficial
Selective	Non-selective
Dependent on the material properties	Less dependent on the material properties

Under microwave heating, mass transfer could be improved by Temperature-Induced Diffusion (TID) because temperature gradient within mixtures; the mixtures of plant material and solvent are heated directly and instantaneously throughout (volumetrically) (Lee et al., 2016; Mao et al., 2021; Taqi et al., 2020). The whole system is heated simultaneously, heating from the inner solvent-matrix mixture to the surface (Figure 2.17.A). Moreover, since microwaves heat selectively, plant material containing various components with different dielectric properties will be heated selectively at different rates. Only polar molecules in the mixture, either solvent or

chemical compounds in the plant matrix, can be heated by microwave heating (Destandau et al., 2013). Plant components, such as water or monosaccharides, which are polar compounds, will be selectively heated because of their strong microwave absorbers (high loss tangent). It can be expected that temperatures within plant matrix may be higher than bulk temperature of solvent. Thus, the dielectric properties drive the selective heating effect on microwave processing.

2.10.2 Dielectric properties

In the microwave heating process, the dielectric properties of a material are a fundamental aspect in microwave heating for describing the material's behaviour to absorb microwave energy and dissipate heat to surrounding molecules. The information regarding the dielectric properties of the solvent is beneficial in choosing the appropriate solvent for dissolving target compounds by microwave heating. Dielectric properties describe the behaviour of a material under the influence of a high-frequency field, which is defined as complex permittivity (ε*) as follows the Equation 2.1 (Metaxas and Meredith, 1983) :

$$\varepsilon^* = \varepsilon' - i\varepsilon'' \qquad [2.1]$$

Dielectric constant (ε') has been assumed real and contributes only to the system's stored energy, while ε" is the effective loss factor relevant to high-frequency heating. The correlation between dielectric constant (ε') and loss factor (ε") is expressed as effective loss tangent or dissipation factor (tan δ), given by Equation 2.2 (Metaxas and Meredith, 1983).

$$\tan\delta = \frac{\varepsilon''}{\varepsilon'} \qquad [2.2]$$

In simple words, the dielectric constant (ε') describes the behaviour of material to absorb microwave radiation, whereas the dielectric loss factor (ε") determines the conversion of the absorbed microwaves to heat. The loss tangent (tan δ) of material

indicates the ability of a material to absorb electromagnetic energy and convert it into heat. The higher the tan δ, the better the material to be heated by microwave (Ibrahim and Zaini, 2018).

On the other hand, the penetration depth is also essential to understand how microwave heating can go inside the materials. Information on penetration depth is also important to evaluate the heating uniformity and to design (scaling up) the electromagnetic heating instrument (Ibrahim and Zaini, 2018). The penetration depth (Dp) is inversely proportional to the dielectric properties of a material, as follows in Equation 2.3:

$$Dp = \frac{\lambda_0 \sqrt{\varepsilon'}}{2\pi\varepsilon''} \qquad [2.3]$$

The free space of microwave wavelength is known as λ_o, which is 12.2 cm for 2.45 GHz. The penetration depth is inverse to the loss factor, even if the material is sensitive to microwaves. If the material thickness is higher than its penetration depth, the material will be heated only at the surface, but the rest will be heated by conduction (Ibrahim and Zaini, 2018). The dielectric properties and penetration depth of some pure solvents are shown in Table 2.8. For instance, in extraction with ethanol solvent with a high loss tangent (0.941, at room temperature) but low penetration depth (0.42 cm), microwave heating will only be effective when using an extraction vessel with a diameter of about 1 cm. If extraction is processed using a vessel with a diameter of more than 1 cm, the rest of the sample (in the vessel's core) will be heated by conduction. However, this penetration problem can be overcome by adding stirring or agitation during the extraction process so that heating will be evenly distributed.

Table 2. 8 Dielectric properties of solvent at room temperature (25 °C) and frequency of 2.45 GHz (Ibrahim and Zaini, 2018)

Solvent	**Dielectric constant (ε')**	**Dielectric loss (ε")**	**Loss tangent (tan δ)**	**Penetration depth (cm)**
Water	80.4	9.89	0.123	1.76
Dimethyl sulfoxide	45.0	37.1	0.825	0.35
Dimethylformamide	37.7	6.08	0.161	1.96
Ethylene glycol	37.0	50.0	1.35	0.24
Methanol	32.6	21.5	0.856	0.52
Ethanol	24.3	22.9	0.941	0.42
Chloroform	4.8	0.437	0.091	9.74
Toluene	2.4	0.096	0.040	31.35
Hexane	1.9	0.038	0.020	70.47

The dielectric properties are also related to the polarity of the material, where the higher dielectric constant shows the higher material's polarity, as following Equation 2.4, which defines the proportionality between the induced dipole moment p and the local electric field F (Pecovska-Gjorgjevich et al., 2012):

$$p = \alpha_{def} . F \qquad [2.4]$$

The dielectric phenomena can be attributed to the polarisation of material due to an external electric field. At microwave frequencies, the main mechanism is orientation polarisation, in which the molecules with equal dipoles of positive and negative charges displaced will tend to orient themselves in the opposite direction to the applied electric field (Figure 2.18) (Tinga and Nelson, 1973). The greater polarisation of the material, the greater the dielectric constant and the amount of potential energy stored in the material. Polar solvents such as water, ethanol, or methanol show higher dielectric properties and loss tangent than nonpolar solvents, so they will be good microwave absorbers generating heat and extracting polar compounds. Based on Table 2.8, the more polar of solvent, the higher the loss tangent, except for water. A polar solvent generally possesses a high dielectric constant and loss tangent that can absorb microwave energy and turns it into heat (Ibrahim and Zaini, 2018), so solvent with a high lost tangent is usually chosen in MAE.

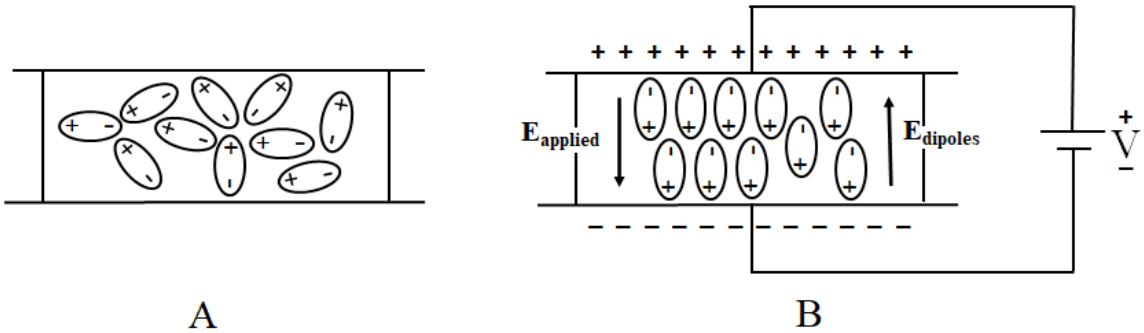


Figure 2. 18 Dipole orientation: (A) Random dipole orientation in an uncharged dielectric capacitor; (B) Dipole ordering by a constant electric field, $E_{applied}$, redrawn with reference to Tinga and Nelson (1973)

2.10.3 Application of MAE in phenolic extraction

Microwave dielectric heating is more effective and selective than conventional heating as it accelerates energy transfer; thus, it will provide shorter extraction using lower amounts of solvent and energy to produce higher-purity products (Parizotto et al., 2019). MAE is widely reported to offer volumetric and selective microwave heating. By volumetric heating, the electromagnetic energy is dissipated to heat directly inside the system, resulting in rapid heating that can significantly reduce the processing time (Metaxas and Meredith, 1983). In addition, direct interaction between the microwaves and mixture system (plant material and solvent) led to plant cell rupture and the quick release of target compounds into the solvent (Gharekhani et al., 2012; Zhou and Liu, 2006). While the selective heating effect of microwaves leads to an increase in extraction yield; it has been shown in phenolic extraction from sea buckthorn leaves in which the plant matrix began selectively heated above 50 °C, so the yield increased at and above 60 °C due to the selective heating effect (Galan et al., 2017).

Many studies reported the applications of MAE in the extraction of phenolic compounds from green tea leaves (Pan et al., 2003), *Myrtus communis* L. leaves (Dahmoune et al., 2015), Chinese bayberry (Duan et al., 2015), *Eucalyptus camaldulensis* leaves (Gharekhani et al., 2012), sea buckthorn leaves (Galan et al.,

2017), blackthorn flower (Lovrić et al., 2017), *Gordonia axillaris* fruit (Li et al., 2017), pomegranate peels (Kaderides et al., 2019), and also cacao pod husk (Nguyen et al., 2020). The summary of these studies is shown in Table 2.6. According to those references, MAE can shorten extraction time and enhance the extraction yield. MAE offers various benefits compared to conventional extraction. Microwave heating is an effective and rapid process because heat is transferred directly to the material (Desai et al., 2010); when maceration required 20-24 hours for extracting phenolic compounds from plant material, MAE only needed 4-5 min (Gharekhani et al., 2012; Pan et al., 2003). Even compared to other advanced extraction methods, MAE could produce a higher yield in less time than UAE (Dahmoune et al., 2015; Huma et al., 2018). In 4.5 min, MAE could generate phenolics yield comparable to those from UAE and CSE for 30 and 120 min, respectively (Huma et al., 2018). On the other hand, Galan et al. (2017) proved that the selective heating effect in MAE could increase 8% phenolics yield of sea buckthorn leaves extract compared to conventional extraction under similar conditions (volumetric heating negated). Those reports confirmed that microwave selective heating could greatly reduce the extraction time and increase the extraction yield.

2.11 Extraction of bioactive compounds from CPH

As discussed above, CPH contains bioactive compounds, such as phenolic compounds, pectin, and pigment compounds. Many studies have been reported to recover these bioactive compounds from CPH, as tabulated in Table 2.9. Maceration and conventional solvent extraction (CSE) are widely used for extracting bioactive compounds from a plant because of their simple methods and low operational cost. As the simplest extraction method, maceration is time-consuming, requires lots of solvents and has a lower yield than other methods. Extraction of phenolic compounds from CPH

in 24 hours maceration could only produce ~3.7 mg GAE/g (Martínez et al., 2012). While by four different sequential extractions using hot 80% ethanol, 60% acetone, warm distilled water, and acidified butanol, Yapo et al. (2013) obtained 69 mg GAE/g dw of phenolics with 85% radical inhibition. At the same time, Karim et al. (2014a) and Teboukeu et al. (2018) revealed the phenolic content of 49.6 mg GAE/g extract (77.6% of radical scavenging) and 150.9 mg GAE/g extract (97.6% inhibition), respectively, by CSE method. Compared with maceration, CSE performed a higher yield and shorter extraction time, but it required a more complicated procedure and still needed high solvent volume to get maximum total phenolic. In general, maceration and CSE were revealed to have environmental issues and were inefficient due to the huge amounts of organic solvent required and the longer extraction time.

Novel extraction methods, such as supercritical fluid extraction (SFE) (Valadez-Carmona et al., 2018) and microwave-assisted extraction (MAE) (Nguyen et al., 2020), have been developed to overcome those limitations. By using CO_2 and 10% ethanol as the extracting solvent, Valadez-Carmona et al. (2018) reported that SFE had good selectivity for antioxidant compounds. In 2.5 hours of extraction, SFE could produce a five-fold higher antioxidants yield (0.213 mmol TE/g) although only recovered half an amount of phenolics (12.9 mg GAE/g extract) compared to the conventional method (23.2 mg GAE/ g extract; 0.04 mmol TE/g). Meanwhile, MAE was reported to obtain 29% higher phenolic content than conventional extraction under 600 W and 5 s/min irradiation time during 30 min of extraction time (Nguyen et al., 2020). As reported by researchers, MAE offers a shorter extraction time and a higher yield compared to conventional extraction, in terms of phenolic extraction from plant materials. MAE offers the main advantage of volumetric heating, in which the electromagnetic energy is dissipated to heat directly inside the system, resulting in rapid heating, so it can

significantly reduce the processing time (Metaxas and Meredith, 1983). MAE can also offer a selective heating effect leading to an increase in TPC yield (Galan et al., 2017). According to those references, MAE will be a promising method to extract the phenolic compounds from CPH in shorter time to produce a higher yield than conventional because of volumetric and selective heating effect. Therefore, a direct comparative study between microwave and conventional heating on phenolic extraction from CPH is needed to understand the potential benefits of microwave volumetric and selective heating.

On the other hand, Nguyen (2015) has successfully extracted the pigment compounds (55% of yield) from CPH using 80% ethanol under conventional heating (thermostatic bath) at 60 °C for 80 min; however, it has not mentioned the major compounds and concentration of pigment compounds in the CPH extract. Vriesmann et al. (2011) reported that the phenolic compounds in cacao were flavan-3-ols (epicatechin, catechin, and procyanidin) with small amounts of anthocyanin. In addition, the anthocyanins in fresh cacao beans were present as free colour-base, free colourless pseudo bases and as ethanol-insoluble colourless complex with other polyphenols. These cacao pigments could be extracted using 70 % ethanol solvent, which main anthocyanins in cacao beans occurred as colourless complexes with other polyphenolic compounds (Forsyth and Rombouts, 1952). Therefore, it can be predicted that CPH may also contain anthocyanin pigment, and this study will then determine the anthocyanin content in the phenolic extract from CPH. However, a very limited number of studies on pigment extraction from CPH led to this work challenging.

Table 2. 9 Summary of solvent extraction of bioactive compounds from CPH

Extraction methods	Target compounds	Plant material preparation	Optimum conditions			Extraction yields	Potential applications	References
			Solvent	Time (min)	Temp (°C)			
Maceration	Fibre, phenolic compound, antioxidants	CPH was dried and ground into a particle size of 220 – 640 micron	Methanol/ acetone (20:1 v/w)	1440 (24 hours)	RT	TDF: 559.9 mg/g TPC: 3.7 mg GAE/g AOA: 33.9 µM TE/g (DPPH); 42.9 µM TE/g (ABTS); 4.5 µM TE/g (FRAP)	Antioxidant	(Martínez et al., 2012)
	Phenolics, flavonoid, flavanol	CPH was blended using water and dried using convective drying, microwave, and freeze-drying	Acetone-water-acetic acid (70:29.5:0.5)	60	RT	TPC: 18.9 ± 1.4 mg GAE/g dw TFC: 6.0 mg EE/g TFLC: 2.1 mg EE/g AOA: 70.8 ± 14 µM TE/g (DPPH); 112.4 µM TE/g (ABTS)	Antioxidant	(Valadez-Carmona et al., 2017)
	Phenolics and antioxidants	CPH was blended into paste, dried, and ground into a particle size of ≤0.5 mm	Ethanol (30:1 v/w)	150	RT	TPC: 23.2 mg GAE/g extract TAC: 0.04 mmol TE/g extract (ABTS)	Antioxidant for food, cosmetic, and pharmaceutical industries	(Valadez-Carmona et al., 2018)
Reflux	Pectin	CPH was dried and milled into powder (<2 mm)	Acidulated water (10:1 v/w) at pH 2.0 using citric acid	90	90	Pectin yield: 15.97% Methoxy content: 13.7%	Functional food	(Marsiglia et al., 2016)

Extraction methods	Target compounds	Plant material preparation	Optimum conditions			Extraction yields	Potential applications	References
			Solvent	Time (min)	Temp (°C)			
Conventional solvent extraction (CSE)	Phenolics, pectins	CPH was dried and ground into powder to pass through a 1-mm sieve (<18 mesh)	80% ethanol (30:1 v/w) for phenolics; water (25:1 v/w) and treated with ethanol (2:1) for pectins	Phenolics: 300 Pectins: 30	Phenolics: 60 Pectins: 100	TDF: 36.6% TPC: 83 mg GAE/g dw	Functional food (antioxidant dietary fibre)	(L. C. Vriesmann et al., 2011)
	Pectin	CPH was minced using 80% ethanol for 25 min and then washed with 70% ethanol. The residue was dried and ground into 12 mm size	Acidified water pH 2 (25:1 v/w). 95% ethanol for precipitating pectin	90	75	Yield: 8.6 % dried material Gel strength: 108 °SAG (moderate efficacious gelling agent) Emulsifying: 35.9% emulsifying activity (effective oil-in-water)	Emulsifier and gelling agent	(Yapo and Koffi, 2013)
	Phenolics, flavonoids, antioxidants	CPH was dried and ground to powder with a particle size of 1 mm	80% ethanol (20:1 v/w)	30	35	TPC: 49.6 ± 3.2 mg GAE/g extract TFC: 22.4 mg RE/g extract AOA: 77.6 % DPPH scavenging; EC_{50}=45.3 mg/mL	Antioxidant for cosmeceutical industry	(Karim et al., 2014a)
	Phenolic compounds	CPH was rinsed with tap water, chopped and dried. Dried CPH was ground into 1 mm size	80% ethanol (20:1 mL/g)	30	40	AOA: 87.1 μg/mL (DPPH); 729.6 μg/mL (FRAP) Elastase inhibition: 23.5 μg/mL Skin whitening: 86.5%	Cosmetic ingredient (Antioxidant, anti-wrinkles, skin whitening, sunscreen effect)	(Karim et al., 2014b)

Extraction methods	Target compounds	Plant material preparation	Optimum conditions			Extraction yields	Potential applications	References
			Solvent	Time (min)	Temp (°C)			
Conventional solvent extraction (CSE)	Pigment compounds	CPH was dried using sun-drying and infrared-drying and ground into fine particles	80% ethanol (10:1 v/w)	80	60	Pigment yield: 55.1 % Absorbance at 436 nm: 2.4	Food pigment or colourant	(Nguyen, 2015)
	Theobromine	CPH was dried and ground into fine particles	70% ethanol (27:1 v/w)	90	80	6.8 mg/100 g dw of theobromine	Nutraceutical, medical, and pharmaceutical industries	(Nguyen and Nguyen, 2017)
	Polyphenol antioxidants	CPH was dried and ground to pass through a 1 mm sieve	100% methanol (21.4:1 v/w)	334	47.48 for TPC; 26.6 for AO	TPC: 150.9 ± 0.5 mg GAE/g extract AOA: 97.6% inhibition	Antioxidant in food industry	(Teboukeu et al., 2018)
	Phenolics, saponin, alkaloids	CPH was dried (by sun-, hot air, vacuum, infrared, and microwave drying) and ground to fine particles (≤1.4 mm)	Distilled water (80:1 v/w)	90 (30 min for maceration and 60 min for CSE)	32 ± 2 for maceration; 50 for CSE	TPC: 12.2 mg GAE/g dw SC: 31.5 mg EE/g dw AOA: 12.3 mg TE/g dw (ABTS), 5.81 mg TE/g dw (DPPH), 12.4 mg TE/g dw (CUPRAC), 8.57 mg TE/g dw (FRAP)	Phytochemical compounds and antioxidants for nutraceutical and functional food industry	(Nguyen et al., 2021)
	Pectin	CPH paste was dried and milled into particle size ≤0.5 mm	4% citric acid solution (pH 3.0). Ethanol for pectin precipitation	95	95	Pectin yield: 8.3 % TPC: 6.2 mg GAE/g dw TDF: 82.1%	Functional food	(Muñoz-Almagro et al., 2019)

Extraction methods	Target compounds	Plant material preparation	Optimum conditions			Extraction yields	Potential applications	References
			Solvent	Time (min)	Temp (°C)			
Four sequential different-solvent CSE	Phenolics, Antioxidant-dietary fibre	CPH was washed with 2% aqueous sodium hypochlorite, dried and ground into powder to pass through a 12 mm sieve	Hot 80% ethanol (25:1 v/w); 60% acetone (25:1); 100% distilled water (25:1); hot acidified butanol (25:1)	320 (20; 60; 60; 180)	bp of 80% ethanol; RT; 37; 100	TDF: 590.2 mg/g TPC: 68.9 ± 5.6 mg GAE/g dw) AOA: 85.4 % (DPPH); 51.9 µmol TE/g (ABTS); 129.5 µmol TE/g (FRAP)	Antioxidant dietary fibre-rich food	(Yapo et al., 2013)
Supercritical Fluid Extraction (SFE)	Phenolics and antioxidants	CPH was blended into paste, dried, and ground into a particle size of ≤0.5 mm	10% co-solvent Ethanol; flow rate: 6 mL/min; Pressure: 200 bar	150		TPC: 12.97 mg GAE/g extract AOA: 0.213 mg TE/g extract (ABTS)	Antioxidants for food, cosmetic, and pharmaceutical industries	(Valadez-Carmona et al., 2018)
Subcritical water (SWE)	Pectin	CPH paste was dried and milled into particle size ≤0.5 mm	96% ethanol (2:1 v/w). Ethanol for pectin precipitation	30	121 with pressure: 103.4 bar	Pectin yield: 8.3 % TPC: 6.2 mg GAE/g dw TDF: 82.1%	Functional food	(Muñoz-Almagro et al., 2019)
Microwave-assisted extraction (MAE)	Catechin, phenolics	CPH was dried and milled into a particle size of 177 micron	Absolute ethanol (100:3 v/w)	10 (1 min on, 2 min off)	70	TPC: 8.7 mg GAE/mL TCC: 51.0 µg/mL	Functional food	(Rahayu et al., 2019)

Extraction methods	Target compounds	Plant material preparation	Optimum conditions			Extraction yields	Potential applications	References
			Solvent	Time (min)	Temp (°C)			
Microwave-assisted extraction (MAE)	Phenolics, saponin, antioxidants	CPH was dried using a microwave oven at 450 W and ground into fine particles	Water (50:1 v/w)	30 min extraction with 5 s/min irradiation	31±2	TPC: 11.0 mg GAE/g dw SC: 97.2 mg EE/g dw AOA: 121.5 mg/g (DPPH)	Antioxidant for functional food	(Nguyen et al., 2020)
	Phenolic compound	CPH was dried by sun-drying and cut into 0.25 – 9.6 mm	85% ethanol (5:1 v/w)	20	Power: 200 W	853.7 mg/L of phenolics	NR	(Mashuni et al., 2020)
	Pectin	CPH was dried and ground to pass through a 200-mesh sieve	Oxalic acid solution (25:1), pH 1.16. Ethanol was used for pectin precipitation	15	Power: 400 W	9.64% of pectin	Gelling agent	(Pangestu et al., 2020)
	Phenolic compounds	CPH was washed using water, chopped into 2 mm, and dried at 50 °C for 24 hours. Dried CPH was ground and sieved into a 100-mesh sieve	96% ethanol (4:1 v/w)	4 min for MAE, then continued with maceration for 24 hours	Power: 180 W	TPC: 453 mg GAE/g extract with a zone of inhibition against E. coli at a concentration of 5 mg/mL	Antibacterial agent for membrane production to prevent biofouling	(Wibisono et al., 2021)

TPC: total phenolic content; TDF: total dietary fibre; TFC: total flavonoid content; SC: saponin content; TCC: total catechin content; AOA: antioxidant activity (by DPPH (2,2-Diphenyl-1-picrylhydrazyl), ABTS 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), CUPRAC (Cupric Reducing Antioxidant Capacity), FRAP (ferric reducing antioxidant power) methods); GAE: gallic acid equivalent; TE: Trolox equivalent EE: escin equivalent; RE: rutin equivalent; RT: room temperature; bp: boiling point; NR: not reported

2.12 Extraction parameter

The extraction yield and quality are affected by extraction methods and their extraction parameter (time, temperature, solvent, material particle size), as discussed below.

2.12.1 Solvent properties

During solvent extraction, an appropriate solvent can dissolve the phenolic compounds without dissolving undesired compounds. Solvent determines the solubility of target compounds and penetrability into the plant matrix. A solvent is a key in extraction; thus, the selection of an appropriate solvent is important to maximise the extraction yield. The selection of solvent should be considered these characteristics: 1) high selectivity for ensures the solubility of the target compound and its purity; 2) not reactive to target compounds; 3) chemically and thermally stable; 4) low viscosity to increase the mass transfer; 5) low boiling is preferable to reduce energy requirement; 6) non-flammable; 7) environmentally and health-friendly; 8) inexpensive (Palma et al., 2013).

In addition, the solvent selection may be different for each extraction method. For example, solvent selection for the MAE experiment should consider the solvent's ability to absorb microwave energy. With the use of absorber solvents (polar solvent), the temperature system increases due to the heat resulting from the interaction of microwave with solvent and plant matrix, while when using transparent solvents (cannot absorb microwave energy), such as hexane or nonpolar solvents, the increasing temperature only causes by the interaction of microwave and plant matrix (Desai et al., 2010). When the plant matrix contains polar compounds, microwave energy heats the plant matrix, and energy is transferred from the plant matrix to the solvent (Mishra et al., 2016). Hence, using an absorber solvent will provide rapid heating to the system.

In the SFE technique, solvents are chosen based on their critical point (critical temperature and pressure); a solvent with a low critical point is preferable. For PLE, solvents are chosen based on their affinity to active components.

In terms of phenolic extraction, as the phenolics are polar compounds, the polar solvents are chosen as solvent extractants. The polarity of the solvent is related to the Hildebrand solubility parameter (HbSP), where the polarity is predicted to increase with the increase of the HbSP. The HbSP is a system developed to explain trends in the miscibility behaviour of solvents that Joel H Hildebrand proposed in 1936. The solubility parameter, δ, is defined as the square root of the cohesive energy density, calculated by dividing the heat of vaporisation by the molar volume, as given by Equation 2.5 (Belmares et al., 2004).

$$\delta = \sqrt{\frac{\Delta H_v - RT}{V_m}} \qquad [2.5]$$

In this regard, the Hildebrand solubility parameter has played an important role in selecting solvents in phenolic extraction. Solvents with similar solubility parameters are predicted to be miscible in significant proportions, whereas different values yield limited solubilities. The Hildebrand solubility parameter of compounds can be calculated using Equation 2.6, proposed by Fedors (1974)

$$\delta = \sqrt{\frac{\sum_i (\Delta e)_i}{\sum_i (\Delta v)_i}} \qquad [2.6]$$

Where $\sum_i (\Delta e)_i$ is the summation of cohesive energies (cal/mol), and $\sum_i (\Delta v)_i$ is the summation of molar volumes (cm^3/mol). The unit for HbSP (δ) is $cal^{1/2}/cm^{-3/2}$, equivalent to 2.045 $Mpa^{1/2}$.

Meanwhile, Hansen (1967) proposed an extension of the solubility parameter approach that involves reviewing three-dimensional systems (δ_d, δ_p, δ_h). The Hansen

solubility parameter (HSP) components are defined as δ_d representing the influence of dispersion, δ_p representing polar effects, and δ_d representing hydrogen bonding contributions (Gao, 2014), where the correlation between HbSP and HSP can be expressed by Equation 2.7.

$$\delta_t^2 = \delta_d^2 + \delta_p^2 + \delta_h^2 \quad [2.7]$$

These solubility parameters, either HbSP or HSP, can predict the most appropriate solvent to extract the target compounds based on their solubility.

Studies related to the use of various solvents as extractants to extract phenolic extraction from plants or biomass have been reported. Extraction of phenolic and flavonoid compounds from *Eucalyptus camaldulensis* Dehn leaves using several solvents: methanol, ethanol, acetone, water, 50% methanol, 50% ethanol, and 50% acetone, showed that 50% ethanol could produce a maximum phenolics, whereas maximum flavonoid could be recovered using methanol (Gharekhani et al., 2012). Other studies evaluated ethanol concentration (0 – 100% ethanol) effect on the phenolic extraction from green tea leaves (Pan et al., 2003) and sea buckthorn leaves (Galan et al., 2017); both demonstrated that TPC yield increased from 0% ethanol and reached maximum at 50% ethanol before then decreasing up to 100% ethanol.

In terms of phenolic extraction from cocoa by-products, Hernández-Hernández et al. (2018) investigated five methods using various solvents to extract the bioactive compounds from cocoa seed and cocoa husk. They prepared homogenised 0.5-µm particle size powder and divided it into five methods: method A was twice extraction of cocoa samples using methanol-water (80:20 v/v) solvent at 70 °C; in method B, cocoa samples were extracted using ethanol-acidified water (30:70 v/v) at pH 3 for 2 hours at room temperature. Then, the residue was re-extracted using acetone:water (70:30 v/v); method C was extracting samples using distilled water at 70 °C for an hour,

which was done twice; method D was twice extraction using methanol-acidified water extraction (80:20 v/v) at pH 3; while method E used acidified water extraction at pH 3. They determined total phenolic content (TPC) by Folin-Ciocalteau spectrophotometric method and HPLC-DAD and then compared the results (Table 2.10). In general, according to the Folin-Ciocalteau method, extract from method B had higher TPC (49.46 ± 2.50 mg/g dw). However, HPLC showed the best phenolic profile for method D extract, which obtained 11.62 ± 0.25 mg/g of theobromine and 17.70 ± 0.03 mg/g of epicatechin. They concluded that extraction with acidified methanol-water was the best quantifying method for theobromine and individual phenols in cocoa. In another study, solvent (methanol) concentrations were optimised for recovering phenolics from CPH; it was reported that solvent was the most important parameter which 100% methanol could recover maximum phenolic from CPH (Teboukeu et al., 2018).

Table 2. 10 Phenolic yields of cacao samples (Hernández-Hernández et al., 2018)

Yield (mg/g dry weight)	**A**	**B**	**C**	**D**	**E**
Folin-Ciocalteau Spectroscopic method					
Total phenolic content	14.64 ± 3.07	49.46 ± 2.50	5.77 ± 0.98	20.39 ± 0.76	9.40 ± 0.25
HPLC					
Theobromine	10.20 ± 0.41	11.00 ± 0.04	8.47 ± 0.01	11.62 ± 0.25	6.66 ± 0.12
Caffeic acid	Traces*	Traces*	Traces*	0.66 ± 0.01	Traces*
Catechin	1.02 ± 0.01	1.97 ± 0.02	1.65 ± 0.03	4.00 ± 0.01	6.16 ± 0.14
Epicatechin	15.84 ± 0.10	9.00 ± 0.30	6.93 ± 0.09	17.70 ± 0.03	7.04 ± 0.05
Derivative I	Traces*	Traces*	Traces*	3.54 ± 0.40	Traces*
Derivative II	Traces*	1.00 ± 0.02	Traces*	1.62 ± 0.04	Traces*
Derivative III	Traces*	Traces*	Traces*	0.87 ± 0.06	Traces*

Traces* ≤0.01 mg/g dry of sample

On the other hand, CPH is rich in low-molecular-weight phenolics and medium-molecular weight-proanthocyanidins. Aqueous methanol and ethanol (60-70%) could extract low-molecular weight phenolics, including proanthocyanidins whilst aqueous acetone was better for getting tannins (Yapo et al., 2013). Lao and Giusti (2018)

declared that a good solvent for pigment extraction would obtain high monomeric anthocyanins and total phenolics extract but low in polymeric colour. Low polymeric colour is an indicator of high anthocyanin quality. The authors investigated the solvent effect on the extraction of anthocyanins from purple corn and revealed that aqueous organic solvent mixtures performed better for anthocyanins and phenolic extraction than water or pure organic solvent. Extracting by 50% ethanol gave the highest monomeric anthocyanins (14.3 mg/g fw) and phenolic (49.8 mg/g fw) compounds with lower polymeric anthocyanins (20.4 mg/g fw). They also reported that acid addition (0.01% 6 N HCl) in 50% ethanol with double cake washing could produce a high level of monomeric anthocyanins and phenolics with a relatively low level of polymeric colour without chemically changing the pigment composition. In addition, Nguyen (2015) has evaluated three different solvents (methanol, ethanol, petroleum ether) for extracting pigment compounds from CPH; the absolute methanol could recover 10.8% pigment with the highest absorbance of 0.597. However, each target compound and each biomass have their properties, so the solvent requirements for extracting them can also differ. Thus, investigating the solvent effect on phenolic extraction from CPH needs to be done.

2.12.2 Particle size

Particle size is one of the most significant factors affecting extraction efficiency as it controls the kinetics of mass transfer. The extraction rate increases with a decrease in the particle size because the contact surface and pore diffusion increase (Patrautanu et al., 2019). In plant material, the bioactive compounds are mostly stored in intracellular spaces, capillaries, or cell structures. Thus, grinding promotes the breakdown of plant cell walls, facilitating the release of active compounds into the extraction solvent and enhancing the yield (Yeop et al., 2017).

Patrautanu et al. (2019) reported that higher yields of TPC were obtained for the smallest particle size (≤250 micron) via UAE of spruce bark. It was because the contact surface and the pore diffusion path increased with decreasing particle size. A similar result was reported by Makanjuola (2017), who extracted phenolic antioxidants from tea, ginger and tea-ginger blends by CSE; they showed at the lowest particle size (425 micron), the antioxidant was maximised for all plant materials. Whereas, Yeop et al. (2017) showed that the particle size of 125-250 micron obtained the highest extraction yield, whereas the smallest particle size (<125 micron) did not give a better yield. Still, these very small particles were more prone to float during extraction because the contact with solvent was limited.

Related to the extraction using microwave heating, the finer particles can improve or much deeper penetration of electromagnetic energy in MAE (Veggi et al., 2013), which also leads to uniform microwave exposure (Desai et al., 2010). For instance, okra with a particle size of 1 mm had a loss tangent of about 0.1 at 20-40 °C (penetration depth of 5 cm) (Lee et al., 2016). The heat generation may not occur in the inner core matrix in a large sample size due to the less penetration depth of microwave heating into the matrix. Thus, material size is also an important parameter in the MAE process in case of a penetration depth for better heat and mass transfer (Ibrahim and Zaini, 2018).

2.12.3 Temperature

Temperature affects the properties of solute and solvent during the process; solubility and diffusivity of solute and viscosity and surface tension of solvent are influenced by changes in temperature (Palma et al., 2013). An increase in temperature will increase the extraction efficiency up to a maximum temperature, which starts to decrease with a further rise in temperature (Veggi et al., 2013). If the temperature is not

selected correctly, it could lead to extract degradation so decrease the yield. For example, the TPC yield increased when the temperature was raised from 40 to 50 °C in solvent extraction using 50% methanol or 50% ethanol but then decreased when the temperature was operated at 60 °C (Lovrić et al., 2017).

In the MAE process, the increasing temperature needs to increase the power level, but it can also increment the solvent loss; increasing the power level may heat the solvent rapidly, causing solvent evaporation and reducing quantity (Desai et al., 2010). While PLE uses elevated temperatures under reduced pressure to break the plant matrix, the elevated temperatures will reduce the solvent viscosity and improve the penetration inside the plant matrix, increasing extraction efficiency. Other than temperature, operating pressure significantly affects PLE; temperature and pressure influence the selectivity and efficiency of extraction by PLE (Ameer et al., 2017).

2.12.4 Extraction time

Extraction time is linked to economising energy and extraction cost. Hence, evaluation of extraction time is important to select the appropriate time because each type of plant material has its own extraction time, depending on the components to be extracted. Extending the extraction time can increase the yield, but prolonged exposure to high temperatures could degrade the target compounds and decline yield (Palma et al., 2013). A longer time of irradiation will disrupt the chemical structure of polyphenol, decreasing the overall extract yield (Ameer et al., 2017). For instance, the optimal extraction time in MAE typically varies from a few minutes to a half-hour, avoiding possible oxidation and thermal degradation in the extraction of sensitive target compounds. The irradiation time is also influenced by solvents' dielectric properties; solvents including ethanol, methanol or water, may heat up extremely on prolonged

exposure, increasing the possibility of overheating, which risking thermolabile compounds (Veggi et al., 2013).

Applying the CSE method to extract the peach fruit revealed that the phenolic yield rose at extraction time from 30 to 180 min before decreasing after that point (Mokrani and Madani, 2016). While Lovrić et al. (2017) showed that extraction using MAE at 50-60 °C in a short extraction time (5 min) was the better TPC yield; the extending time from 5 to 25 min did not seem to have a significant effect on the yields. Other studies reported the MAE of phenolic from *eucalyptus* leaves obtained maximum yield at 11 min extraction time (Gharekhani et al., 2012), while phenolic extraction from green tea leaves (Pan et al., 2003) and pomegranate peels (Kaderides et al., 2019) only required 4 min to reach maximum yield. It is clear that different plant material needs different extraction time to achieve maximum yield.

2.12.5 Solvent-to-feed (S/F) ratio

Solvent-to-feed (S/F) ratio is essential to investigate during extraction because a sufficient solvent is needed to immerse the whole samples. Although the use of much solvent volume could increase the extraction recovery (Pan et al., 2003), studies reported that it must not exceed 30-34% (Veggi et al., 2013); too high an S/F ratio can also cause a decrease in the yield and need more time and energy. On MAE, increasing solvent loading could decrease the microwave radiation per particle of plant material, giving a relatively low dielectric heating effect; this means the penetration depth will also be reduced (Desai et al., 2010). In several applications, a ratio of 20:1 (v/w) was found to be the best ratio to get maximum phenolic yield (Galan et al., 2017; Gharekhani et al., 2012; Li et al., 2017; Pan et al., 2003). However, in some cases, a larger amount of solvent volume was needed to reach the maximum yield; Nguyen et

al. (2020) and Kaderides et al. (2019) used 50:1 (v/w) and 60:1 (v/w) of S/F ratio, respectively, to attain maximum phenolic yield.

CHAPTER 3. EXPERIMENTAL METHODOLOGY

3.1 Materials

Fresh cacao pod husk (CPH) was sourced from local farmers in Malang and Blitar, Indonesia, from November 2018 to January 2020. Before being shipped to the UK and used as research material, fresh CPH was dried and ground into powder, as explained in Section 3.2. Solvents used for extractants were methanol (Fisher Chemical, UK), ethanol (Romil, UK), 1-propanol (Honeywell, Germany), 1-butanol (Honeywell, Israel), 1-pentanol (Honeywell, Germany), and deionised water (Mili-Q). Folin-Ciocalteu 2 N (Sigma-Aldrich, Switzerland) and sodium carbonate (Honeywell Fluka, Germany) were used as reagents for total phenolic content (TPC) analysis.

Potassium chloride (KCl) (SLS lab, UK) and sodium acetate ($CH_3COONa.3H_2O$) (SLS lab, UK) were used to analyse the total monomeric anthocyanin (TMA). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) (Sigma Aldrich, USA) was used as an oxidant for antioxidant activity (AOA) analysis. Gallic acid monohydrate (Sigma-Aldrich, China), (+)-catechin hydrate (Sigma-Aldrich, China), (-)-epicatechin (Sigma-Aldrich, China), quercetin (Sigma-Aldrich, China), p-coumaric acid (Sigma Aldrich, China), and Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (Sigma-Aldrich, Switzerland) were used as standard compounds. Furthermore, the solvents (mobile phase) used for HPLC analysis were formic acid 90% (Fisher Chemical, UK), orthophosphoric acid 85% (SLS lab, UK), and acetonitrile (Fisher Chemical, UK). Ethylene glycol anhydrous (Sigma-Aldrich, USA) was used for a heating bath for the conventional extraction method.

3.2 Preparation of CPH

The average weight of fresh cacao pod husk (CPH) was 553.7 ± 24 g, with the ratio of epicarp, mesocarp and endocarp to the whole CPH being 17.23 ± 1.7% (w/w), 69.58 ± 4.0% (w/w), and 13.20 ± 4.1% (w/w), respectively. CPH samples have been prepared in several forms: fresh CPH and dry CPH, either with or without size reduction. The dry CPH samples were prepared in two categories: CPH powder (with size reduction) and CPH chip (without size reduction). Furthermore, the dry samples were also prepared for each layer of CPH. Dry CPH was then packed in vacuum plastic and stored at room temperature. Every step of the preparation is presented in Table 3.1 and Figure 3.1. Sample preparation was carried out in Laboratory of Food and Agricultural Product Processing, Universitas Brawijaya, Indonesia.

Table 3. 1 Preparation of CPH sample

CPH sample		**Sample preparation**	**CPH size**
Fresh CPH	Without size reduction	The fresh CPH was cut into 2-4 cm sizes and chopped into the size of 0.5 x 0.5 x 0.5 cm	Fresh CPH with size of 0.5 x 0.5 x 0.5 cm
	With size reduction	The fresh CPH was cut into 2-4 cm sizes and blended with the solvent (once before the extraction process)	Blend fresh CPH
Dry CPH	Without size reduction	- Drying the CPH slices (2-4 cm) using a forced air dryer at 50 °C - Cutting the dry CPH into a size of 0.5 x 0.5 cm	CPH chip with size of 0.5 x 0.5 cm
	With size reduction	- Drying the CPH slices (2-4 cm) using a forced air dryer at 50 °C - Grinding the dry CPH and sifting sequentially onto 150, 125, 90, 63, and 38-micron sieves to obtain a uniform particle size	CPH powder with size: ≤150; 125-150; 63-90; 38-63; and ≤38 micron
CPH layer	Without size reduction	- The whole CPH was divided into three layers: epicarp, mesocarp, and endocarp. Firstly, the endocarp part was scraped off. Then, the epicarp was peeled off using a knife from the mesocarp. The mesocarp was then cut into small sizes (2-4 cm). - Each part was dried using a forced-air dryer at 50 °C. - Cutting the dry CPH layers into the size of 0.5 x 0.5 cm	CPH layers (epicarp, mesocarp, endocarp) chip with a size of 0.5 x 0.5 cm

CPH sample	Sample preparation	CPH size
With size reduction	- The whole CPH was divided into three layers: epicarp, mesocarp, and endocarp. Firstly, the endocarp part was scraped off. Then, the epicarp was peeled off using a knife from the mesocarp. The mesocarp was then cut into small sizes (2-4 cm). - Each part was dried using a forced-air dryer at 50 °C. - Grinding the dry CPH layers and sifting sequentially onto 150, 125, 90, 63, and 38-micron sieves to obtain a uniform particle size	CPH layers (epicarp, mesocarp, endocarp) powder with size: 125-150; 63-90; 38-63; and ≤38 micron

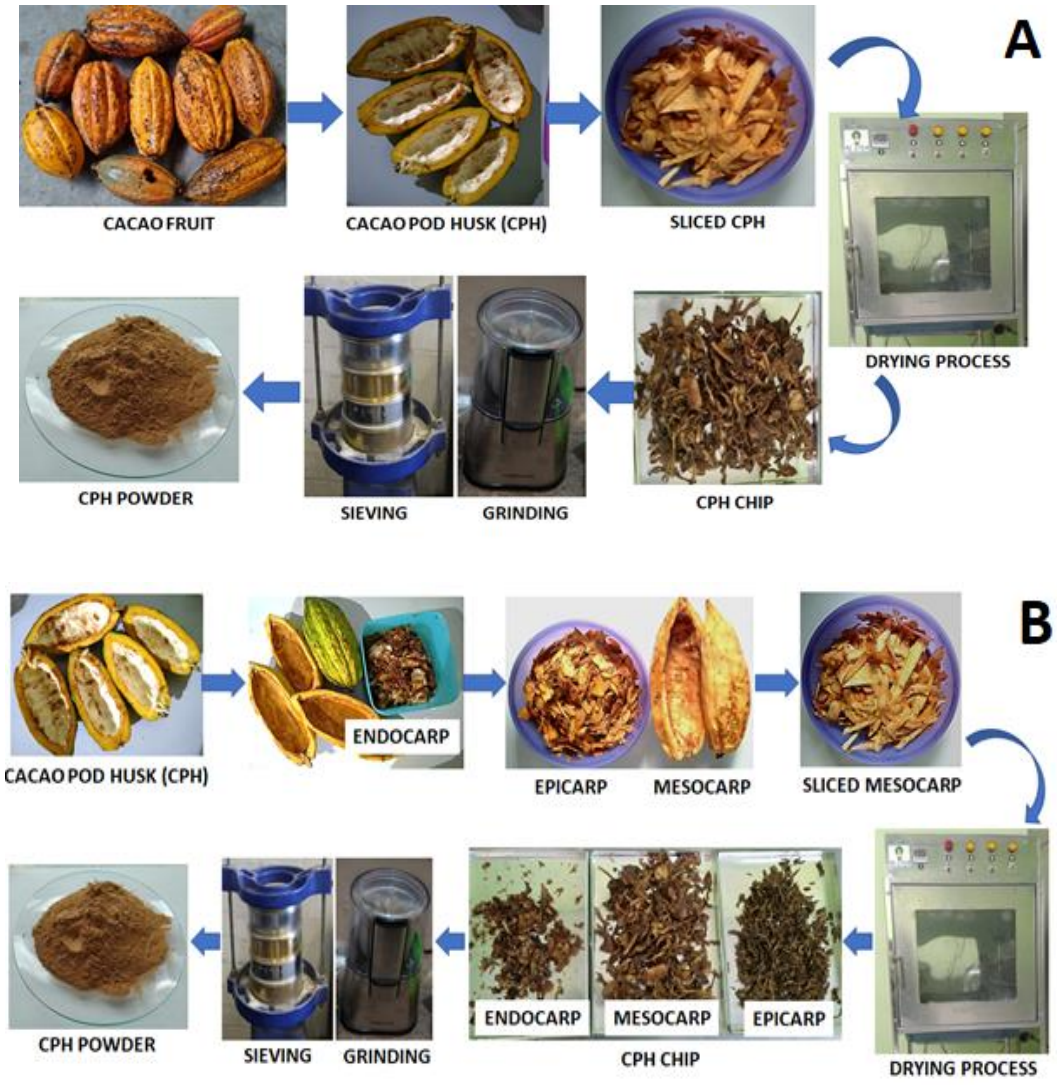


Figure 3. 1 Preparation of CPH samples: (A) dry CPH powder, (B) different layers of CPH: epicarp, mesocarp, and endocarp

The moisture content of the CPH layer before and after drying were measured using oven (gravimetric method) and the results can be seen in Table 3.2. The dry CPH yielded by drying was 13.3 ± 0.7% (w/w) with a moisture content of 8.46 ± 0.89 % (w.b); the moisture content was measured as wet basis to describe the amount of water present in agricultural materials, in this case, CPH. Wet basis content can only range from 0 to 100 %.

Table 3.2 Moisture content of fresh and dry CPH

CPH sample	Ratio part/whole CPH (%)		Moisture content (%)	
	Fresh CPH layer (%, w.b)	Dry CPH layer (%, w.b)	Fresh CPH layer (%, w.b)	Dry CPH layer (%, w.b)
Whole CPH	100.00 ± 0.0	100.00 ± 0.0	86.71 ± 0.68	8.46 ± 0.89
Epicarp	17.23 ± 1.71	18.27 ± 4.39	75.44 ± 0.38	13.81 ± 0.51
Mesocarp	69.58 ± 4.04	69.67 ± 3.54	82.56 ± 0.19	15.36 ± 0.48
Endocarp	13.20 ± 4.11	12.05 ± 2.83	92.00 ± 0.58	14.76 ± 0.54

Mean±S.D. (n= 3, triplicate measurements)

3.3 CPH characterisation (work for CHAPTER 7)

CPH was characterised using a surface area and porosity analyser, thermogravimetric analysis (TGA), and scanning electron microscope (SEM). CPH was analysed using Brunauer-Emmett-Teller (BET) method and SEM image to understand the differences in pore characteristics and surface area before and after extraction. At the same time, TGA analysis was carried out to provide a proximate analysis of CPH before and after extraction. The lignocellulosic compounds (lignin, cellulose and hemicellulose) and chemical composition of the dry CPH powder were confirmed using the Chesson and gravimetric methods, respectively.

3.3.1 Pore characteristics

The CPH powder has a porous inhomogeneity structure, including mesopores and macropores. In the context of physisorption, there are three pore classifications: macropores (pores width > 50 nm), mesopores (pores width of 2 – 50 nm), and micropores (pores width <2 nm). The texture of porous solids and fine powders can be measured by gas adsorption using various subcritical fluids (such as nitrogen at -195.85 °C), organic vapour or supercritical gases. Adsorption is the accumulation of atoms, ions, or molecules (adsorbate) on the material interface (adsorbent). The inverse adsorption process is desorption, in which the amount adsorbed progressively decreases. Adsorption can be divided into physical (physisorption) or chemical (chemisorption) adsorption; gas adsorption is physisorption, in which an adsorbable gas is brought into contact with solids' surface (adsorbent) via intermolecular forces. The solids' surface can be defined at different levels: van der Waals surface (outer part sphere of surface atom at the atomic scale), physisorption (known as Connolly surface that is a probe-accessible surface), and r-distance (located r from Connolly surface, which is accessible). Adsorption (physisorption) isotherm can be displayed in graphical form by plotting the adsorbed amount on the y-axis against the relative pressure (P/Po) on the x-axis at constant temperature and above the adsorptive's critical temperature. Adsorption hysteresis will occur if only the adsorption and desorption curves do not coincide (Thommes et al., 2015). There are six types of physisorption isotherms associated with hysteresis loops, as given in the following Figure 3.2, Tables 3.2 and 3.4.

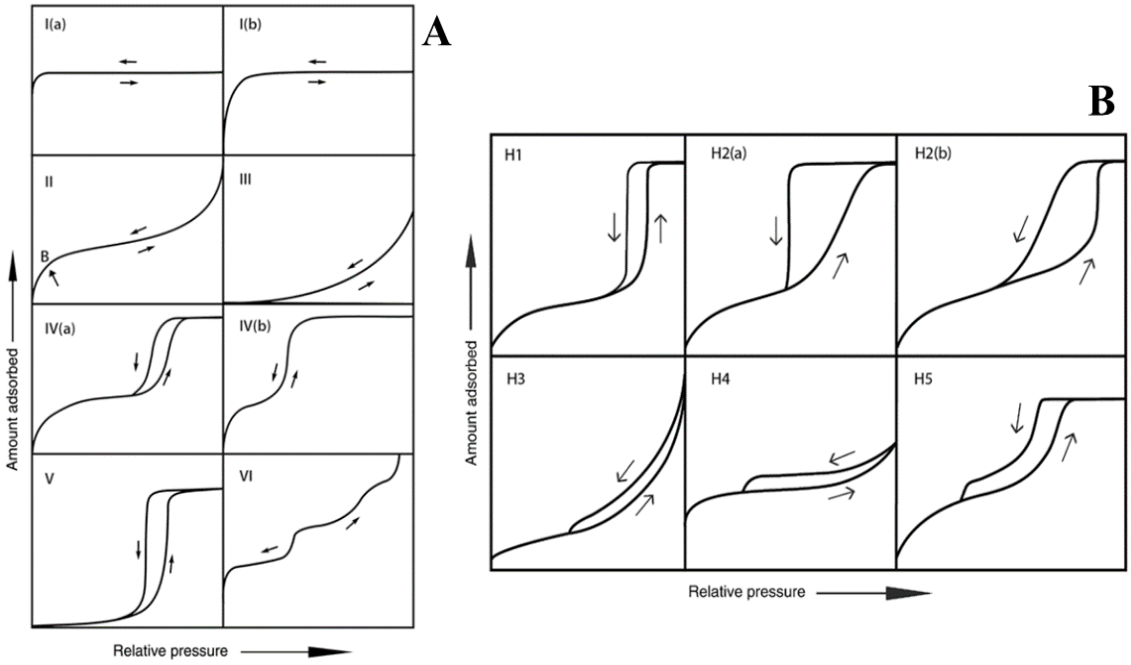


Figure 3. 2. Classification of (A) physisorption isotherm and (B) hysteresis loops (Thommes et al., 2015)

Table 3. 3 Classification of physisorption isotherm (Thommes et al., 2015)

Type	Material classification
I	Isotherm occurs on microporous solids with relatively small external surfaces. Type I(a) is found in microporous materials with mostly narrow micropores (width < 1 nm), while Type I(b) is common for wider micropores and narrow mesopores (< 2.5 nm).
II	Isotherm type for unrestricted monolayer-multilayer adsorption that occurs in nonporous or microporous adsorbents.
III	Isotherm type for a nonporous or macroporous solid with weak adsorbent-adsorbate interactions.
IV	Isotherm type for mesoporous adsorbent; monolayer-multilayer adsorption initially occurs on mesoporous walls, followed by pore condensation. In Type IV(a), capillary condensation is accompanied by hysteresis, while type IV(b) is found in conical and cylindrical mesopores that are closed at the tapered end.
V	Isotherm type for hydrophobic microporous and mesoporous adsorbent. It is caused by relatively weak adsorbent-adsorbate interaction, followed by pore filling.
VI	Isotherm type for layer-by-layer adsorption on a highly homogeneous nonporous surface.

Table 3. 4 Classification of hysteresis loops (Thommes et al., 2015)

Type	Hysteresis classification
H1	Type H1 can be found in materials with a limited number of homogeneous mesopores.
H2	Type H2 is given by complex pore structure material. Type H2(a) is implicated to pore-blocking or percolation in a limited number of pore necks, while Type H2(b) is also attributed to pore-blocking but in a much larger neck size distribution.
H3	Type H3 is found in non-rigid aggregates of plate-like particles.
H4	Type H4 is found in micro-mesoporous carbons, which are associated with micropore filling.
H5	Type H5 can be found in materials that have specific pore structures with open and partially clogged mesopores.

3.3.1.1 Brunauer-Emmett-Teller (BET) analysis

Nitrogen adsorption/desorption isotherms at -195.85 °C were used to measure surface area and pore characteristics of CPH using an automated Surface Area and Porosity Analyser (Micromeritics ASAP 2420). Prior to measurements, approximately two grams of CPH were put in a sample tube with a glass filler rod and seal frit. Next, the airdried CPH was degassed at 90 °C for 24 hours, as explained by Melia et al. (2018). The Nitrogen isotherms were monitored from 0.010 to 0.998 relative pressure (P/Po) and back. The data of nitrogen adsorption/desorption were calculated using the BET (Brunauer-Emmet-Teller) equation for surface area and BJH (Barrett-Joyner-Halenda) model with Harkins-Jura curve correction for pore volume and size distribution. The CPH surface area, S_{BET}, was determined by applying BET theory on the adsorption isotherm between 0.05 and 0.30 of relative pressure (P/Po), giving positive BET 'C' constants. Nitrogen is commonly applied for surface area measurement due to the availability of user-friendly commercial equipment with built-in data processing. The amount of N_2 molecules that had been adsorbed onto the sample was measured by the volumetric method (Tan et al., 2012). In addition, the pore

characteristic was analysed by the BJH method (Harkins-Jura correction), and data analysis was processed using MicroActive Software 5.0, as presented in Appendix D.

3.3.2 Thermogravimetric analysis (TGA)

Thermogravimetric analysis (TGA) is one technique for studying thermal decomposition of biomass. TGA enables the simultaneous measurement of a sample's mass, temperature, and time in a controlled dynamic atmosphere. The measurement principle is the sample's weight variation because of the thermal treatment so that some mass is lost because of volatiles or compounds decomposition. The temperature was measured using thermocouples in direct contact with the sample pans, and temperature changes were controlled by a customised temperature program which may involve isothermal and ramp steps with varying heating rates (De Blasio, 2019). In this work, TGA was used to analyse the proximate content and to investigate the decomposition of the CPH samples as a temperature function. The biomass's moisture (MC), volatile matter (VM), fixed carbon (FC), and ash contents have all been determined as part of the proximate analysis (García et al., 2013). TGA could also be used to understand the pyrolytic behaviour of biomass: lignin, cellulose, and hemicellulose. The primary peaks of hemicellulose (200-300 °C), a shoulder in response to cellulose (250-350 °C), and peaks of lignin at 200-500 °C were all visible on differential thermogravimetric (DTG) curves (Carrier et al., 2011).

TGA was performed using a TGA Q500 (TA Instrument) Instrument. CPH powder (30 mg) was placed on platinum (Pt) pans and heated from room temperature to 105 °C at 5 °C/min of heating rate under a Nitrogen gas (100 mL/min at 1 bar pressure). This temperature was then sustained for 30 min to remove moisture. Following this process, the temperature was increased to 950 °C while continuing a ramp rate of 5 °C/min under Nitrogen conditions (100 mL/min, 1 bar) and remained

heated for 30 min to get the volatile matter content. The nitrogen was then switched to air at 100 mL/min (1 bar) and held for another 30 min to get the fixed carbon and ash content. Data were processed using TA Universal Analysis 2000 Software 4.5A, as presented in Appendix E.

Weight changes were recorded, and the proximate contents (moisture (MC), volatile matter (VM), fixed carbon (FC), and ash contents) were determined. Moisture was calculated by determining the sample mass loss when heated at controlled conditions of 105 °C for 30 min. The mass percentage moisture in CPH samples can be calculated as follows Equation 3.1 (ASTM E-871) (ASTM, 1998a):

$$\text{Moisture}\,(\%) = \frac{W_i - W_f}{W_i - W_p} \text{x}100 \qquad [3.1]$$

where: W_p is the pan weight (g), W_i is initial weight (g), and W_f is final weight (g).

Volatile matter (VM), the gaseous products, was determined by establishing weight loss resulting from heating biomass (after 105 °C), corrected for moisture content according to ASTM E-871. Volatile matter content can be calculated as follows ASTM E-872 (ASTM, 1998b). The percentage of weight loss was determined by Equation 3.2:

$$\text{Weight loss, A, (\%)} = \frac{W_i - W_f}{W_i - W_p} \text{x}100 \qquad [3.2]$$

where: W_p is the pan weight (g), W_i is initial weight (g), and W_f is final weight (g). Then, the volatile matter was determined as follows Equation 3.3.

$$\text{Volatile matter (\%)} = A - B \qquad [3.3]$$

where, B = moisture content (%), according to ASTM E-871

The mass percentage of residue left over from dry oxidation (oxidation at 575 ± 25 °C) was used to express the ash content. Ash content is the percentage of minerals and other

inorganic substances in biomass. The results were reported to 105 °C dried mass of sample (ASTM E-1755)(ASTM, 2015). Ash content can be calculated by Equation 3.4.

$$\text{Ash (\%)} = \frac{m_{ash} - m_{pan}}{m_{sample}} x100 \quad [3.4]$$

Where m_{ash} is final weight (g); m_{pan} is pan weight (g); and m_{sample} is sample weight (g).

In addition to this, the fixed carbon (FC) content was determined by difference using the balance in Equation 3.5 (García et al., 2013):

$$\text{Fixed carbon (\%)} = 100 - (\text{\% Ash} + \text{\% Volatile Matter}) \quad [3.5]$$

Those proximate contents can be calculated as wet, dry, and ash-free basis. The dry basis was computed by dividing each value (VM, FC, ash contents) by (1 – %moisture), while the ash-free basis was estimated from dry basis contents by dividing VM and FC by (1 – %ash).

3.3.3 Lignocellulosic analysis

The major compounds in lignocellulosic biomass are lignin, cellulose, and hemicellulose, which have different chemical structures. The chemical differences between these three components affect their reactivity in biomass conversion. Therefore, analysis of each lignocellulosic compound in CPH solid residue will aid in identifying its potential application. Lignin, cellulose, and hemicellulose were analysed using the Chesson method (Chesson, 1978) by sequential reflux procedures. One gram (a) of CPH sample was refluxed with 150 mL H_2O at 100 °C for one hour. The mixture was filtered, and the leftover material was washed with 300 mL of hot water and dried until it reached a constant weight (b). Next, the dried residue was added with 150 mL of 1N H_2SO_4 and refluxed for 2 hours at 100 °C. The residue was filtered and washed using 300 mL of hot water to get pH neutral, then dried and weighed (c). The dried

residue was treated with 10 mL H_2SO_4 72% at room temperature (25 °C) for 4 hours. Lastly, the dried residue was repeatedly refluxed using 150 mL of 1N H_2SO_4 for 2 hours at 100 °C. The residue was filtered, washed with hot water to neutral (400 mL), and dried at 105 °C. The dried residue was weighted (d) and ashed (e). The lignocellulosic contents were calculated using Equations 3.6:

$$\begin{aligned} \%\text{Hemicellulose} &= \frac{b-c}{a} x100 \\ \%\text{Cellulose} &= \frac{c-d}{a} x100 \\ \%\text{Lignin} &= \frac{d-e}{a} x100 \end{aligned} \quad [3.6]$$

3.3.4 Chemical composition analysis (INS 01-2891-1992)

Moisture content: Moisture content was measured by the gravimetric method. Briefly, two grams of CPH powder (particle size ≤38 micron) or CPH chip was dried in the 105 °C oven for 1 hour, immediately cooled in a desiccator, and then weighed. The process was continued for further drying, cooling, and weighing at hourly intervals until a constant weight was obtained. The percentage of moisture content can be calculated as follow Equation 3.7.

$$\text{Moisture content (\%)} = \frac{W_2 - W_3}{W_2 - W_1} x100\% \quad [3.7]$$

Where W_1 is weight of empty moisture crucible (gram); W_2 is weight of moisture crucible with sample before drying (gram); W_3 is weight of moisture crucible with dried sample (gram)

Protein content: The crude protein was analysed by macro Kjeldahl method. Two grams of CPH powder were placed in a Kjeldahl digestion flask and added with 10 g anhydrous sulphate, 0.7 g copper sulphate, and 20 mL concentrated sulphuric acid. The mixture was digested in a fume cupboard until frothing ceased. The digest was then

cooled and diluted up to 50 mL with distilled water. The diluted mixture was poured into a distillation apparatus: the receiving conical flask containing 50 mL of boric acid 3% and indicator mixture (methyl blue/methyl red (1:2) in 95% ethanol). After distillation of the mixture for 15 min, sodium hydroxide 60% was added until the boric acid solution turned clear to brown. Last, the solution was titrated against hydrochloric acid 0.1 N. A blank solution (distilled water) was taken using the same procedure. The crude protein content was calculated using Equation 3.8.

$$\text{Crude protein (\%)} = \text{\%nitrogen x conversion factor (6.3)}$$

$$\text{\%Nitrogen} = \frac{(\text{mL standard acid - mL blank})}{\text{sample weight}} \text{ x } 0.0014 \text{ x N} \quad [3.8]$$

Crude fat content (Soxhletation/Weibull): A combination of Soxhlet and gravimetric methods were used to measure the fat content in CPH. Two-grams of CPH was put in a 250 mL beaker, and 30 mL of hydrochloric acid 8 N and 20 mL of distilled water were added. The mixture was heated and boiled for 15 min and then filtered. Neutralise the solid residue using distilled water and dried at 105 °C for 1 hour. The dried residue was extracted by Soxhlet apparatus using diethyl Ether solvent for 2 hours. Afterwards, the diethyl Ether solvent was vaporised from the extract, and the solid extract was dried at 105 °C for 1 hour, immediately cooled in a desiccator, and then weighed. The sample was returned to the oven for further drying, cooling, and weighing at hourly intervals until it reached a constant weight. The fat content was calculated as follows Equation 3.9.

$$\text{\%Fat} = \frac{W_2 - W_1}{\text{sample weight}} \text{x}100 \quad [3.9]$$

Where W_1 is weight of empty extraction flask (gram), and W_2 is the weight of flask + fat extract (gram)

Carbohydrate: Carbohydrate was determined using a mathematical calculation (%) as follows Equation 3.10.

%Carbohydrate = 100% - %(protein + fat + ash + moisture) [3.10]

3.3.5 Scanning electron microscope (SEM) analysis

A scanning electron microscope (SEM) is a versatile advanced instrument to observe the phenomena of materials' surface. SEM works on the premise that primary electrons are generated from the source to provide the specimen's atomic energy, allowing them to release secondary electrons (Ses), which may then be collected from the specimen's various points to create an image. SEM is operated under a vacuum to avoid electron interactions with gas molecules to obtain high resolution. SEM images provide information about materials' topography, morphology, composition, and crystallography. Topography indicates surface features and texture, such as smoothness or roughness, while morphology provides information about a material's shape and size. Thus, SEM images can be used to characterise any morphological changes in particles or material after modifying process. By using SEM, samples must be electrically conductive to avoid overcharging on the surface, which causes extreme brightness and poor images. Hence, non-conductive samples, such as polymer or carbon, must be sputter coated with a conductive surface, such as gold and platinum (Akhtar et al., 2018).

Microstructural analysis of dry CPH was observed using Scanning electron microscope (SEM) analysis (JSM-6510LA, JEOL, Japan) at high vacuum condition. The dry CPH (particle size of 38-63 micron) was mounted onto a sample holder with double-adhesive conducting tapes. Following that, gold particles were applied to coat each sample in an auto-coater (JEOL JEC-3000 FC) at 3.3 Pa. Images of each sample

were captured at an accelerating voltage of 15 kV at various magnifications of 500, 1000, and 5000 with a working distance (WD) of 11 mm.

3.4 Solvent extraction procedure

General descriptions of the solvent extraction procedure used in this thesis can be found below. Sections 3.4.1 – 3.4.3, on the other hand, describe detailed sequential extraction to evaluate the effect of each processing parameter. The CPH sample and the extraction solvent were mixed in a borosilicate (Pyrex) flask and extracted for the required conditions while stirring at 1200 rpm. Each extract was vacuum filtered through a Whatman No 1 paper filter and centrifuged (Sigma 2-6E, Germany) at 3900 rpm for 20 min. All experiments, as well as extract analysis, were performed in triplicate. The systematic extraction process and detailed conditions for each processing variable are presented in Figure 3.3 and Table 3.4. The appropriate solvent, material condition and extraction method were then applied for maximising the extraction yields (total phenolic content, total monomeric anthocyanin, and antioxidant activity).

Reflux: The dry CPH and solvent were mixed in a 250 mL three-neck borosilicate (Pyrex) flask. A condenser was then attached to the middle neck of the boiling flask and clamped in an upright position (Figure 3.4.A). The cooling water must flow from the bottom pipe of a condenser and leave the top pipe to ensure the condenser is working. A thermometer was placed in the flask, and reflux was conducted for one hour at the solvent's boiling point.

Maceration: The dry CPH and solvent were placed in a Pyrex TE cavity flask (80 mL, inner dia. 39 mL, height 7 cm) and enclosed with a rubber stopper. Maceration lasted one hour at room temperature (20 °C).

Microwave-assisted extraction (MAE): The MAE was operated using a microwave system consisting of a MiniFlow 200SS (Sairem, France) batch reactor (Figure 3.4.B)

at 2.45 GHz. The mixture of CPH powder and solvent in a Pyrex TE_{10} reactor (80 mL, inner dia. 39 mL, height 7 cm) was enclosed with a rubber stopper and placed in a single-mode cavity in the WE340 waveguide, ending in a short circuit. The sample mixture was stirred at 1200 rpm using an external magnetic stirrer (IKA magnetic stirrer) at the bottom of the microwave reactor. The microwave was operated at 120 W of power. A temperature optical fibre was used to measure and control the temperature inside the reactor. The temperature recorded during heating was bulk temperature; the temperature of biomass or solvent could be colder or hotter than the bulk temperature, depending on their dielectric properties (Galan et al., 2017). The bulk temperature, forward power (FP) and reflected power (RP) were recorded by the system during microwave heating. The absorbed power (AP = FP – RP) and bulk temperature profile are illustrated in Figure 5.6.A. The setting temperature (60 °C) could be reached in 75 s (heating phase), and energy absorbed during the heating and holding (5 min) phases were 8.97 and 1.15 kJ, respectively, for a total of 10.12 kJ.

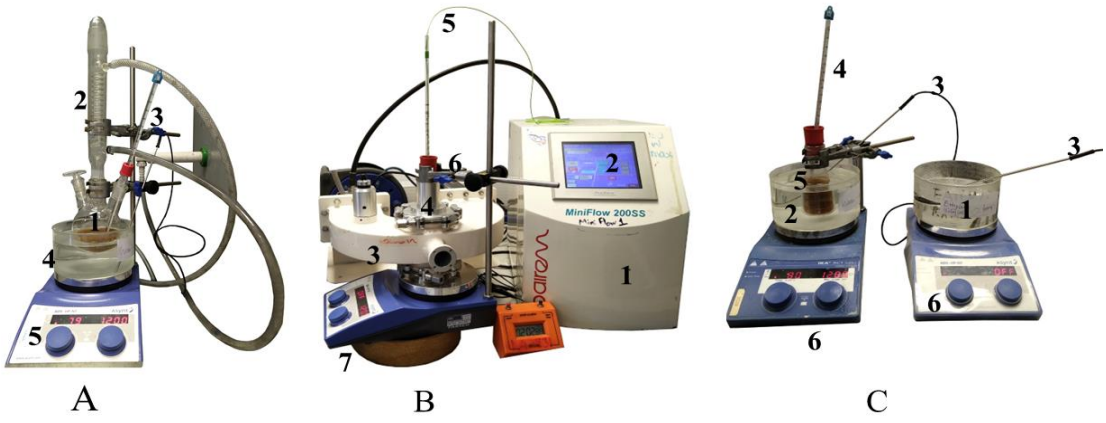


Figure 3. 3 (A) Schematic diagram of reflux extraction: 1-three-neck round flask for sample; 2-condenser; 3- thermometer; 4-water-bath; 5-hotplate stirrer; (B) Schematic diagram of Miniflow 200SS: 1-Microwave generator; 2-Touch screen digital front panel; 3-WE340 waveguide; 4-TE_{10n} single-mode cavity with aluminium lid and quick release clamp, including Pyrex reactor with rubber stopper; 5-Temperature optical fibre; 6-sliding short circuit; 7-magnetic stirrer; (C) Schematic diagram of CSE: 1-ethylene glycol bath; 2-water bath; 3-thermocouple; 4-thermometer; 5-Pyrex reactor with rubber stopper; 6-hot plate stirrer

Conventional solvent extraction (CSE). In order to comprehend heating rate effect, a water bath (WB) and ethylene glycol bath (EgB) were first used to set up the CSE experiments. The bulk heating profiles of both were then compared to that of MAE, and the result showed that the bulk heating profile of CSE-EgB was similar to MAE (Figure 5.6.B). Therefore, the CSE-EgB (hereinafter referred to as CSE) for subsequent experiments was designed to replicate the heating rate of MAE. The mixture of CPH sample and solvent was also placed in the same reactor flask as MAE (an 80 mL-Pyrex TE, inner dia. 39 mL, height 7 cm). To have a very similar bulk heating profile to MAE, the sample mixture was immersed in a 120 °C–EgB until reaching the operating temperature; the sample was then immediately transferred to a water bath (WB) for the extraction process. The sample temperature inside the reactor was measured using an alcohol thermometer (Figure 3.4.C).

3.4.1 Preliminary experiments to identify the best solvent, extraction methods and CPH particle size (work for CHAPTER 4)

The preliminary study for CPH extraction was evaluated based on extract's total phenolic content (TPC). To begin the screening experiment, reflux was selected as the extraction method because it works with reproducible evaporation and condensation of solvent at constant boiling temperature and ambient pressure without significant solvent loss. Reflux is a popular method as it is a simple technique and more efficient than maceration in terms of processing time and solvent requirements (Zhang et al., 2018). The study was started by comparing the extraction of phenolics from fresh and dry CPH to understand material drying effect, then continue with the investigating of the appropriate solvent for extracting the phenolic from CPH. The most appropriate solvent selected was used to evaluate the extraction methods and CPH particle size effect. All

experiments and extracts analysis were performed in triplicate. In simple way, these detailed experiment can be found in Figure 3.3 and Table 3.5.

a. Influence of drying

To understand the effect of drying, extraction of phenolic from fresh CPH was compared to that from dry CPH. CPH samples were prepared in four conditions: fresh CPH without grinding (0.5 x 0.5 x 0.5 cm), fresh CPH with grinding (fresh CPH was blended with the solvent), dry CPH without grinding (0.5 x 0.5 cm), and CPH powder (size ≤ 150 micron). A reflux system (Figure 3.3.A) was selected to extract the phenolic from all CPH, and briefly, ±5 g of fresh CPH and 50% (v/v) aqueous-ethanol solvent (with ratio 20:1 and 40:1 mL/g) were loaded into 250 mL three-neck borosilicate glass round bottom flask. Extraction was carried out at boiling point of 50% (v/v) ethanol/water solvent (±77°C) for 60 min. Experiments were conducted in Laboratory of Food and Agricultural Product Processing, Universitas Brawijaya, Indonesia to minimise sample damage. Dry CPH was chosen as sample for next experiments.

b. **Influence of solvent type**

Eight solvents were selected for phenolic extraction, including methanol, ethanol, 1-propanol, 1-butanol, 1-pentanol, deionised water, 50% (v/v) methanol/water, and 50% (v/v) ethanol/water. The extracts were prepared using reflux (Figure 3.3.A). Briefly, 1 g of dried CPH (particle size ≤ 150 micron) and 40 mL of solvent were refluxed for 60 min at the boiling point of solvents. According to the results, 50% (v/v) ethanol/water was selected as solvent for subsequent extractions.

c. **Comparison of extraction method**

Phenolic extractions were carried out using reflux, maceration, conventional solvent extraction (CSE) and microwave-assisted extraction (MAE). Briefly, 1 g of dry CPH (particle size ≤ 150 micron) and 40 mL of 50% (v/v) ethanol/water were loaded into a

Pyrex reactor which then was covered with a rubber stopper. Each extraction method was run for 60 min. MAE was then selected as a proper method for extracting the phenolic from CPH.

d. Influence of CPH particle size

One gram of each CPH group of particle size (CPH without grinding (0.5x 0.5 cm), 125-150, 63-90, ≤ 38 microns) was mixed with 40 mL of 50%(v/v) ethanol/water and then extracted using MAE method (Miniflow 200S, Sairem) at 60 °C for 30 min. CPH with particle size of ≤ 38 micron was chosen for next extractions.

3.4.2 Maximising the extraction yields (work for CHAPTER 5)

Following the preliminary study, the next step was assessing the processing parameters to maximise the extraction yield. Therefore, the influence of extraction time and temperature, ethanol concentration, and solvent-to-feed (S/F) ratio on total phenolic content (TPC), total monomeric anthocyanin (TMA), and antioxidant activity (AOA) were investigated. The CPH sample and solvent used for extraction were dry CPH with size ≤38 micron and ethanol/water mixture, respectively, that were obtained from previous works in Section 3.4.1d. MAE was selected as the extraction method (Section 3.4.1c) and compared with the CSE method. All experiments and extract analyses were performed in triplicate. Simple way to see the detailed variables for each study of extraction parameters are provided in Figure 3.3 and Table 3.5.

a. Influence of extraction time and temperature

Experimental was begun to evaluate the influence of extraction time and temperature. One gram of dry CPH (≤ 38 micron) was mixed with 40 mL of 50% (v/v) ethanol/water in a Pyrex reactor. The mixture was then extracted using MAE and CSE methods at different temperature (50, 60, and 70 °C) for various extraction time (1, 5, 10, 15, and 30 min). The best conditions (time and temperature) were selected based on either the

maximum TPC and/or AOA values and were used to investigate the ethanol concentration and solvent-to-feed (S/F) ratio effects. Maximum time was found at 5 min extraction. For subsequent MAE and CSE experiments, 60 and 70 °C were selected to maximise the phenolic yield, respectively, while temperature of 50 °C was chosen to maximise antioxidant yield.

b. Influence of ethanol concentration

The effect of ethanol concentration was studied based on the highest of both phenolic and antioxidant yields. To maximise the phenolic yield, one gram of dry CPH (≤ 38 micron) was added with 40 mL of ethanol/water solvent at various concentrations (0, 10, 30, 40, 50, 60, 70, 90, 100% v/v). Each mixture was extracted using MAE at 60 °C and CSE at 70 °C for 5 min. On the other hand, for maximising the antioxidant yield, a mixture of one gram dry CPH and 40 mL of ethanol water (0, 10, 30, 40, 50, 60, 70, 90, 100% v/v) was extracted using MAE and CSE at 50 °C for 5 min. According to both studies, 50% (v/v) ethanol/water was then selected to evaluate the effect of solvent-to-feed (S/F) ratio.

c. Influence of solvent-to-feed (S/F) ratio

In a Pyrex reactor, one gram of dry CPH (≤ 38 micron) was mixed with a various of solvent volumes (20, 30, 40, and 50 mL). Each mixture was then extracted for 5 min using MAE and CSE methods. MAE at 60 °C was compared to CSE at 70 °C to maximise phenolic yield, whereas to maximise antioxidant yield, MAE was compared to CSE at 50 °C.

d. Extraction of bioactive compound at maximum conditions

Extraction of bioactive compounds at maximum conditions was carried out using CPH with various particle sizes: CPH without grinding (0.5 x 0.5 cm), 125-150, 63-90, 38-63, and ≤ 38 microns. One gram of each CPH group was extracted using 40 mL of 50%

(v/v) ethanol/water at 50 °C for 5 min extraction. Extraction was run using MAE and CSE methods.

3.4.3 Comparison of bioactive compounds in CPH layers (work for CHAPTER 6)

Extraction of each CPH layer: epicarp, mesocarp, and endocarp was designed using both MAE and CSE at maximum conditions found in Sections 3.4.1 and 3.4.2. Each CPH layer with different particle sizes (0.5x0.5 cm, 125-150, 63-90, 38-63, and ≤38 micron) was extracted using 50% (v/v) ethanol/water (40:1 mL/g) at 50 °C for 5 min. The TPC, TMA and AOA among three distinct layers are compared to understand the distribution of bioactive compounds in CPH layers.

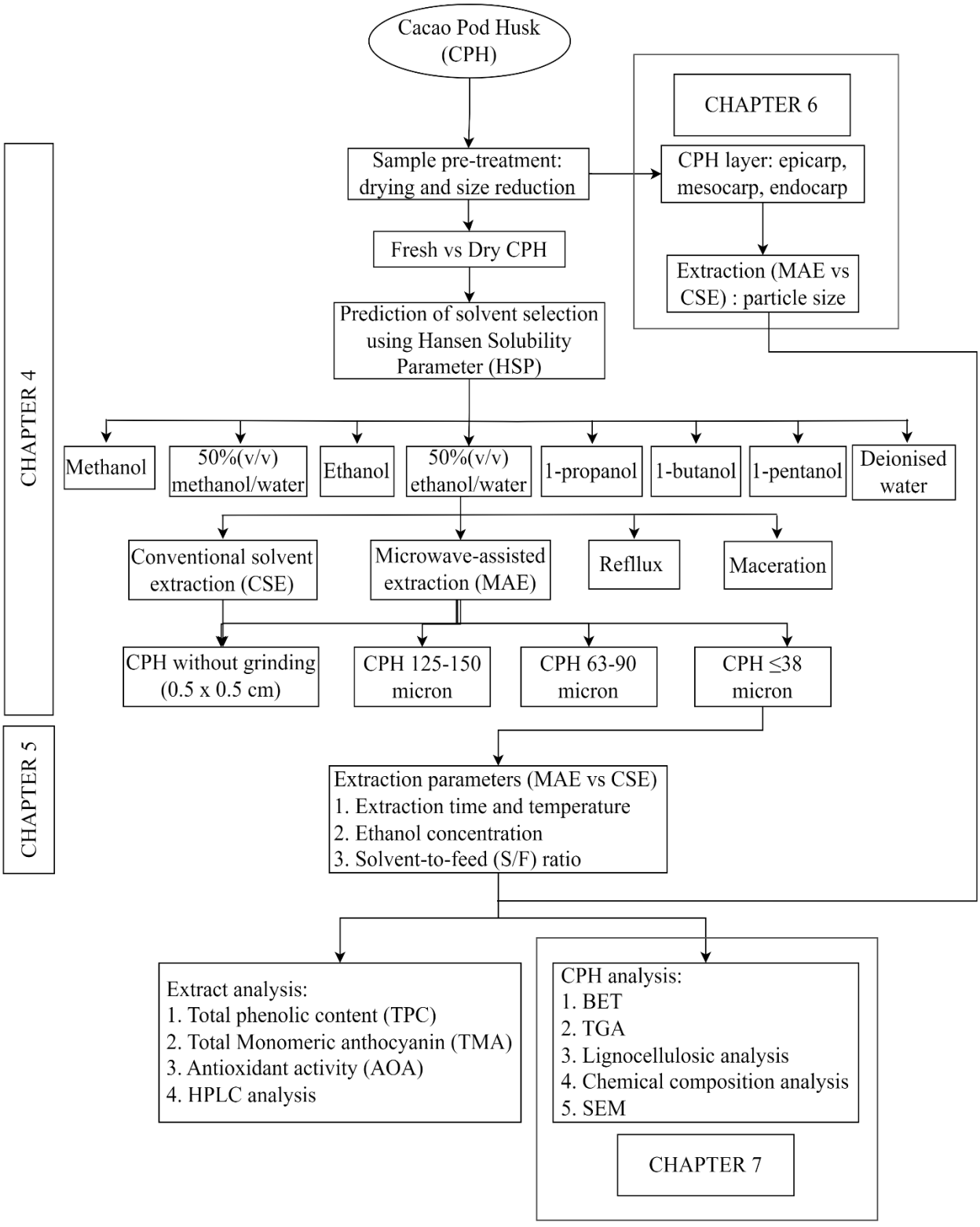


Figure 3. 4 A systematic study of bioactive extraction from cacao pod husk (CPH)

Table 3. 5 Conditions for bioactive extraction from CPH

Parameter	CPH sample	Extraction method	Solvent	S/F ratio (mL/g)	Time (min)	Temperature (°C)
Preliminary experiments to identify the best CPH pretreatment, extraction solvent, and extraction methods (CHAPTER 4 work)						
Drying	Fresh CPH with size 0.5x0.5x0.5 cm; Blend fresh CPH; CPH chips; dry CPH powder ≤150 micron Sample weight: 5 g	Reflux	50% (v/v) ethanol/water	20:1 40:1	60	bp of solvent (±77 °C)
Various solvents	Dry CPH ≤150 micron Sample weight: 1 g	Reflux	• Absolute methanol • Absolute ethanol • Absolute 1-propanol • Absolute 1-butanol • Absolute 1-pentanol • Deionised water • 50% (v/v) methanol/water • 50% (v/v) ethanol/water	40:1	60	bp of solvent
Various methods	Dry CPH ≤150 micron Sample weight: 1 g	• CSE • MAE • Reflux • Maceration	50% (v/v) ethanol/water	40:1	60	• CSE: 70 °C • MAE: 70°C • Reflux: at b.p. of solvent • Maceration: RT (20°C)
Particle size	Dry CPH with particle size: • 0.5x0.5 cm • 125-150 micron • 63-90 micron • ≤38 micron Sample weight: 1 g	MAE	50% (v/v) ethanol/water	40:1	30	60°C
Maximising extraction yields (CHAPTER 5 work)						
Extraction time and temperature	Dry CPH ≤38 micron Sample weight: 1 g	• MAE • CSE	50% (v/v) ethanol/water	40:1	1,5,10, 15,30	50, 60, 70°C
Ethanol concentration	Dry CPH ≤38 micron Sample weight: 1 g	• MAE • CSE	• Deionised water • 10% (v/v) ethanol/water • 30% (v/v) ethanol/water • 40% (v/v) ethanol/water • 50% (v/v) ethanol/water • 60% (v/v) ethanol/water • 70% (v/v) ethanol/water • 90% (v/v) ethanol/water • 100% (v/v) ethanol (absolute ethanol)	40:1	5	• MAE:50, 60°C • CSE: 50, 70°C
Solvent-to-feed (S/F) ratio	Dry CPH ≤38 micron Sample weight: 1 g	• MAE • CSE	50% (v/v) ethanol/water	20:1; 30:1; 35:1; 40:1; 50:1	5	• MAE: 50, 60°C • CSE: 50, 70°C

Parameter	CPH sample	Extraction method	Solvent	S/F ratio (mL/g)	Time (min)	Temperature (°C)
Extraction at maximum conditions (CHAPTER 5 work)						
Maximum condition	Dry CPH with particle size: • 0.5x0.5 cm • 125-150 micron • 63-90 micron • 38-63 micron • ≤38 micron Sample weight: 1 g	• MAE • CSE	50% (v/v) ethanol/water	40:1	5	50 °C
Extraction of CPH layers (CHAPTER 6 work)						
Particle size	Dry epicarp, mesocarp, endocarp layers of CPH with particle size: • 0.5x0.5 cm • 125-150 micron • 63-90 micron • 38-63 micron • ≤38 micron Sample weight: 1 g	• MAE • CSE	50% (v/v) ethanol/water	40:1	5	50 °C

3.5 Quantitative analysis of CPH extract

Quantitative analysis of CPH extracts: phenolic, anthocyanin, and antioxidant contents, was determined using a spectrophotometric method based on Beer-Lambert Law. Cell path length and sample concentrations are directly proportional to the light's absorbance, as follows in Equation 3.11.

$$I = I_o e^{-aCl} \qquad [3.11]$$

Where I is intensity; I_o is initial intensity; a is constant of proportionality; C is molar concentration of solute (mol/L or mg/L); l is cell path length (cm).

UV/Vis spectrophotometer analysis was conducted at UV (180 – 400 nm) and visible (400 – 700 nm) spectrum regions. As a consequence, each sample should have a chromophore group, a functional group which absorbs UV or visible radiation, such as saturated aldehyde (–CHO) and ketone (–C=O–), which will correspond to maximum absorption wavelength (λ_{max}). Thus, samples containing compounds that are not

chromophores (including $–CH_3$, –Cl, $–NH_2$, –OH) should be modified or complexed with a chromophore reagent or complex solution (Gordon and Macrae, 1987). For example, phenolic compounds in a sample solution are not chromophores, so they should be complexed with a Folin-Ciocalteau reagent which has chromophore groups.

Quantitative measurements are performed by setting the absorbance to zero with a reference solution and then replacing this with an identical cuvette containing the clear sample solution (free from dispersed solid particles). For the visible region, glass or plastic cuvette can be used, but a quartz cuvette must be required for UV ranges. To calculate the concentration of target compounds in the sample, a calibration curve must be prepared and used to convert the absorbance value to the sample's concentrations according to Equation 3.11 of the Beer-Lambert Law.

3.5.1 Determination of total phenolic content (TPC) by colourimetric method

The TPC of each CPH extract was measured using a colourimetric method with the Folin-ciocalteu reagent, as described by Galan et al. (2017). A colourimetric reaction is widely used because of its ease, rapid, applicable and low cost (Blainski et al., 2013). A specific redox reagent known as Folin-Ciocalteau was prepared by initially diluting 100 g of sodium tungstate ($Na_2WO_4.2H_2O$) and 25 g of sodium molybdate dihydrate ($Na_2MoO_4.2H_2O$) in 700 mL of distilled water. Then, 50 mL of concentrated HCl and 50 ml of 85% phosphoric acid were added to acidify. When 150 g of $LiSO_4.4H_2O$ was added after the mixture had boiled for 10 hours and cooled, a yellow solution of Folin-ciocalteau reagent was produced containing heteropoly-phosphotungstates/molybdates complex (Sanchez-Rangel et al., 2013). All phenolic compounds in extract solution would be oxidised by Folin-Ciocalteau reagent, resulting blue chromophore complex that could be measured using a UV/Vis Spectrophotometer (Figure 3.5). The amount of reactive phenolic compounds in the sample is proportional

to the intensity of the blue colour. To complete this reaction, sodium carbonate must be added to achieve a pH of ~10 condition. Under basic conditions, the phenolic proton dissociates to generate a phenolate ion, which can decrease the Folin reagent (Sanchez-Rangel et al., 2013). According to Blainski et al. (2013), the colourimetric method requires a reference compound to calculate the total phenolic concentration in the extract; gallic acid is frequently used as a reference substance for determining TPC as it is affordable, water-soluble and stable in dry form, with a maximum wavelength of 760 nm.

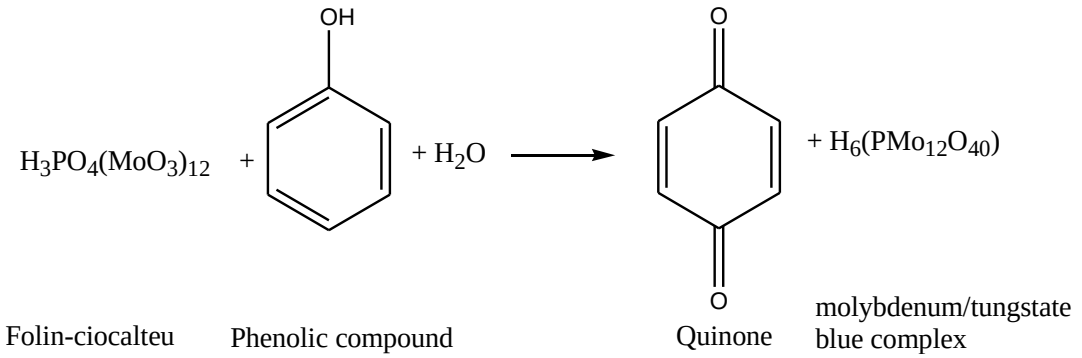


Figure 3. 5 Reaction between phenolic compounds and Folin-ciocalteu reagent

TPC analysis was started by mixing 0.5 mL of the sample with 0.5 mL of Folin-Ciocalteu reagent 1 N and 7.5 mL of ultrapure water in a 15 mL vial while stirring for 3 min at 300 rpm. The mixture was left in the dark (room temperature) for 1 hour after adding 1.5 mL of sodium carbonate solution 200 g/L. Finally, using a UV/Vis Spectrophotometer (Cecil CE-1021, UK; Cecil CE-1020S, UK; Shimadzu UV-1280, Japan), the absorbance of the extract was read at 760 nm, which was selected as the maximum wavelength based on scanning the spectrum of gallic acid standard, as shown in Figure 3.6.

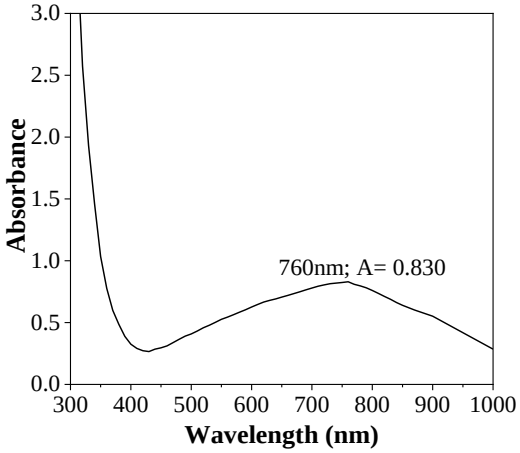


Figure 3. 6 Spectrum of gallic acid 100 mg/L

The extract concentration was calculated using the gallic acid calibration curve (Figure 3.7), and each extract's TPC was determined following Equation 3.12. All analyses were performed in triplicate, and the TPC was quantified as milligram gallic acid equivalents per gram dry weight of CPH sample (mg GAE/g dw).

$$TPC = c \times \frac{V_{extract}}{W_{sample}} \qquad c = \frac{y - intercept}{slope} (\text{from calibration curve}) \qquad [3.12]$$

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 a litre, and W is weight of dry CPH in gram. The TPC of each extract was calculated by the steps presented in Appendix B.1.

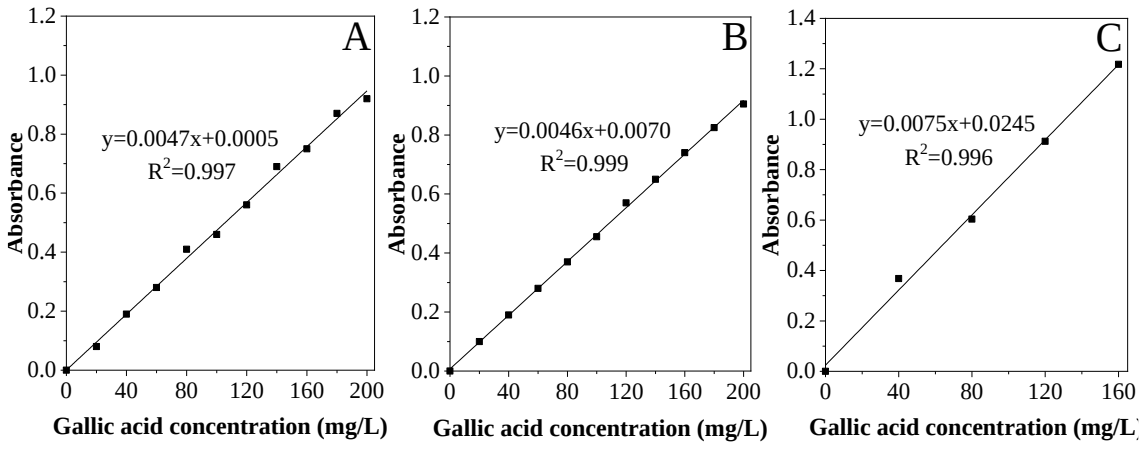


Figure 3. 7 Calibration curve of gallic acid standard using (A) UV/Vis Spectrophotometer Cecil CE-1021; (B) UV/Vis Spectrophotometer Cecil CE-1020S; (C) UV/Vis Spectrophotometer Shimadzu UV-1280

3.5.2 Determination of total monomeric anthocyanin (TMA) using pH differential method

TMA was measured using pH differential method, which is derived from the structural transformation of anthocyanin chromophore at pH 1.0 and 4.5 (Figure 3.8), as described by Lee et al. (2005). Monomeric anthocyanins reversibly transform in response to pH, becoming coloured oxonium structure (orange to purple) at pH 1.0 and colourless hemiketal structure at pH 4.5. The sample was read at two wavelengths: 520 nm and 700 nm, of which 520 nm detecting monomeric anthocyanin and 700 nm correcting for haze (Figure 3.9).

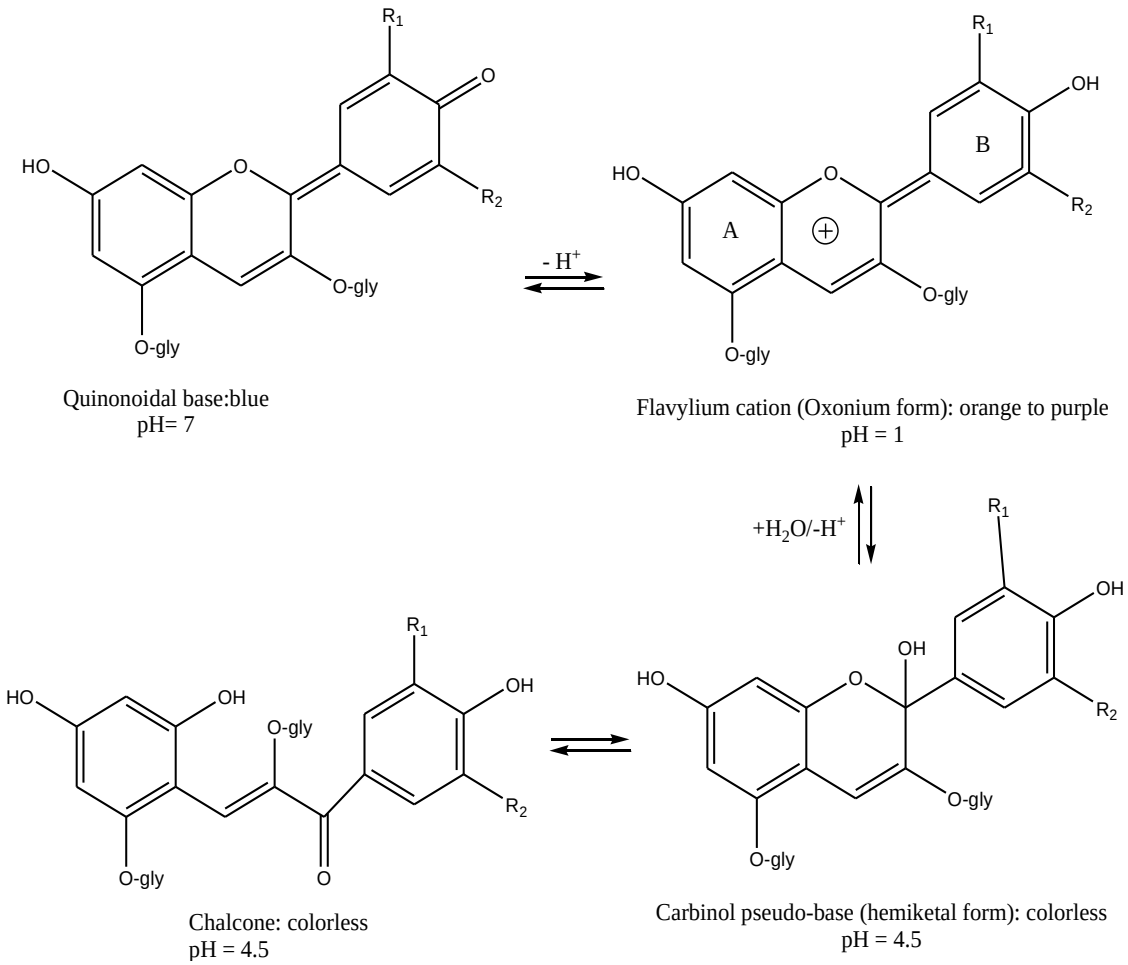


Figure 3. 8 Predominant anthocyanin structural forms present at different pH levels (Lee et al., 2005)

For conditioning extract, two buffer solutions of pH 1.0 and 4.5 were used to dissolve the extract. To prepare pH 1.0 buffer solution, 1.864 g potassium chloride (KCl) was dissolved in 960 mL deionised water, adjusted to pH 1.0 using HCl, and diluted into 1 L to produce pH 1.0 buffer solution containing KCl 0.025 M. Using the same procedure as pH 1.0 buffer solution, a pH 4.5 buffer solution (CH_3COONa) was prepared. Sodium acetate (32.814 g) was dissolved in 960 mL of deionised water and added HCl to bring the solution to pH 4.5 before being diluted to 1 L.

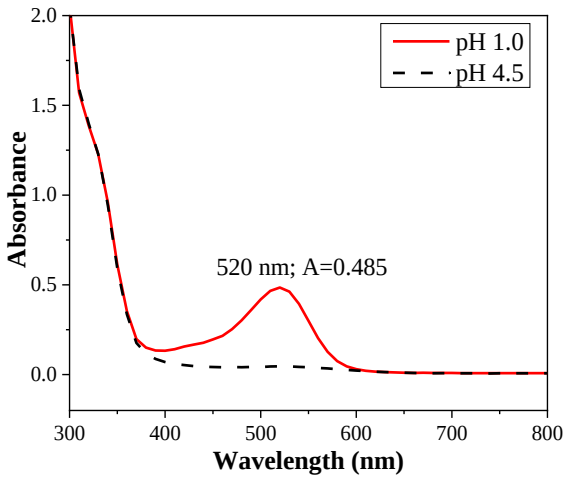


Figure 3. 9 Spectrum of anthocyanin (blueberry extract) in pH 1.0 and 4.5

In order to determine the extract's TMA, 0.5 mL of each extract was diluted in 4.5 mL of each buffer solution. Afterwards, UV/Vis Spectrophotometer measured each sample's absorbance at 520 nm and 700 nm. Analysis was performed in triplicate. TMA is quantified as cyanidin-3-glucoside equivalent per gram dry weight of CPH sample (mg Cy_3GE/g dw), as follows Equation 3.13:

$$TMA = \frac{A \times MW \times df \times 10^3}{\varepsilon \times l} \text{ x } \frac{V_{extract}}{W_{sample}} \qquad [3.13]$$

$$A = (A_{520} - A_{700})_{pH\ 1.0} - (A_{520} - A_{700})_{pH\ 4.5} \qquad [3.14]$$

Where, A is the difference in absorbance of each sample that can be calculated using Equation 3.14; MW is molecular weight for Cy_3Glu = 449.2 g/mol; df is diluting factor;

10^3 is the conversion factor from g to mg; molar extinction coefficient (ε) = 26,900 L/cm.mol; l is pathlength in cm (1 cm); V is volume of extract in litre; and W is weight of dry CPH in gram. The TMA of each extract was calculated by the steps presented in Appendix B.2.

3.5.3 Determination of antioxidant activity (AOA) using DPPH radical scavenging assay

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) method is selected to assess extracts' AOA as it is a quick and simple method with low toxicity and inexpensive to quantify antioxidant inhibition. DPPH assay is most popular and frequently used to test the ability of compounds to act as free radical scavengers or hydrogen donors and to evaluate the antioxidant activity of foods (Hangun-balkir and Mckenney, 2012; Pyrzynska and Pękal, 2013) and has been validated by the following publications: (Brand-Williams et al., 1995; Hangun-balkir and Mckenney, 2012; Plank et al., 2012; Pyrzynska and Pękal, 2013; Teboukeu et al., 2018). DPPH is a stable free radical with a delocalised electron, so it requires a proton to be non-radical. In the meantime, antioxidants have a proton to donate to DPPH free radicals. The reaction between DPPH free radicals and antioxidants results in proton transfer, which is characterised by a colour change from purple (DPPH free radical) to yellow (DPPH-H) (Figure 2.6).

Each phenolic extract's AOA was measured using DPPH radical scavenging assay, modified from the procedure described by Brand-Williams et al. (1995). To begin, DPPH stock solution was first prepared by dissolving 24 mg of DPPH in 100 mL of absolute ethanol. Following that, 250 mL of absolute ethanol was added to 100 mL of DPPH stock solution to produce DPPH working solution that had an absorbance of 1.1 ± 0.05 at 517 nm. A 3.9 mL of DPPH working solution was reacted with 0.1 mL of phenolic extract and the mixture was left in the dark for 30 min. The incubation time

(30 min) was selected from the preliminary observations shown in Figure 3.10; the decrease in absorbance started at 15 min and was slightly reduced to 30 min before remaining stable after 30 min. As a result, 30 min was picked as the incubation time for this study. After 30 min incubation, the absorbance of free radical scavenging activity in sample was read using a UV/Vis Spectrophotometer (Cecil CE-1020S, Shimadzu UV-1280) at 517 nm. This maximum wavelength (517 nm) was selected from the spectrum scan of the DPPH solution (Figure 3.11). All analysis was performed in triplicate.

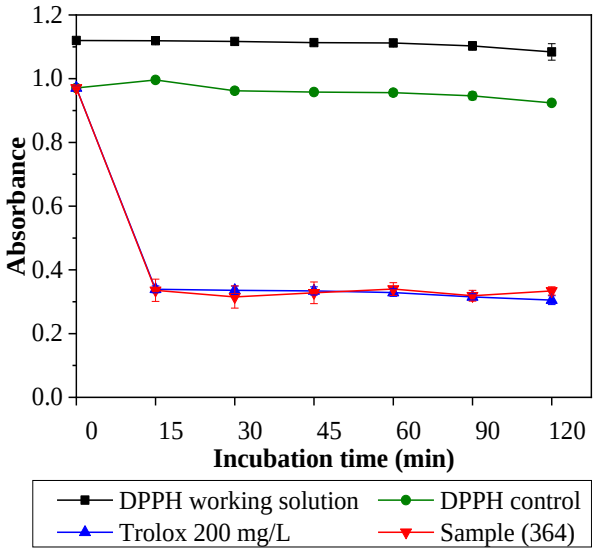


Figure 3. 10 Preliminary study for selecting the incubation time

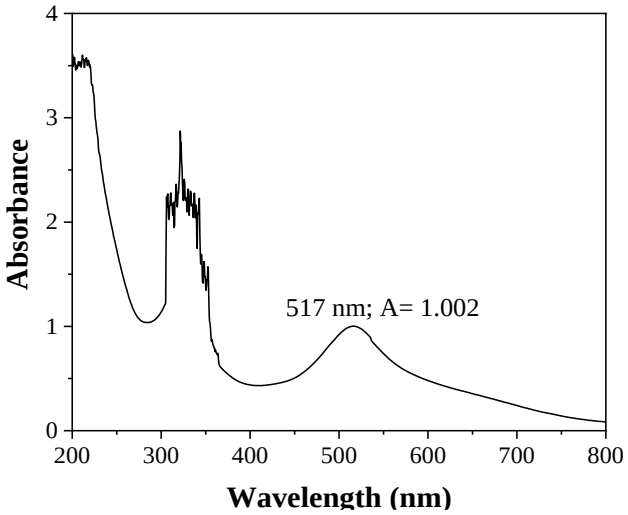


Figure 3. 11 Spectrum of DPPH working solution

The AOA of each extract was calculated following Equation 3.15, and sample concentration (C) was determined using a calibration curve of Trolox standards (Figure 3.12).

$$AO = \frac{C \times V_{extract}}{W_{sample}} \quad \text{and} \quad A = A_{DPPHcontrol} - A_{sample} \qquad [3.15]$$

Where AO is antioxidant activity in mg TE/g dry weight; C is concentration of DPPH with sample (mg/L); V is extract volume (L); W is weight of CPH (g); $A_{DPPHcontrol}$ is absorbance of DPPH working solution after 30 min incubation; A_{sample} is absorbance of DPPH with sample. The AOA was expressed as mg Trolox equivalent per gram dry CPH weight (mg TE/g dw). The AOA of each extract was calculated by the steps presented in Appendix B.3.

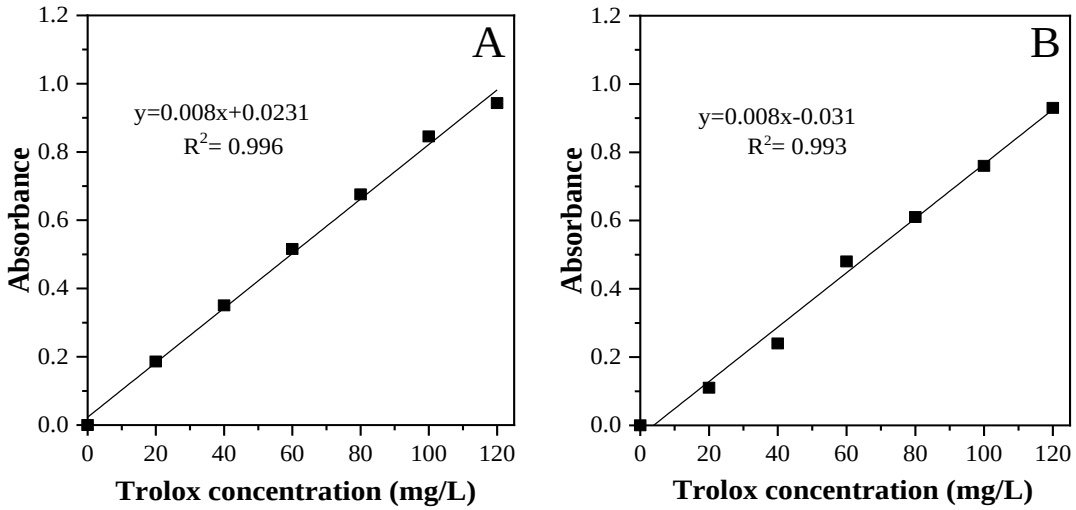


Figure 3. 12 Calibration curve of Trolox standard using UV/Vis Spectrophotometer at 517 nm: (A) UV/Vis Spectrophotometer Cecil CE-1020S; (B) UV/Vis Spectrophotometer Shimadzu UV-1280

3.5.4 Identification of phenolic compounds using High-Performance Liquid Chromatography (HPLC)

Using High-Performance Liquid Chromatography (HPLC), individual phenolic compounds in CPH extract were identified. The fundamental chromatographic process involves the partitioning of target molecules between the mobile phase and stationary

phase, in which the liquid mobile phase flows over the stationary phase in a vertical column. The sample molecules within the mobile phase will travel down the column, and the separation occurs between the phases at different extents. Molecules with similar polarity to the column will interact with the active compounds in the stationary phase and have a longer retention time, and vice versa. The chromatographic column is the "heart" of the system, where separation occurs; only when columns are efficiently packed will a good resolution be obtained. The most column used is 4.6 mm i.d. and 15-25 cm in length, packed with stationary phases of 5-10 µm particle size. (Gordon and Macrae, 1987).

A normal-phases, reserved-phase or ion exchange chromatographic mode is typically required for an HPLC examination of any crude mixture. The most widely used to separate phenolic compounds in plant extracts is reserved-phase HPLC (RP-HPLC), which uses the stationary phase that is less polar than the eluting solvent. RP-HPLC employs a nonpolar column, frequently made of surface-modified silica (Rme_2SiCl, R is a straight chain alkyl group), such as C_{18} silica. The mobile phase is typically composed of water and miscible organic solvents, such as acetonitrile (AcN), methanol, or tetrahydrofuran (THF). Buffers, acids, or bases are sometimes added to suppress compound ionisation or control the degree of ionisation of free unreacted silanol groups in order to reduce peak tailing and improve chromatography (Sarker and Nahar, 2015).

The main components of a simple HPLC chromatograph are presented in Figure 3.13. The solvent or mobile phase must be degassed and be free of particulate material. The sample is initially injected via an injector into a holding loop which is then connected to the mobile phase stream. They are pumped with specific flow rates, depending on the column dimensions; for example, a column with 4.6 mm internal

diameter uses a flow rate of 1-2 mL/min in HPLC. UV is undoubtedly the most widely used as the detector; the wavelength used was selected not always from the λ_{max} (maximum wavelength) value, but it should be chosen which is good sensitivity for target compounds and good selectivity over the interfering compounds (Gordon and Macrae, 1987).

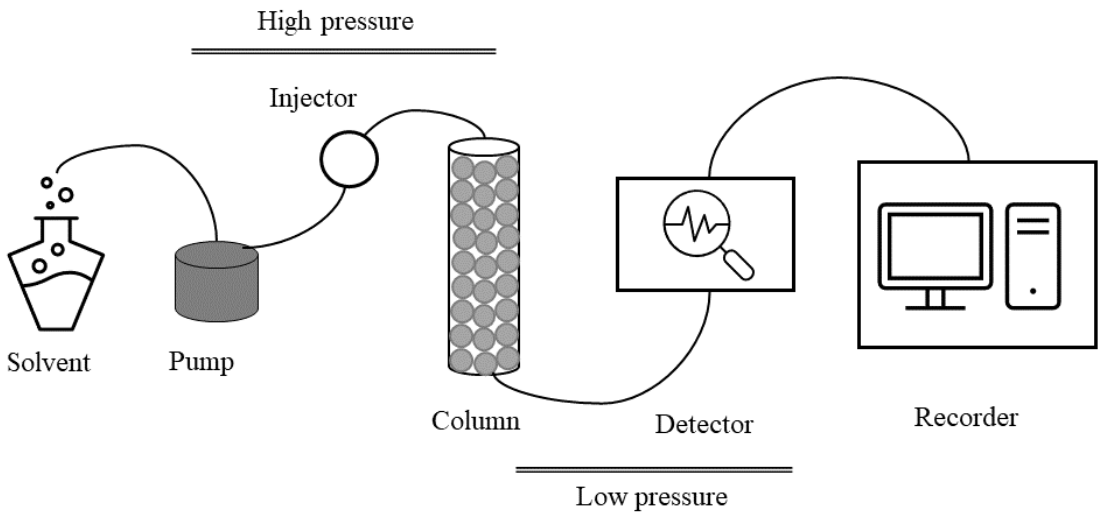


Figure 3. 13 Basic High-Performance Liquid Chromatography (HPLC) system (Gordon and Macrae, 1987)

The RP-HPLC, Agilent HPLC series 1260 Infinity II (Agilent Technologies Co, Ltd, USA) with a variable wavelength detector (HPLC-VWD), was used to identify phenolic compounds in CPH extracts according to the procedure described by Gottumukkala et al. (2014) with modifications. Prior to analysis, each CPH extract (2 mL) was filtered through a 0.2 μm syringe filter (Regenerated Cellulose Membrane, Sartorius Minisart). Ten-microliters of each sample was individually injected into a reverse-phase column (Water Sunfire C18, 250 x 4.6 mm in diameter, 5 μm particle size) by an automatic injector, and the temperature was maintained at 30 °C using a column oven. Analysis was run for 50 min using mobile phase: 0.01% orthophosphoric acid in ultrapure water (A) and acetonitrile (B), with a flow rate of 1 mL/min under the

following gradient elution: 0.01 min, 11% B; 30 min, 25% B; 35 min, 100% B; and 40 to 50 min, 11% B. Orthophosphoric acid was used as acid solution to reduce the peak tailing due to compound ionisation. The phenolic compounds were monitored by UV detector at 280 nm.

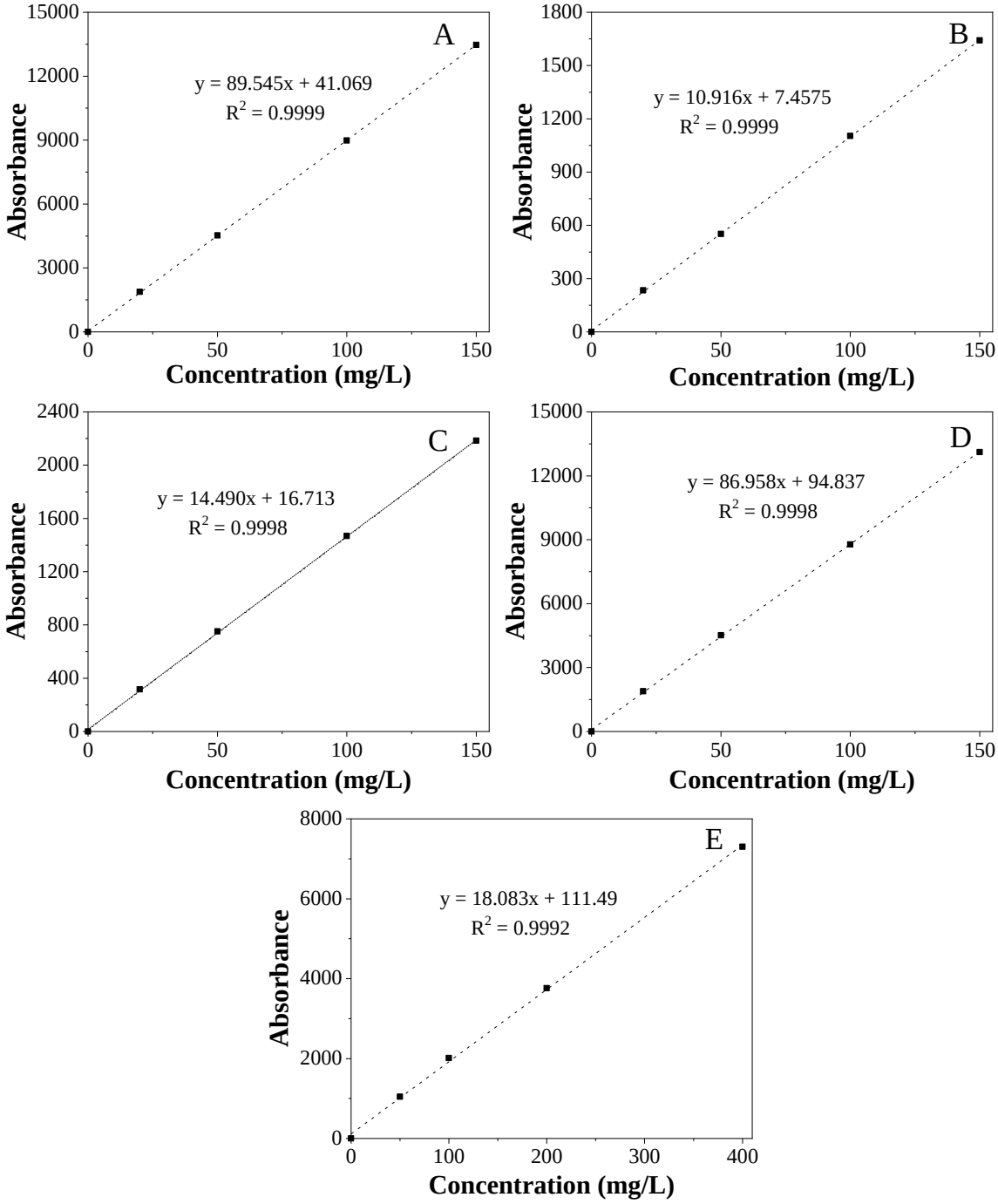


Figure 3. 14 Calibration standard curve used for chromatographic analysis: (A) Gallic acid; (B) Catechin; (C) (-)-Epicatechin; (D) p-Coumaric acid; and (E) Quercetin

The retention time of the following standard solutions: gallic acid, (+)-catechin, (-)-epicatechin, p-coumaric acid, and quercetin (Sigma Aldrich) was used to identify the individual phenolic compound, and their concentrations were calculated using the calibration curve of each standard, with concentrations ranging from 50 to 400 µg/mL for quercetin and 20 to 150 µg/mL for other standards (Figure 3.14).

3.6 Dielectric properties measurement

3.6.1 Solvent

The solvent's dielectric properties were performed using an 85070E Dielectric Probe Kit, following the reported method by Galan et al. (2017). The instrument is made of a performance probe equipped with an electronic calibration (Ecal) module connected to the Agilent PNA-L Network Analyzer N5232A (300 kHz-20 GHz) via a high-quality coaxial cable. Each 40 mL of solvent was heated at a temperature ranging from 20 to 70 °C. After the solvent was taken out of the heating process, the dielectric properties were read immediately at 500 kHz – 5 GHz by immersing the performance probe in the solvent. Dielectric constant (Ɛ') and dielectric loss factor (Ɛ") were recorded using Agilent Technologies 85070 software. The measurements were carried out in triplicate.

3.6.2 Solvent with CPH

The dielectric properties of the CPH-solvent mixture were also determined using an Agilent 85070E Dielectric Probe Kit. A 100 mL beaker containing one gram of CPH powder and 40 mL solvent was heated between 20 and 70 °C. The mixture's dielectric properties were then measured using the same procedure described in Section 3.6.1.

3.7 Statistical Analysis

All extraction experiments and extracts analyses were carried out in triplicate and the results are expressed as the mean ± standard deviation (SD). The statistical analyses were performed using JASP 0.17.1 Software and the significant differences were evaluated using analysis of variance (ANOVA) with Tukey test at the 5% probability level. P-values below 0.05 ($p_{value} < 0.05$) were considered to be significant differences between treatments. All ANOVA calculations are presented in Appendix F.

CHAPTER 4: RESULTS AND DISCUSSION – PRELIMINARY EXTRACTION STUDY

4.1 Introduction

CPH has been widely reported to contain phenolic compounds with antioxidant activity (Karim et al., 2014a; Martínez et al., 2012; Nguyen et al., 2020; Valadez-Carmona et al., 2017), which has been tested to prevent lipid oxidation in food products (Teboukeu et al., 2018). The first step in investigating the CPH's phenolic compounds and their functionalities is solvent extraction. It should be noted that there are lots of important extraction variables affecting the yield and activity of phenolics, thus, the best extraction process must be decided. A preliminary study to select the appropriate solvent, the most effective extraction method and material pretreatments (drying and size reduction) for extracting the bioactive compounds from CPH is discussed in this chapter.

As part of the extraction process, sample pretreatment is an essential step in conditioning samples and improving the accessibility of bioactive compounds during extraction. Drying is a sample pretreatment to reduce the moisture content in fresh plant material, which aims to extend shelf life, prevent microbial activity, decrease plant respiration (Nguyen et al., 2021), and minimise transportation costs. Since CPH was reported to be highly perishable, the drying effect was the first factor had been monitored in this phenolic extraction. Dehydration or drying on CPH can prevent spoilage and extend shelf life (Valadez-Carmona et al., 2017), but it can also degrade phytochemical compounds, lowering extraction yields. Therefore, thermal drying techniques with relatively low temperature and/or short time could be recommended for drying industry (Si et al., 2016). On the other hand, sample pretreatment with size reduction can help break down the plant cell and so increase the extractability of

phenolic compounds during the extraction process. Brewer et al. (2014) found that size reduction on wheat bran markedly increased the total phenolic (TPC), flavonoid, anthocyanin and carotenoid contents but decreased the extracts' antioxidant properties (DPPH assay). They assumed that DPPH's redox potential is different from molybdenum (VI)'s Folin-Ciocalteu reagent for TPC analysis, which would explain why DPPH and TPC did not always exhibit the same trend.

The extraction process was the next investigation because extraction conditions might affect the extraction yield and its functionality. Solvent selection is a crucial aspect of extraction's success because the use of appropriate solvent will solubilise all phenolic compounds while leaving unwanted compounds. The solvent was reported to be the most influential factor in extraction of phenolic content and antioxidant compounds from CPH (Teboukeu et al., 2018). Hansen solubility parameter (HSP) value can predict the proper solvent for extracting phenolic compounds in order to increase solvent's selectivity. Charles M. Hansen (1967) developed HSP theory on the basis of "like dissolve like" principle, which includes three major interactions contributing to the cohesive energy density: dispersion forces, polar forces, and hydrogen bonding (Hansen, 1967). In terms of solubility parameter, cohesive energy is the amount of energy required to overcome the intermolecular force between two molecules. In a simple way, the HSP (δ) represents the influence of dispersion (δ_d), polar effects (δ_p), and hydrogen bonding contributions (δ_d) in the solubility of materials. Two materials are more likely to dissolve one another when their solubility parameters are similar.

In addition, selecting an effective extraction method is also essential to enhance the extraction yield. A variety of solvent extraction techniques have been observed to extract the phenolics from CPH: maceration (Martínez et al., 2012), sequential

conventional solvent extraction (CSE) (Karim et al., 2014a; Nguyen et al., 2021; Teboukeu et al., 2018; Yapo et al., 2013), microwave-assisted extraction (MAE) (Mashuni et al., 2020; Nguyen et al., 2020). The phenolic content obtained by MAE was reported to be ~29% higher than conventional extraction (Nguyen et al., 2020). Others (Dahmoune et al., 2015; Li et al., 2017; Pan et al., 2003) pointed out that MAE achieved a high phenolic yield while performing extraction in a shorter time and at a low temperature. MAE offers the main advantage of volumetric heating, in which the electromagnetic energy is dissipated to heat directly inside the system, resulting in rapid heating, so it can significantly reduce the processing time (Metaxas and Meredith, 1983). Direct interaction between the microwave energy and mixture system (plant material and solvent) led to plant cell rupture and the quick release of intracellular products into the solvent (Gharekhani et al., 2012; Zhou and Liu, 2006). MAE can also offer a selective heating effect leading to an increase in TPC yield. The plant matrix of sea buckthorn leaves has been reported to be selectively heated above 50 °C, so microwave heating offers an increased yield at and above 60 °C due to the selective heating effect (Galan et al., 2017).

The works of literature indicate that CPH contains phenolic compounds that may be of value as antioxidants (Karim et al., 2014a; Martínez et al., 2012; Nguyen et al., 2020; Valadez-Carmona et al., 2017); CPH extract also contains pigments (Nguyen, 2015) but its pigment type and concentration have not been reported. While Vriesmann et al. (2011) mentioned that flavan-3-ols (epicatechin, catechin, and procyanidin) with small amounts of anthocyanin pigment were present in cacao. Therefore, it is possible that pigment content in CPH extract is in the form of anthocyanin. Anthocyanin is a class of phenolic compounds which is essential as a natural food colourant. Hence, investigating anthocyanin amounts is also important to understand the possibility of

CPH extract for food colourants. Moreover, phenolic and anthocyanin compounds have antioxidant properties which can be applied in the food industry (Khoo et al., 2017; Teboukeu et al., 2018). Therefore, a preliminary study of various extraction conditions that affect the phenolic extraction from CPH needs to be done. Not only the total phenolic content (TPC) yield would be assessed as a parameter of extract quality in this experiment, but their total monomeric anthocyanin (TMA) and antioxidant activity (AOA) were also evaluated. The objectives of this chapter, therefore, are to (1) evaluate the influence of CPH pretreatment (drying and size reduction) on the extract yields and functionality; (2) evaluate the appropriate solvent for selective phenolic extraction based on HSP estimation; (3) investigate the effect of extraction method (conventional and microwave) on the yield and functionality of the extract.

4.2 The influence of drying on extraction yields

Section 4.2 will be part of a manuscript prepared for Journal Biomass and Bioenergy:

Dewi, S.R, Stevens, L.A, Ferrari, R., Irvine, D.J, Binner, E.R., Extraction of phenolic-based antioxidants from cacao pod husk (CPH): implications for scaling-up process and potential applications of CPH solid residue

Since fresh CPH is very perishable due to high moisture content (86.71 ± 0.68%), a preliminary extraction study began with understanding the influence of CPH drying on the extraction yields. Conventional drying, such as sun drying and hot-air drying, are most commonly used due to their affordability. By hot-air drying, the heat generated will be transferred to the plant material through conduction and convection heating. The effect of size reduction and solvent loading in the experiments were included as part of this preliminary study. Firstly, CPH was dried using a force-air dryer at 50 °C in order to stabilise it and extend its shelf-life. The low temperature was set during drying to minimise the degradation of bioactive compounds in CPH. The yield of CPH drying was about 13% (w/w) dried sample per gram fresh sample. This means that 10 kg of

fresh CPH will obtain 1.3 kg of dry CPH. Nguyen et al. (2021) reported that the fresh CPH has a high moisture content of about 89.11% (w.b) and the drying yield ranged from 13.7 to 17.32 g dried sample per fresh sample.

Prior to comparing the extraction yields of fresh and dry CPH to look into the drying effect, how the size reduction and solvent loading affected the extraction of fresh CPH has been investigated. Fresh CPHs with and without size reduction (grinding) were extracted using reflux at different solvent-to-feed (S/F) ratios. Reflux is a simple extraction with reproducible evaporation and condensation of solvent at constant boiling temperature and ambient pressure (Zhang et al., 2018).

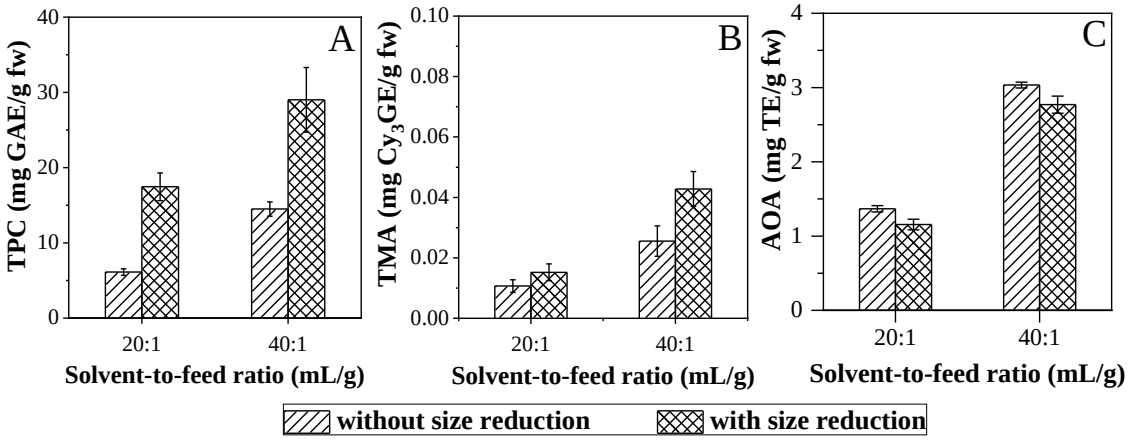


Figure 4. 1 Extraction of fresh CPH (Reflux extraction, fresh CPH without size reduction (0.5x0.5x0.5 cm) and with size reduction (blend), 50% (v/v) ethanol/water solvent, 60 min): (A) Total phenolic content (TPC); (B) Total monomeric anthocyanin (TMA); (C) Antioxidant activity (AOA), mean ± S.D (n= 9, triplicate extraction and triplicate analysis)

The results (Figure 4.1) showed that size reduction could increase phenolic yields but decrease antioxidant activity (AOA). This data coincides with the finding of Brewer et al. (2014) that antioxidant properties of wheat bran extract declined because of grinding. Size reduction could increase the contact surface of biomass, so improving the bioactive yields, but at the same time, the thermolabile antioxidant compounds could be degraded due to heat. Due to the crucial impact of size reduction on extraction yields, further experiments to evaluate the effect of different CPH particle sizes on the

extraction process will be addressed in Section 4.5. On the other hand, a comparison of the S/F ratio of 20:1 and 40:1 mL/g showed that the TPC, TMA and AOA yields increased in high solvent volume. Valadez-Carmona et al. (2017) also used an S/F ratio of 40:1 mL/g to get phenolic compounds from CPH. Hence, a ratio of 40:1 mL/g was selected for this preliminary study. Detailed experiments of the solvent loading effect are shown in Chapter 5 (Sections 5.2.3 and 5.3.2).

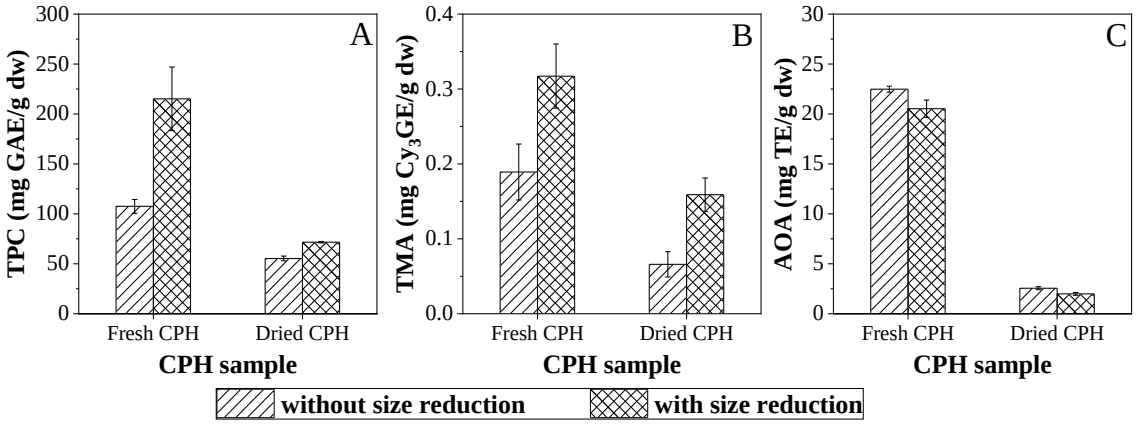


Figure 4. 2 Comparison of bioactive extraction from fresh and dry CPH (Reflux extraction, 50% (v/v) ethanol/water solvent, S/F ratio of 40:1 mL/g, 60 min): (A) Total phenolic content (TPC); (B) Total Monomeric anthocyanin (TMA); (C) Antioxidant activity (AOA), mean ± S.D (n= 9, triplicate extraction and triplicate analysis)

Figure 4.2 presents the comparison of extraction yields of fresh and dry CPH (drying effect). The extraction yields reduced significantly after drying ($p_{value} < 0.001$). TPC decreased by 49% due to drying and up to 66% by combining drying and size reduction; in contrast, the antioxidant activity of extract declined by 89%. During drying process, some volatile compounds might have been evaporated or degraded from CPH. According to Rodriguez-campos et al. (2011), drying the cocoa reduced a number of volatiles and polyphenols while developing flavour during the elimination of those volatiles; they found that alcohols (phenylethyl alcohol and benzyl alcohol), aldehyde (pentanal, phenylacetaldehyde, and 2,3-butanedione), and volatile acids (isovaleric acid, hexanoic acid, octanoic acid and nonanoic acid) decreased after the drying

process. Thus, it is reasonable to expect that a decrease in volatiles also occur during CPH drying. Another study by Kaškoniene et al. (2015) also reported a reduction of phenolic amount up to 3.5 times and antioxidant activity up to 4.5 times in *C. angustifolium* L. extract due to the drying effect. They suggested that loss of phenolic content may be attributed to the degradation of phenolics due to enzymatic or non-enzymatic reactions. It may also be caused by transformation in the chemical structure of polyphenols. Slow drying may cause alcohols oxidation, resulting in several aldehydes, such as trans-2-hexenal, phenylacetaldehyde, nonanal, decanal, terpinene-7-al, detected only in dried samples (Kaškoniene et al., 2015).

Furthermore, the study of drying (hot air oven drying and industrial rotary drying (IRD)) effect on blackcurrant pomace extraction revealed that IRD could significantly reduce extracts' total phenolics, particularly p-coumaric and ferulic acid. While IRD treatment for different particle sizes of material had various effects on the extraction yields (total phenols, anthocyanin content, and flavonols content); anthocyanin was affected by material's particle size, which the maximum anthocyanin was found from the largest particle size (> 5 mm). In contrast, total phenol and flavonols were not significantly influenced by the various particle size (Azman et al., 2021).

HPLC results (Figure 4.3 and Table 4.1) confirmed that fresh CPH extract contained gallic acid and quercetin, while in dry CPH extract, gallic acid, epicatechin and quercetin were found. In both extracts, catechin and p-coumaric could not be identified. Valadez-Carmona et al. (2017) reported the presence of gallic acid, catechin, epicatechin, p-coumaric acid, quercetin, and protocatechuic acid in both CPH extracts. These differences in results may be due to differences in analysis conditions, the extraction process or CPH clone used. Several cacao clones/varieties exist worldwide, such as Criollo, Forastero, and Trinitario, with different primary components. For

instance, the Criollo clone contained high caffeic acid aspartate but low in anthocyanin, whereas Forastero clone had high anthocyanin but low caffeic acid. However, both clones had similar epicatechin amounts (Elwers et al., 2009). Another study reported that CPH with different clones/varieties or geographical origin, ripening stages, location of materials collected, collection periods, climate and storage conditions might have different physicochemical and biochemical compositions, including phenolic compounds (Ouattara et al., 2021).

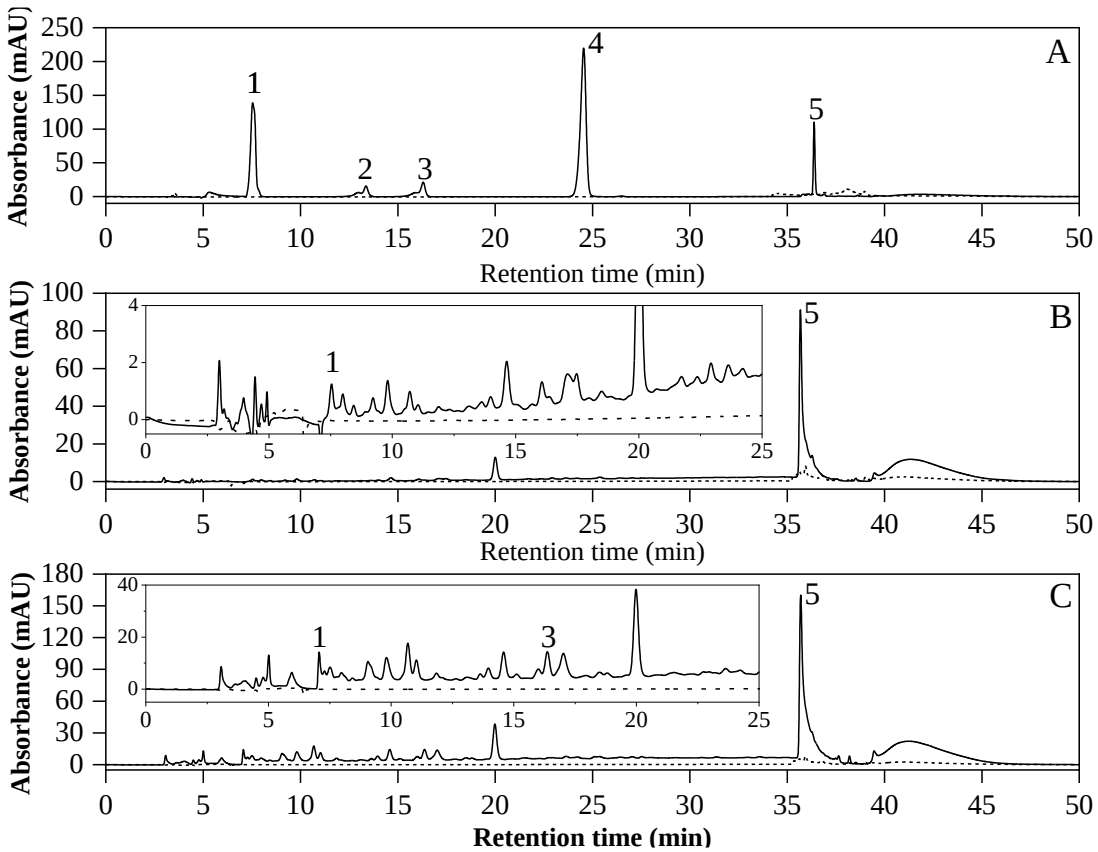


Figure 4. 3 HPLC chromatograms of phenolic compounds at 280 nm: (A) Standard compounds; (B) Fresh CPH extract; (C) Dry CPH extract: [1] Gallic acid; [2] Catechin; [3] (-)-Epicatechin; [4] p-Coumaric acid, [5] Quercetin; the dash line (---------) is blank solvent

According to the HPLC chromatogram in Figure 4.3, the fresh CPH extract had more peak numbers, which indicated that there were more compounds, including phenolics. This also confirmed that the drying process could degrade or evaporate some individual thermolabile compounds, so those compounds were not found in dry CPH

extract. Moreover, those compounds still need to be confirmed using standard compounds or other instruments. Kaškoniene et al. (2015) reported that drying of *C. angustifolium* had two effects on the herb's composition: quantitative changes in compounds and the loss/appearance of some compounds.

Table 4. 1 HPLC phenolics profile of extract from fresh and dry CPH (µg/g CPH)

Compound name	**Fresh CPH (µg/g)****		**Dry CPH (µg/g dry CPH)**
	(µg/g fresh CPH)	**(µg/g dry CPH)**	
Gallic acid	1.31 ± 0.63	9.7 ± 4.7	9.5 ± 0.37
Catechin	nd	nd	nd
Epicatechin	nd	nd	307.0 ± 11.8
p-Coumaric acid	nd	nd	nd
Quercetin	2544.8 ± 257.6	18861.5 ± 1909	5176.9 ± 2365
Total	2546.11	18871.20	5541.0
TPC*	29.02	215,090.9	72,205.2

Mean ± S.D (n= 3, triplicate extraction); nd: not defined; *TPC, analysed by Folin-ciocalteau method; **concentration of individual compounds in fresh CPH is converted from µg/g fw to µg/g dw

In this study, the phenolics, anthocyanins and their antioxidant activity in dry CPH extract were lower than in fresh CPH. Fresh CPH contained 107.5 to 215.1 mg GAE/g dw with antioxidant activity ranging from 20.5 to 22.5 mg TE/g dw; while the phenolic content in dry CPH was about 55.3 to 77.2 mg GAE/g dw with antioxidant activity of 2.6 – 2.7 mg TE/g dw. The findings also confirmed that both CPH extracts contained anthocyanins (0.07 – 0.32 mg Cy_3GE/g dw), which were still relatively low. Thus, maximising the bioactive compounds in extract needs to be investigated. According to the results, it was clear that the bioactive content in fresh CPH was higher than in dry CPH. However, by considering the properties of fresh CPH, which was perishable, dry CPH was preferred as sample selected for studying the extraction of bioactive compounds from CPH. Moreover, drying can cut production or distribution costs, prevent sample oxidation or decay, and increase the quality of final products.

Even so, the extraction of bioactive compounds from fresh CPH is possible for future applications, for example, to process the CPH directly at cacao waste collection sites.

4.3 The influence of solvent type on extraction yields

Sections 4.3 – 4.5 were part of the manuscript published in the Journal of Food and Bioproducts Processing*:*

Dewi, S.R., Stevens, L.A., Pearson, A.E., Ferrari, R., Irvine, D.J., Binner, E.R., 2022, Investigating the role of solvent type and microwave selective heating on the extraction of phenolic compounds from cacao (Theobroma cacao L.*) pod husk, Food and Bioprocess Processing, 134, July 2022, 210-222. https://doi.org/10.1016/j.fbp.2022.05.011*.

Selecting a high-selectivity solvent is critical in the extraction process since the solute's solubility in the solvent is directly responsible for the extraction yield. Since phenolic compounds are known as polar compounds, the deionised water and the following group of alcohols (methanol, ethanol, 1-propanol, 1-butanol, 1-pentanol, 50% (v/v) methanol/water, and 50% (v/v) ethanol/water) were initially investigated as solvents to extract the phenolics from CPH. It may be hypothesised that phenolic compounds become more soluble from 1-pentanol to methanol with the increasing alcohol relative polarities of 0.568, 0.586, 0.617, 0.654, and 0.762, respectively (Reichardt, 1984).

Prior to extracting phenolic from CPH, the solubility of phenolic compounds in those solvents could be predicted using the Hildebrand solubility parameter (HbSP), where compounds with similar solubility parameters are miscible in most proportions. The HbSP of each solvent and gallic acid (as a phenolic standard) were calculated using Equation 1.6 proposed by Fedors (1974), and the results can be seen in Table 4.2. The detailed calculation of Hildebrand Solubility Parameter is presented in Appendix C.1. The HbSP value of gallic acid (included as a phenolic compound standard) was close

to that of 50% (v/v) methanol/water and 50% (v/v) ethanol/water. Thus, it would be theoretically predicted that either the 50% (v/v) methanol/water and/or the 50% (v/v) ethanol/water mixture would be the best solvents for phenolic extraction of the solvents surveyed. The solubility of anthocyanin was not discussed particularly here because anthocyanin is one of the phenolic compound classes.

Table 4.2 Hildebrand solubility parameter (HbSP) of solvents and phenolic compounds (gallic acid) at 25 °C

Compound	**δ ($Mpa^{1/2}$)**	**Compound**	**δ ($Mpa^{1/2}$)**	**Compound**	**δ ($Mpa^{1/2}$)**
Methanol	29.4	1-Butanol	23.8	50% (v/v) methanol/water	38.6
Ethanol	26.6	1-Pentanol	23.0	50% (v/v) ethanol/water	37.3
1-Propanol	25.0	Water	47.9	Gallic acid (phenolic standard)	39.1

Figure 4.4 shows the solubility of phenolic compounds, including anthocyanin, was affected by the solvent type ($p_{value} < 0.001$); they 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 and TMA yields compared to both the pure alcohol solvent and deionised water alone. The TMA is in line with TPC yield (Pearson's $r = 0.910$; $p_{value} = 0.012$) because anthocyanin is part of phenolic compounds. The maximum TPC and TMA were obtained using 50% (v/v) ethanol/water, 72.2±0.47 mg GAE/g dw and 0.17±0.01 mg Cy_3GE/g dw, respectively. This value was significantly different ($p_{value} < 0.001$) from the TPC yield by 50% (v/v) methanol/water, which was predicted to have approximately the same yield based on the HbSP calculations. In addition, methanol has a HbSP that was significantly lower than that of model materials, gallic acid, but delivered extraction levels that were similar to the 50% (v/v) methanol/water mixtures. It can be seen that HbSP of gallic acid compared with that of the extraction solvent alone cannot completely explain the high

solubility of phenolic compounds in 50% (v/v) methanol/water and/or the 50% (v/v) ethanol/water mixture. Therefore, in an attempt to get a better correlation, the more complex Hansen solubility parameter (HSP) was applied.

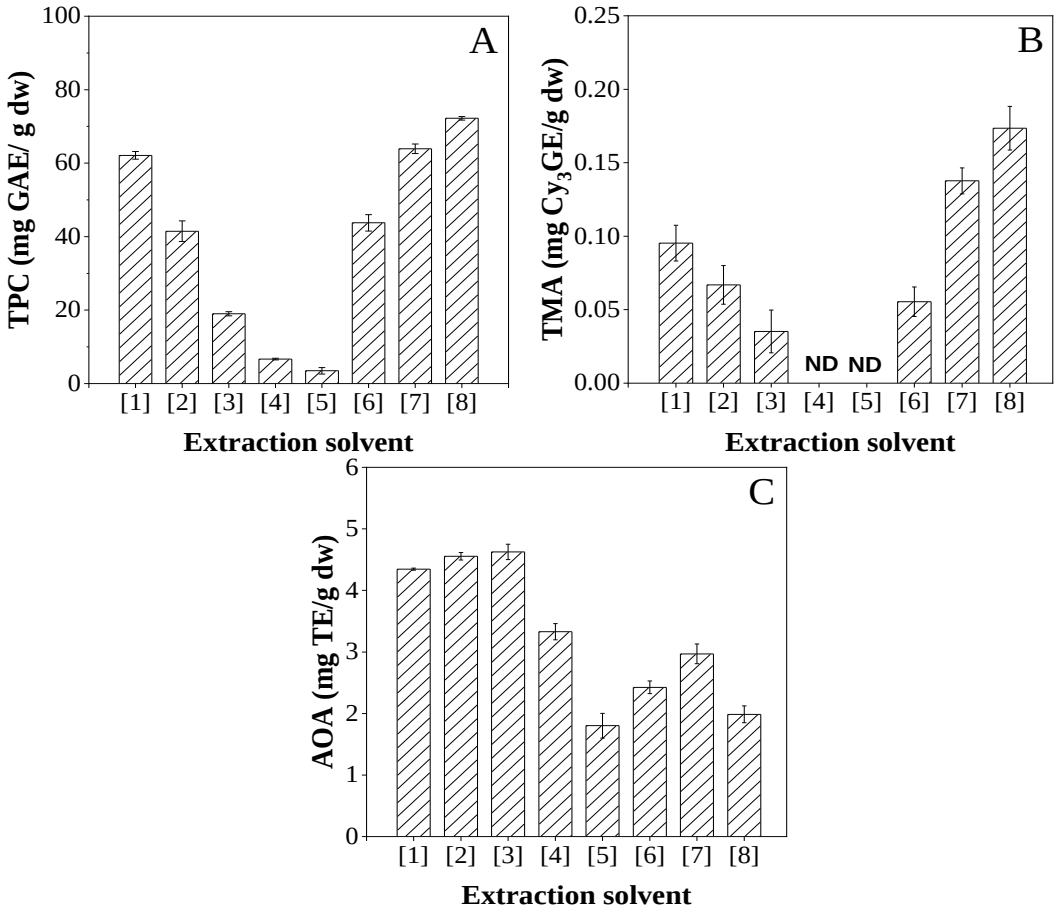


Figure 4. 4 Effect of solvent type on TPC (A), TMA (B), AOA (C) yields (Reflux extraction, dry CPH with particle size ≤150 micron, S/F ratio 40:1 mL/g, 60 min extraction time): [1] methanol; [2] ethanol; [3] 1-propanol; [4] 1-butanol; [5] 1-pentanol; [6] deionised water; [7] 50% (v/v) methanol/water; and [8] 50% (v/v) ethanol/water solvent, mean ± S.D (n= 9, triplicate extraction and triplicate analysis); ND: not determined

Hansen solubility parameter (HSP) divides HbSP into three components: δ_d indicating the dispersion effect (related to van der Waals), δ_p describing polar effects (related to dipole moment), and δ_h representing hydrogen forces (Gao, 2014), where the correlation between HbSP and HSP can be expressed by Equation 1.7. HSP value was calculated using HSPiP Sofware by entering the target molecule's SMILES (Simplified Molecular Input Line Entry System) notations, resulting in the cohesion energy in terms

of dispersion (δ_d), polar (δ_p) and hydrogen bonding (δ_h). Detailed information for calculation the HSP value can be found in Appendix C.2. The HSP values for solvent extractants and phenolic compounds are represented in Table 4.3.

Table 4. 3 Hansen solubility parameter (HSP) of solvents and phenolic compounds (gallic acid) at solvents' boiling points

Solvent	**Temp (°C)***	**HSP of Solvent (Mpa$^{1/2}$)**				**HSP of gallic Acid (phenolic compound exemplar standard) (Mpa$^{1/2}$)**				**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) Methanol/water	72	18.8	12.0	25.4	33.8	21.8	8.8	22.8	32.7	7.3
50% (v/v) Ethanol/water	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)

The higher solubility of the phenolic in 50% (v/v) ethanol/water was rationalised by using the HSP value in Table 4.3. A single component, gallic acid was used as an exemplar material to determine if this trend in solvents was predicted by the HSP method, 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 Equation 4.1, and the results are shown in Table 4.3.

$$Ra^2 = 4(\delta_{d1}-\delta_{d2})^2 + (\delta_{p1}-\delta_{p2})^2 + (\delta_{h1}-\delta_{h2})^2 \quad [4.1]$$

Gallic acid (as a phenolic standard compound) exhibits the hydrogen bonding part (δ_h) that is similar to the part of 50% (v/v) ethanol/water at 77 °C, as shown in Table 4.3. Thus, as the principle of 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 computed 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 less providing good extraction yields.

On the other hand, Figure 4.4 shows that antioxidant properties of extracts have different behaviour from phenolics (Pearson's r = 0.075; p_{value} = 0.861) or anthocyanins (Pearson's r = -0.542; p_{value} =0.267). Antioxidant activity in the lighter alcoholic extracts (methanol to propanol) was higher than in butanol, pentanol and aqueous solvents. It seems that there was no linear correlation between the presence of phenolics and the antioxidant activity of the extract when using alcoholic solvents. The possibility of a high AOA in the propanoic extract may be caused by other compounds extracted that are not phenolic classes. Therefore, other antioxidant analysis methods, such as ABTS and/or FRAP, should be tested as comparison for determining the antioxidant activities of CPH extracts.

In addition, Saito et al. (2004) reported that the reactivity of antioxidants, also known as radical scavenging activity, to the antiradical compound (methyl protocatechuate) increased in protic solvents, such as methanol, ethanol and 1-propanol, because they triggered further oxidation of the antiradical compound. The alcohol molecule or group might be added to the antiradical compound and undergo deprotonation, which then reacts with two radicals. Otherwise, the sterically bulky alkyl group on alcoholic groups such as 1-butanol or 1-pentanol could reduce the ability of compounds to scavenge the radicals. This literature then supports our findings that the high antioxidant activity of CPH extract in methanol, ethanol, or 1-propanol may also

be due to the protic solvent effect. Yet, the decrease in AOA of extract in butanol or pentanol was due to their steric effect.

According to calculated solubility (HSP) and experimental results, the data, therefore, suggests that 50% (v/v) ethanol/water was the preferable solvent for the following experiments, even though the ethanol concentration effect still needs to be addressed to maximise the anthocyanin and antioxidant yields. Furthermore, ethanol is a water-soluble solvent that is GRAS (Generally Recognised as Safe) for human consumption; it can be easily recovered through reduced pressure distillation. This section has shown that by applying the more complex HSP model, which solvent and solvent mixture types are likely to give the best extraction at specific temperatures have been predicted. Thus, it points to the use of this system going forward to choose appropriate solvents and processing temperatures.

4.4 Comparison of extraction methods

Selecting an effective extraction method that maximises phenolic yield while minimising phenolic degradation is also essential. In this section, the extraction of phenolic compounds using various conventional methods, including CSE, reflux, and maceration, was compared to MAE. As previously mentioned in several references, MAE offered a higher yield than conventional methods (Galan et al., 2017; Li et al., 2017; Nguyen et al., 2020) because of its volumetric and selective heating. Therefore, the bulk heating profile of MAE and CSE were compared in order to understand the MAE volumetric heating, as illustrated in Figure 4.5. The MAE heating time to reach 70 °C set temperature was fourfold quicker (100 seconds) than that of CSE-WB (375 seconds), confirming the microwave volumetric heating effect. In contrast, the heating process on CSE just involved conductive heating, so they needed a longer time to reach the same point as MAE. Therefore, to decouple the microwave volumetric heating, the

subsequent CSE experiments were designed to replicate the MAE bulk heating rate by using an ethylene glycol bath (CSE-EgB); samples were immersed in an ethylene glycol bath (EgB) until they achieved the set temperature before then immediately transferred to a water bath (WB) at the processing temperature. This procedure attained a similar bulk heating profile between CSE and MAE (Figure 4.5.A), negated the impact of bulk heating rate, and thus any differences observed between MAE and CSE-EgB experiments can be attributed to selective microwave heating effects. The results (Figure 4.5.B) explained that the TPC yield on MAE was 15% higher than CSE-EgB (hereinafter referred to as CSE). Therefore, we can believe that high TPC on MAE is affected by both volumetric heating/heating rate and selective heating. Thus, the ethylene glycol bath was used for further CSE experiments.

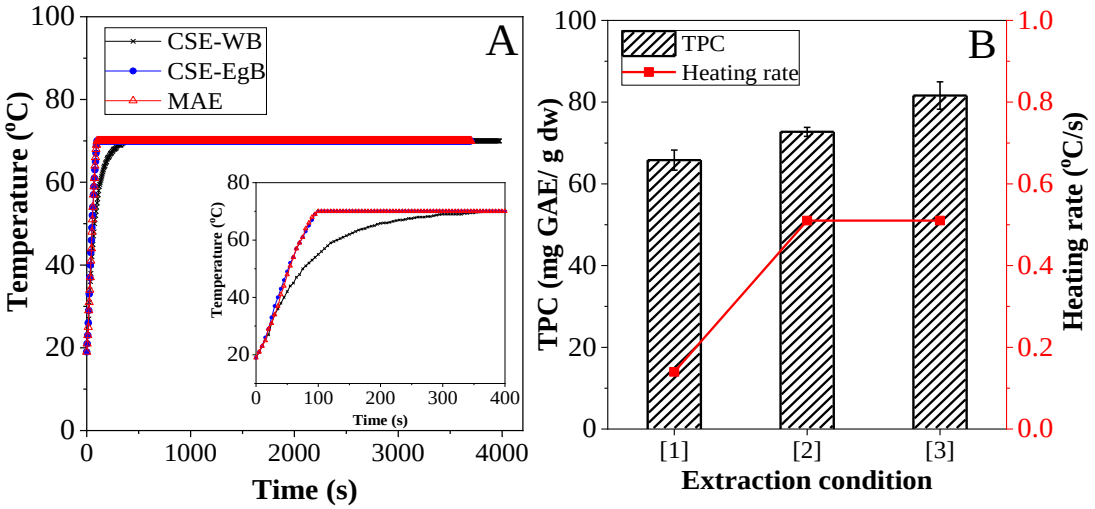


Figure 4.5 Comparison of MAE and CSE (CPH with particle size ≤150 micron, 50% (v/v) ethanol/water, S/F ratio 40:1 mL/g, 70 °C, 60 min): (A) Bulk temperature profile for MAE and CSE experiments at 70 °C set-point; (B) TPC yield and heating rate of MAE and conventional experiments: [1] CSE, water bath; [2] CSE, 120 °C ethylene glycol bath (to reach set temperature) then transferred to water bath; [3] MAE, 120 W; mean ± S.D (n= 9, triplicate extraction and triplicate analysis)

The comparison of extraction methods is shown in Figure 4.6, with significant differences in TPC and TMA (p_{value} < 0.001) among the four methods but not in antioxidant activity (p_{value} = 0.589). MAE produced the highest TPC (81.61±3.35 mg GAE/g dw) and TMA (0.26 mg Cy_3GE/g dw) compared with all conventional methods.

As previously stated, this is believed to be due to MAE's volumetric and selective heating effect. Compared to CSE with the same heating rate (volumetric heating was neglected), the TPC and TMA yields in MAE increased by 15% and 43%, respectively; this indicates the selective heating effect on MAE leads to an increase those both yields. The finding was consistent with previous work by Nguyen et al. (2020), which found that extraction of CPH using MAE yielded ~29% higher TPC over CSE. Additionally, using MAE to extract phenolic from sea buckthorn leaves led to an 8% increase in TPC compared to CSE (Galan et al., 2017). They found that the plant matrix of sea buckthorn leaves had been selectively heated at and above 60 °C.

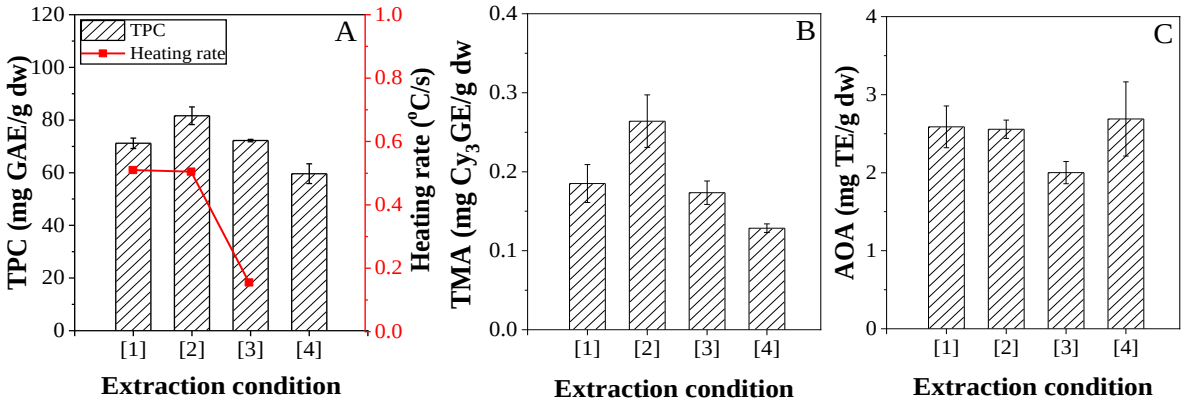


Figure 4. 6 The TPC (A), TMA (B), AOA (C) yields by using various extraction methods (CPH with particle size ≤150 micron, 50% (v/v) ethanol/water, S/F ratio 40:1 mL/g, 60 min): [1] CSE at 70°C; [2] MAE at 70 °C, 120 W; [3] Reflux at 77 °C; [4] Maceration at room temperature (20 °C), mean ± S.D (n= 9, triplicate extraction and triplicate analysis)

Reflux showed the same TPC (p_{value} = 0.853) and TMA (p_{value} = 0.688) yields as CSE, although it had a lower heating rate (400 seconds; 0.17 °C/s). It was probably because the extraction temperature on reflux (77 °C) was higher than that of CSE (70 °C). Galan et al. (2017), who extracted phenolics from sea buckthorn leaves using MAE and CSE, indicated that the phenolic content was significantly increased by increasing the extraction temperature from 40 to 80 °C. In contrast, maceration yielded the lowest TPC and TMA yields since no heating involved. It was proven that heating improved extraction rates and enhanced yields by increasing diffusion rates and solubility

(Chanioti et al., 2014). However, extraction at 70 °C (CSE and MAE) yielded the same AOA as maceration (no heating). During extraction, the phenolic yield might increase by heating, but at the same time, heat might degrade the extracted phenolic compounds which have high antioxidant activity. Hence, the AOA values of both CSE and MAE showed the same as maceration (p_{value} = 0.589), which had a low TPC. Heating seems to increase the phenolic content yet also decrease the antioxidant activity of the extract. On the other hand, the lowest AOA yield in reflux extraction also might be because degradation of the antioxidant compounds due to higher temperature (77 °C). Therefore, based on the TPC and TMA yields, MAE was selected for the subsequent experiments in order to maximise the yields on bioactive extraction from CPH.

4.5 The influence of particle size on extraction yields

Particle size is another critical factor that should be observed in phenolic extraction from biomass. In this section, the effect of CPH particle size on the extraction yield was studied using MAE, an extraction method selected from the previous discussion. According to Veggi et al. (2013), finer particle size improved extraction's contact surface area, increasing the extraction efficiency. Because phenolic compounds were found in the cell wall (Hutzler et al., 1998) and vacuole of plant cells (Ferreres et al., 2011), it was hypothesised that size reduction (grinding) would break the plant cell wall and increased the particle surface area. As a result, the bioactive compounds are more accessible to extract.

The results (Figure 4.7) showed that the size reduction has increased the TPC and TMA yields but tended to decrease their antioxidant activity (AOA) (p_{value} < 0.001). The maximum TPC and TMA yields were reached by the smallest particle size (≤38 micron), accounting for 101.8±1.3 mg GAE/g dw and 0.24±0.03 mg Cy_3GE/g dw, respectively. However, this improving extracts' yields did not increase their antioxidant

activity; AOA yields for all CPH particle sizes were around ±3.0 mg TE/g dw. This might be because: 1) the extracted antioxidant compounds have reached their maximum level at around 3 mg TE/g dw, or 2) the TPC (Folin-ciocalteu method) and AOA (DPPH assay) tests analysed different compounds, explaining why the findings of the two tests did not always follow the same trend.

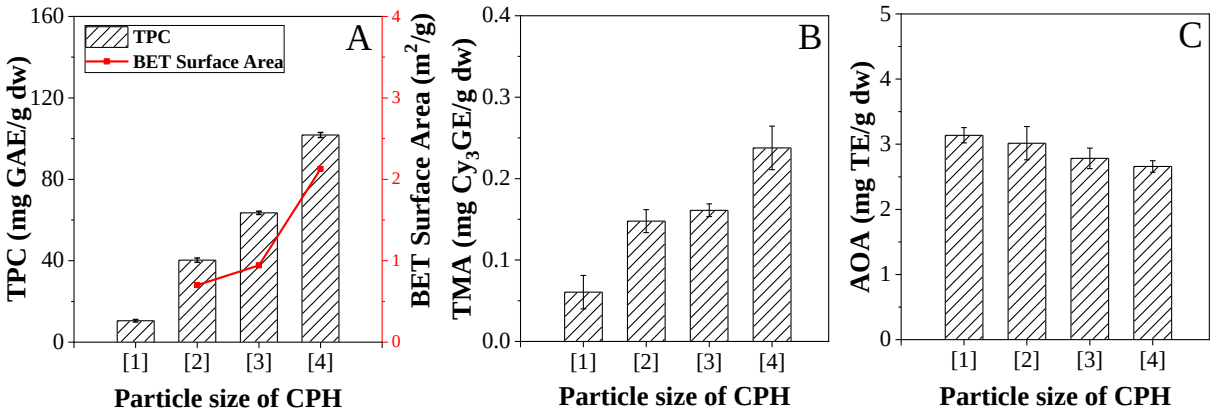


Figure 4. 7 Effect of CPH particle size on TPC (A), TMA (B), AOA (C) yields (50% (v/v) ethanol/water, S/F ratio 40:1 mL/g, 30 min): [1] CPH with size 0.5x0.5 cm (without grinding); [2] CPH with particle size 125-150 micron; [3] CPH with particle size 63-90 micron; [4] CPH with particle size ≤38 micron, mean ± S.D (n= 9, triplicate extraction and triplicate analysis)

Table 4. 4 BET surface area and pore volumes 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 (4V/A) (nm)
125-150 micron	0.77 ± 0.07	0.22 ± 0.01	1.54 ± 0.08	5.00 ± 0.53	26.12 ± 5.05
63-90 micron	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)

Following the size reduction pretreatment, the CPH powder was subjected to the BET analysis to understand the changes in surface area and pore characteristics caused by size reduction. The changes can be seen in Table 4.4, and based on data the average pore diameter (19-26 nm), it can be clearly understood that CPH powder is predominately mesoporous and macroporous materials. The adsorption-desorption isotherm graphs (Figure 4.8) closely represented Type IV behaviour (Figure 3.2.A), as

expected for mesoporous material. A “knee” point was observed between 0.01 and 0.06 relative pressure (P/Po), yet it was not sharp, indicating limited micropore filling, and the multi-layer adsorption inside the mesopores was observed at high pressure. The hysteresis loops found in the isotherms exhibited the Type H4 (Figure 3.2.B), which was frequently identified in micro-mesoporous carbons; monolayer-multilayer adsorption might start at low relative pressure followed by pore condensation (Thommes et al., 2015).

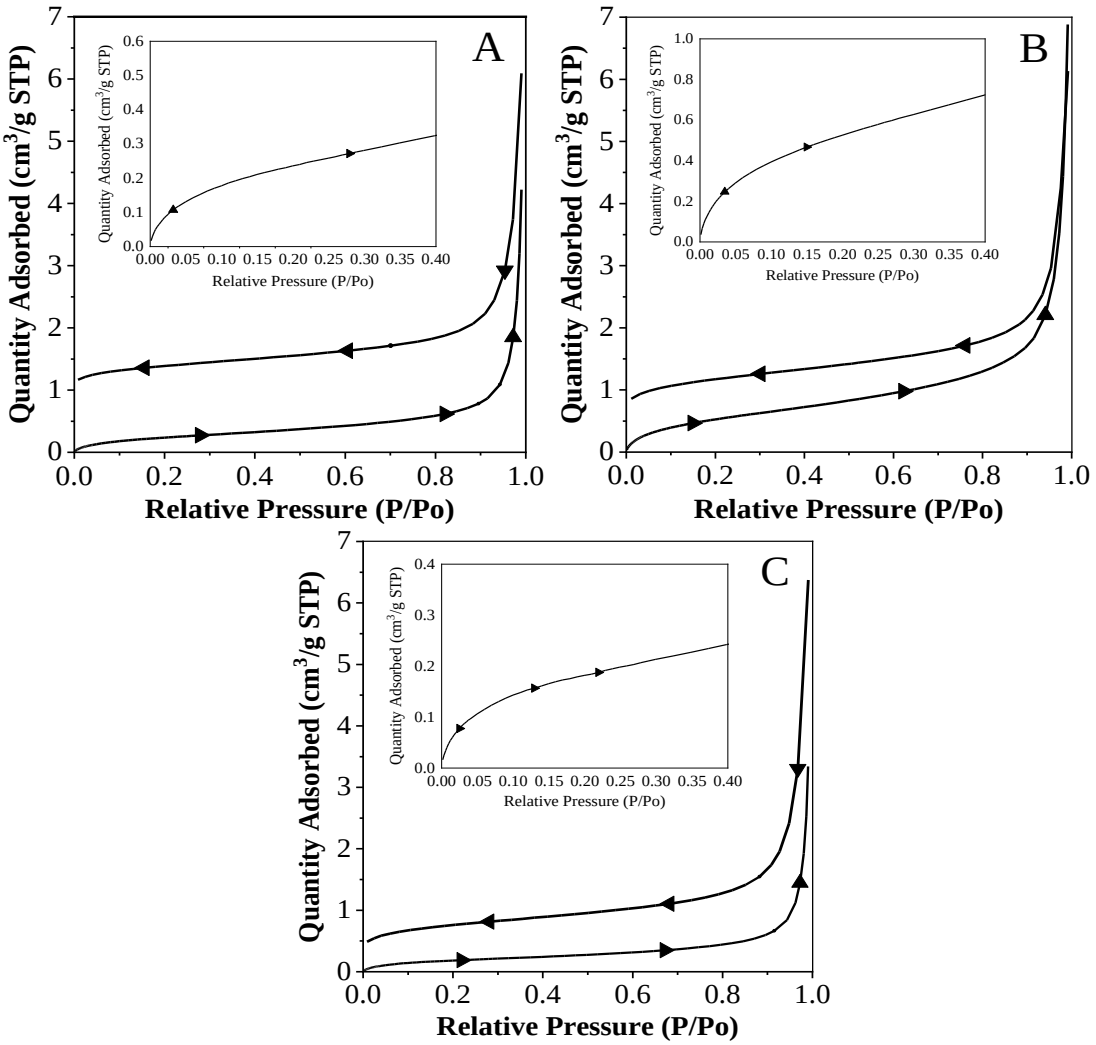


Figure 4. 8 BET Isotherm graph: (A) CPH particle size 125-150 micron; (B) CPH particle size 63-90 micron; (C) CPH particle size ≤38 micron

The following data in Table 4.4 shows a reduction in CPH size from 150-micron to 38-micron has improved the surface area, micropore and mesopore volumes. Surface

area and micropore volume both increased 2.5 times from 0.77 to 1.96 m^2/g and 0.22 to 0.56 mm^3/g, respectively, whereas mesopore volume increased twice as much from 1.54 to 3.35 mm^3/g. As a result, the TPC yield increased 2.5-fold from 40.3 to 101.8 mg GAE/g dw, while the TMA increased 1.5-fold from 0.15 to 0.24 mg Cy_3GE/g dw. The data suggests that as particle size reduced, micropore volume and surface area improved, enhancing the extraction rate of phenolic from CPH and finally increasing the TPC yield. Increasing the contact surface area could facilitate the interaction between the CPH matrix and extraction solvent. One could argue that size reduction is also crucial for phenolic extraction, and therefore, CPH with the smallest particle size (≤38 micron) was selected for the subsequent experiments. Moreover, a study of the impact of the extraction treatment on those surface areas and pore volume of CPH is explained in Chapter 7 through BET and SEM results.

4.6 Summary

This chapter addresses the impact of sample pretreatment, solvent selection and extraction methods on the bioactive yields and their functionality. The preliminary study provides evidence that CPH contains phenolic and anthocyanin compounds that can be applied as natural antioxidants. Firstly, we found that drying can decrease extraction yields that may be because of the degradation or evaporation of some volatile or thermolabile bioactive compounds during the drying process. The comparison of bioactive extraction from fresh and dry CPH confirmed that phenolic content in fresh CPH extract was higher than in dry CPH. However, considering the distribution cost and the properties of fresh CPH that was very perishable (moisture of 86.7% w.b), the dry CPH was selected as a biomass sample in this study. While for scaling-up process, the drying process may be considered in terms of energy requirement; the processing of fresh CPH may cut the energy-intensive process (drying) in a large-scale experiment.

For future research, the influence of processing parameters (solvent, extraction method, temperature) and drying methods (microwave drying, freeze drying) on the extraction of fresh CPH may be of interest to investigating the on-site process.

According to the experimental results, the extraction solvent (η^2 = 0.99) and particle size (η^2 > 0.94) are critical parameters to consider in phenolic extraction from CPH; both parameters significantly affect (p_{value} < 0.001) the TPC and TMA yields, but they do not significantly affect the antioxidant activity of CPH extract. Both experimental data and Hansen solubility parameter (HSP) suggested that an ethanol/water mixture was the most appropriate solvent to extract the phenolic compounds from CPH. The 50% (v/v) ethanol/water had similar solubility to the phenolic compounds, in which the solubility of target compounds in the solvent was one of the key factors of the whole extraction process. Meanwhile, the size reduction of dry CPH significantly increased the yields; the highest TPC and TMA yields were recovered from dry CPH with the smallest particle size of ≤38 micron which has the highest surface area of 2.13 m^2/g and micropore volume of 0.56 mm^3/g, while the maximum antioxidant activity of CPH extracts was about ±3.0 mg TE/g dw. Nevertheless, the use of fine particles of CPH must be considered when the scale-up process is carried out because the separation process would need more energy. Sample pretreatment (drying and size reduction) could also be used as input for the scaling-up process because it affects energy requirements and equipment size, so both must be considered whether to be included in the CPH valorisation flowsheet. Lastly, although Microwave-Assisted Extraction (MAE) has been shown to produce higher yields compared to conventional methods and indicates to have the potential to extract bioactive compounds, it remains to be studied how this interacts with other system variables to maximise the potential benefits of microwave heating over conventional.

Therefore, further comparisons of MAE and CSE need to be addressed in maximising the extract yields under similar extraction conditions.

CHAPTER 5: RESULTS AND DISCUSSION – MAXIMISING THE EXTRACTION YIELDS

5.1 Introduction

In the previous chapter, it has been proved that CPH contains phenolic compounds, including anthocyanin, which have potential as natural antioxidants. It has also been reported that the extraction method adopted had a significant impact on the extraction yields. It was proposed that MAE would enhance the TPC and TMA yields due to its volumetric and selective heating. For example, Galan et al. (2017) found that the plant matrix of sea buckthorn leaves had been selectively heated at and above 60 °C during phenolic extraction. However, no standard extraction method is ideal to extract phenolic compounds from plant material due to the diversity of phenolic compounds; each method has its advantages and drawbacks. Therefore, investigating the extraction conditions is important to maximise the extraction yield.

A number of factors have been reported to affect the extraction efficiency of phenolic-antioxidant compounds, including solvent type, solvent concentration, extraction time, temperature, and solvent-to-feed (S/F) ratio (Galan et al., 2017; Gharekhani et al., 2012; Mokrani and Madani, 2016; Pan et al., 2003). For instance, an increase in extraction temperature could increase the TPC yield of sea buckthorn leaves extract (Galan et al., 2017), but on the contrary, the TPC yield of peach fruit extract decreased with increasing temperature (Mokrani and Madani, 2016). Moreover, the effect of solvent type on peach fruit and *Eucalyptus camaldulensis* Dehn leaves were different; a comparison of aqueous acetone, aqueous ethanol, aqueous methanol, and water showed that aqueous acetone was the best solvent to extract phenolics antioxidant from peach fruit (Mokrani and Madani, 2016), while 50% (v/v) aqueous ethanol was more suitable for the phenolic extraction of *Eucalyptus camaldulensis* Dehn leaves

(Gharekhani et al., 2012). It was also reported that the maximum ethanol concentration to extract the polyphenol was 50% (v/v), above which the yield decreased (Galan et al., 2017; Gharekhani et al., 2012; Pan et al., 2003). They also revealed that the longer extraction time and increasing solvent volume could enhance the extraction yields. Another study reported the extraction of phenolic antioxidants from CPH by MAE was affected by extraction time, irradiation time, solvent-to-feed ratio, and microwave power (Nguyen et al., 2020). Microwave power and irradiation time during the MAE process directly affected the diffusivity and solubility of phenolics from CPH in the solvent, which enhanced extraction efficiency. However, plant material type was also noted to directly affect phenolic compounds' extractability.

In this study, several extraction parameters still need to be investigated to identify the extraction conditions that would maximise the extraction yield of CPH. MAE method was compared with CSE at the same extraction conditions: extraction time, temperature, ethanol concentration, and solvent-to-feed (S/F) ratio to understand the influence of the microwave selective heating effect in maximising the extraction yield. The CSE was designed to have the MAE's heating profile for negating the volumetric heating effect. In the first section (Section 5.2), the comparison of MAE and CSE was assessed based on the TPC yield and the appropriate extraction conditions were chosen based on the highest TPC value. While in Section 5.3, the antioxidant activity of the CPH extract was used as a parameter to define extract quality so as to select the best extraction conditions. The outcomes are intended to be useful inputs into a decision-making process for scaling up the process.

5.2 Comparison of MAE and CSE on maximising the extraction yield based on the total phenolic content (TPC)

Section 5.2 was revised from the manuscript published in Conference Paper:

Dewi, S.R., Stevens, L., Irvine, D., Ferrari R., Binner E., 2021, Microwave-assisted extraction of phenolic from cacao pod husks- an alternative for valorisation, AMPERE 2021 Proceeding – 18th International Conference on Microwave and High Frequency Applications, 179-186, Virtual, 13-16 September 2021, https://zenodo.org/record/5645566#.Ywccz3Fby3A.

5.2.1 Effect of extraction time and temperature

It has been reported that extraction yields depend on temperature and extraction time, so investigating the extraction time and temperature was critical to maximising the extraction of TPC and preventing the degradation of phenolic compounds. Phenolic compounds are thermolabile compounds, so temperature condition and extraction time have to consider to minimise the degradation of phenolic yield. Moreover, in the MAE process, temperature and microwave power work together to control how much energy is converted to heat in the dielectric material. At the same time, extraction time is crucial in defining the energy demand for the process and so the cost efficiency of the extraction process.

The initial series of extractions which involved the combination of extraction temperatures of 50, 60 and 70 °C and extraction times of 1, 5, 10, 15, and 30 min were studied, and the results are presented in Figure 5.1. According to the results, the extraction temperature and time did have an effect ($p_{value} < 0.001$) on the amounts of extraction yields of TPC, TMA, and AOA, in both MAE and CSE experiments ($p_{value} < 0.001$). The TPC values ranged from 86.01 to 107.32 mg GAE/g dw (Figure 5.1), with 5 min extraction producing the highest yields for both MAE and CSE experiments. Given that TPC, TMA and AOA yields tended to decline when the extraction time was extended up to 30 min, this finding suggested that the best time for extracting phenolic

from CPH was 5 min. Therefore, it was postulated that the final equilibrium interaction between phenolic compounds and solvent was reached after 5 min, whilst the degradation of extracted phenolic after 5 min was attributed to more prolonged exposure to heating. Long extraction times at high temperature might reduce TPC due to hydrolysation and oxidation of some phenolic compounds (Galan et al., 2017). Therefore, it was concluded that a long time was not required to extract more phenolic antioxidants.

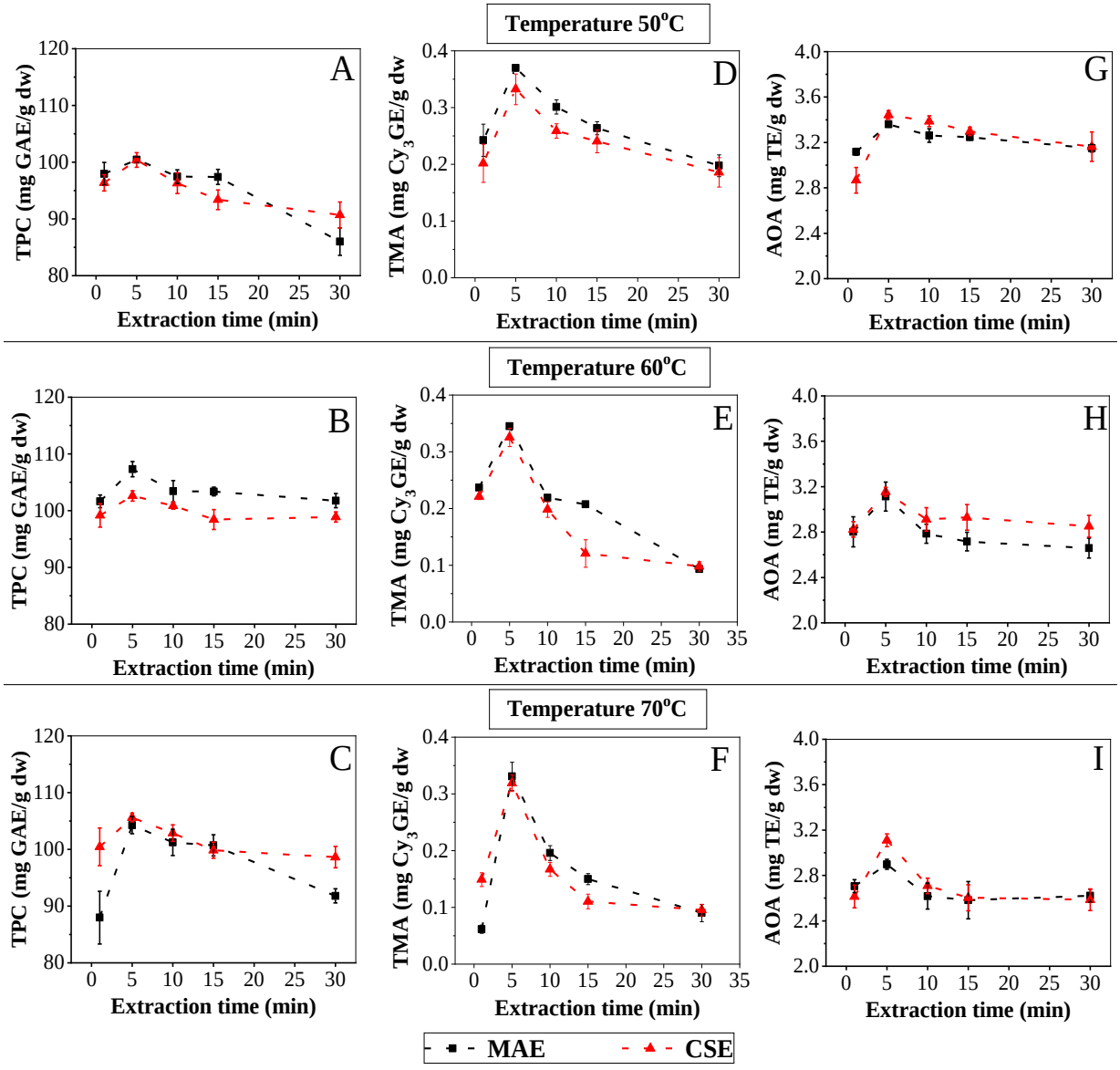


Figure 5. 1 Effect of extraction time and temperature on TPC (A-C), TMA (D-F), and AOA (G-I) yields (CPH with particle size ≤38 micron, 50% (v/v) ethanol/water, S/F ratio 40:1 mL/g, 50 – 70 °C, 1 – 30 min), mean ± S.D. (n= 9, triplicate extraction and triplicate analysis)

Meanwhile, the influence of extraction temperature on MAE and CSE showed a distinct trend ($p_{value} < 0.001$). While the TPC yield on MAE (Figure 5.1 A-C) increased from 50 to 60 °C and then decreased at 70 °C, the TPC obtained by CSE increased as temperature rose. According to Daneshfar et al. (2008), gallic acid (phenolic compound exampler) became more soluble as temperature increased from 25 to 60 °C. Thus, the heating process may increase the extraction efficiency of phenolic compounds because of the increase in phenolic solubility and mass transfer. Moreover, Galan et al. (2017) reported that increasing temperature decreased the solvent's surface tension and viscosity, allowing deeper penetration into the sample matrix and solubilising more phenolic compounds. The reduction in TPC yield at 70 °C was attributed to the degradation of extracted phenolic at a higher temperature (i.e. overheating).

Figure 5.2.C shows the loss tangent of 50% (v/v) ethanol/water from 20 to 70 °C both with and without CPH, which represents a good indication of the dielectric properties of the mixture during the extraction process.

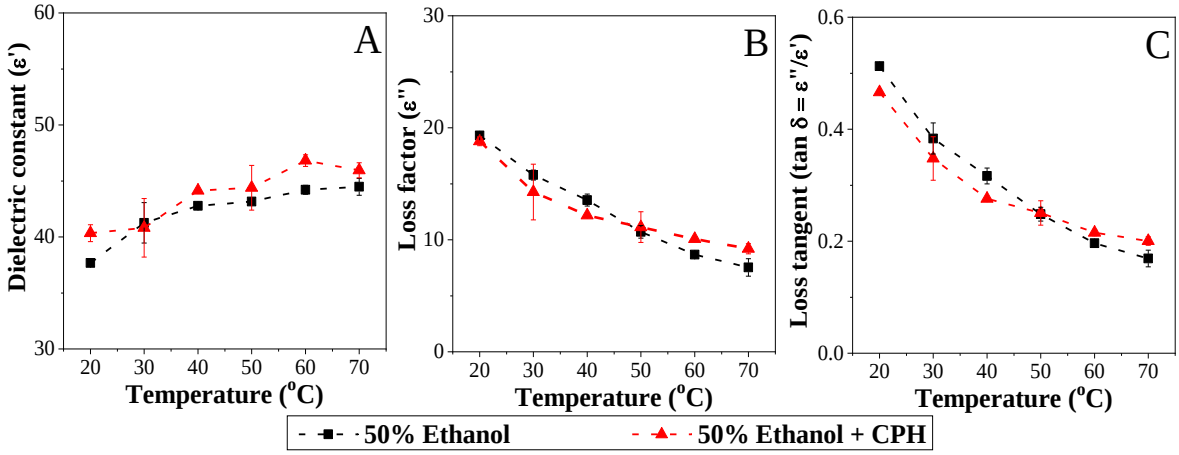


Figure 5. 2 Dielectric properties: (A) Dielectric constant; (B) Loss factor; and (C) Loss tangent of extraction solvent (50% (v/v) ethanol/water) with and without CPH at 2.45 GHz, mean ± S.D (n= 3, triplicate measurements)

Loss tangent (tan δ) represents the relationship between the dielectric constant and loss factor, indicating the materials' ability to absorb and convert electromagnetic energy into heat (Ibrahim and Zaini, 2018). The correlations among loss tangent, dielectric

constant and loss factor are expressed by Equation 1.2 (Metaxas and Meredith, 1983). In Figure 5.2, compared to the solvents, the loss tangent for the solvent–CPH mixtures were lower up to 40 °C, then hit the same level as the solvent at 50 °C, and went higher at and above 60 °C. Although these differences are relatively small, it may indicate a potential for the CPH to be selectively heated above 50 °C and support the conclusion that the decreasing of the TPC yield at 70 °C may be due to overheating, causing the degradation of the extracted phenolics. Moreover, Volf et al. (2014) reported that gallic acid and catechin from grape seed were thermally degraded when heated to 60, 80 and 100 °C. At 60 and 100 °C, gallic acid degraded at 12% and 20%, respectively, while catechin dropped by 13% at 60°C and up to 25% at 100 °C.

Otherwise, both TMA and AOA of the extract decreased by increasing the temperature from 50 °C to 70 °C. During MAE, the antioxidant activity decreased by 14%, from 3.36 ± 0.02 mg TE/g dw to 2.9 ± 0.04 mg TE/g dw, while on CSE decreased by 10% from 3.44 ± 0.04 mg TE/g dw to 3.11 ± 0.06 mg TE/g dw. The study on anthocyanin extraction from blueberry showed that anthocyanin yields increased with increasing temperatures, but at 41 °C anthocyanin began degrading under hot reflux extraction. When using MAE, degradation of anthocyanin occurred above 53.6 °C (Yu et al., 2016). Then, it was concluded that increasing temperature might improve the phenolics' solubility. Still, at the same time, a high temperature can lead to the degradation of extracted phenolic compounds (other than gallic acid, such as anthocyanin or other thermolabile compounds) that have high antioxidant activity.

In general, comparing extraction at 60 °C for 5 min, MAE produced 5% higher TPC yield than CSE, even though both had very similar heating rates. This indicated that the microwave selective heating effect might be responsible for a higher TPC. This finding was validated by Galan et al. (2017), who reported that MAE yielded 8% higher

TPC over CSE due to the selective heating effect. They suggested that phenolic extraction from sea buckthorn leaves by MAE offered a higher yield at and above 60 °C. Nguyen et al. (2020) also reported the increment of MAE yield was about 29% compared to the conventional method. In this study, the highest amount of TPC was performed in 5 min at 60 °C (107.3 ± 1.4 mg GAE/g dw) for MAE and 70 °C (105.6 ± 0.76 mg GAE/g dw) for CSE. While the highest anthocyanin (TMA) and antioxidant activity (AOA) were achieved at 50 °C, either in MAE (0.370 ± 0.0 mg Cy_3GE/g dw and 3.36 ± 0.02 mg TE/g dw, respectively) or CSE (0.332 ± 0.03 mg Cy_3GE/g dw and 3.44 ± 0.04 mg TE/g dw, respectively). Therefore, based on the TPC yields, the following experiments in Section 5.2 were observed using both MAE at 60 °C and CSE at 70 °C for 5 min extraction time. The performance of both methods was then compared at their maximum conditions. The results were in accordance with that of Lovrić et al. (2017), who demonstrated that 5 min produced the highest phenolic and antioxidant activity by the MAE experiment.

5.2.2 Effect of ethanol concentration

Solvent for MAE process should be selected based on the target compounds' solubility, solvent and plant matrix interaction, solvents' dielectric properties and their penetration depths (Veggi et al., 2013). Solvents' dielectric properties are a crucial parameter because different solvents have different dielectric properties and, thus, different heating rates. Solvents with high dielectric loss tangent (tan δ) are highly capable of absorbing and dissipating microwave energy into heat (Ibrahim and Zaini, 2018). In this study, ethanol/water mixtures were used as extraction solvents since they are polar solvents which show good solubility for phenolic compounds (Section 4.3). In addition, ethanol/water mixtures will be good microwave absorbers as ethanol and water are good microwave adsorbers (Table 2.8), so the mixtures will exhibit good

potential for dissolving phenolic compounds. The effect of solvent concentration (0 to 100% (v/v) ethanol/water) on TPC yields for MAE at 60 °C and CSE at 70 °C was compared. The following results (Figure 5.3) were clear both methods had comparable trends and close yields (p_{value} = 0.579); the TPC and TMA contents for both methods increased rapidly and reached a maximum at 50% (v/v) ethanol/water before decreasing with increasing ethanol percentage.

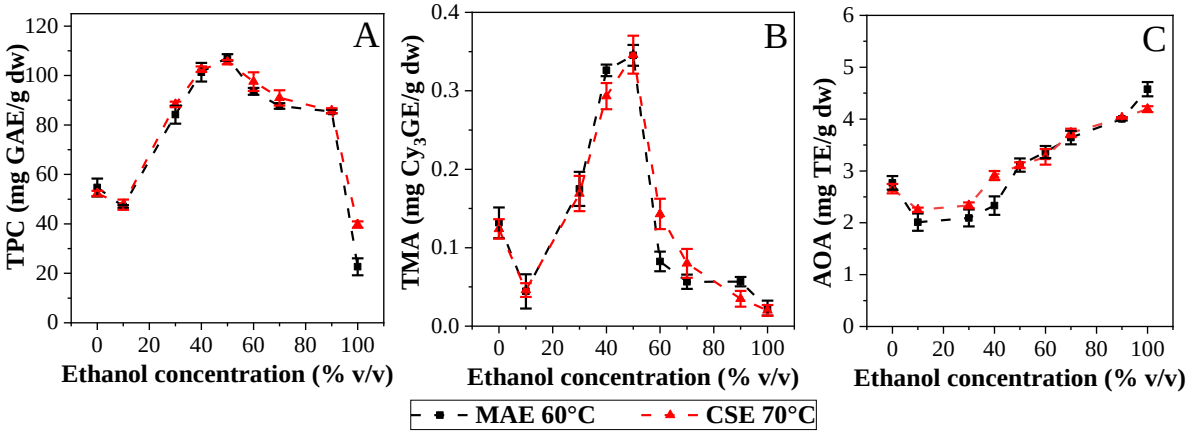


Figure 5. 3 Effect of ethanol concentration on TPC (A), TMA (B), AOA (C) yields (CPH with particle size ≤38 micron, S/F ratio 40:1 mL/g, temperatures 60 °C for MAE and 70 °C for CSE, 5 min), mean ± S.D. (n= 9, triplicate extraction and triplicate analysis)

At ethanol levels lower than 50% (v/v), the TPC and TMA of extracts increased with an increase in the ethanol concentration due to an increase in their solubility and achieved a maximum of 107.3 mg GAE/g dw of phenolics with 0.35 mg Cy3GE/g dw of anthocyanin at 50% (v/v). The TPC and TMA yields then dropped significantly when the ethanol concentration was raised to 100% (v/v). This is because the solubility of phenolic compounds, including anthocyanins, decreases in higher ethanol concentrations. It should be noted that phenolic compounds (with gallic acid as an exemplar) have similar Hansen solubility parameter to 50% (v/v) ethanol/water solvent (Section 4.3), thus their solubility is maximised in that solvent. The maximum TMA yields at 50% (v/v) ethanol/water coincide with the expected TPC yields, given that anthocyanins are a class of phenolic compounds (Pearson's r = 0.663; p_{value} < 0.001).

This result was in agreement with the microwave extraction of phenolics from green tea leaves (Pan et al., 2003), *Eucalyptus camaldulensis Dehn* leaves (Gharekhani et al., 2012), sea buckthorn leaves (Galan et al., 2017), blackthorn flowers (Lovrić et al., 2017), and pomegranate peels (Kaderides et al., 2019). In each case, it was reported that the appropriate solvent to reach the maximum phenolic compound was 50% (v/v) ethanol/water. Chew et al. (2011a) also demonstrated that in phenolic extraction from *Orthosiphon stamineus,* the binary-solvent of ethanol and water was more favourable than the mono-solvent system (deionised water or absolute ethanol).

In contrast, increasing ethanol concentration from 10 to 100% (v/v) significantly increased the total AOA yield of the extract. This result was significantly different from the TPC trend (Pearson's r = -0.153; p_{value} = 0.053), which reached a maximum at 50% (v/v). The antioxidant activity still increased at above 50% (v/v) concentration and reached a maximum in 100% (v/v) ethanol, although the phenolic content experienced low. This result was consistent with the AOA trend discussed in Section 4.3, in which the antioxidant activity in alcoholic extract (pure methanol or ethanol) was higher compared to aqueous alcohol (50% (v/v) methanol/water or ethanol/water) even though the TPC experienced low at that points. Apart from the protic effect, this might be because compounds with high AOA, such as quercetin or catechin, were extracted more in high ethanol concentrations. It could be explained by identifying the individual phenolic compound in the extract using HPLC. Daneshfar et al. (2008) reported that gallic acid (included as a phenolic compound standard) had twelve times higher solubility in ethanol than in water at 25°C, and this solubility was still three times with the increasing temperature to 60°C. Besides, the catechin as a phenolic group also had a high solubility in ethanol/water instead of in water (Cuevas-valenzuela et al., 2014). Overall, after comparing the MAE at 60 °C to CSE at 70 °C, it was clear that MAE

could produce the same amount of extraction yields (TPC, TMA, AOA) as CSE with a lower temperature, which in turn should reduce the degradation of the product.

As previously mentioned, solvents' dielectric properties influence the MAE process. Thus, the correlation between solvents' dielectric properties and extraction yields is also addressed. The loss tangent of various solvents used is presented in Figure 5.4. The loss tangent of both solvent and solvent-CPH mixture increased as ethanol percentage increased, with the loss tangent of the solvent-CPH mixture being higher than the solvent itself. The presence of plant material in the mixture could increase the loss tangent because it might also absorb microwave energy which was later dissipated as heat (Singh et al., 2014). Theoretically, a solvent with a high-loss tangent is a proper solvent for MAE as it is a better microwave absorber.

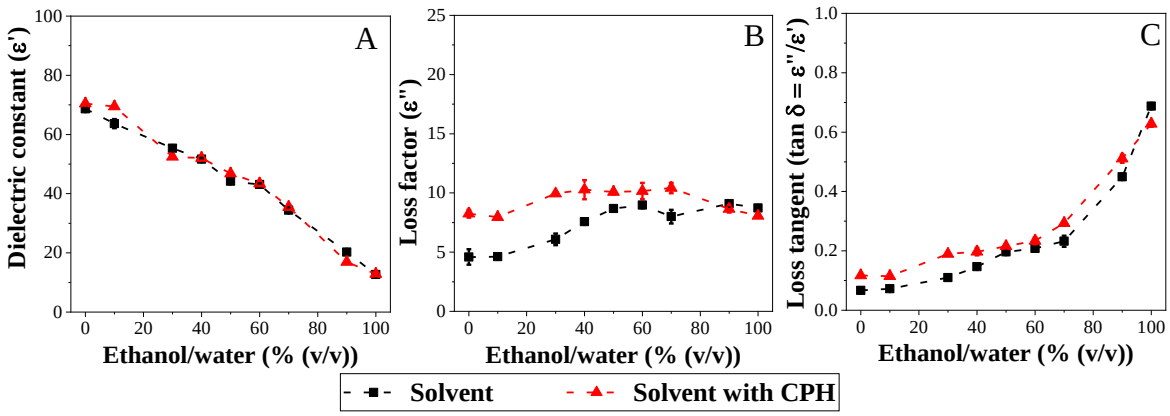


Figure 5. 4 Dielectric properties: (A) Dielectric constant; (B) Loss factor; and (C) Loss tangent of extraction solvents (ethanol/water) at 60 °C and 2.45 kHz, mean ± S.D (n= 3, triplicate measurements)

The experimental results (Figure 5.3), however, demonstrated that the TPC and TMA declined above 50% (v/v) ethanol/water solvents when the loss tangent raised. Hence, it was clear that dielectric loss tangent was not responsible for extraction yield. In addition, the trend of bioactive yields on MAE was similar to on CSE. This data suggested that phenolic compounds' solubility in solvents could be more affected by the solvent solubility parameters instead of their dielectric properties. The basic principle for solvent extraction is "like dissolves like, " meaning that components with

similar chemical characteristics will dissolve one another. Of all the ehanol concentrations used, it was observed that the 50% (v/v) ethanol/water mixture has the closest Hansen solubility parameter (HSP) value (hydrogen part) to gallic acid (phenolic compound exampler), as previously reported in Section 4.3; thus, it was proposed that it would more easily dissolve phenolics than other any concentration. The practical results proved that 50% (v/v) ethanol/water was the most extracted solvent for total phenolics, which means that phenolic compounds have similar properties to 50% (v/v) ethanol/water, and therefore, 50% (v/v) ethanol/water was chosen as the best extraction solvent for subsequent experiments.

5.2.3 Effect of solvent-to-feed (S/F) ratio

The S/F ratio is also an essential factor in extraction experiments; solvent volume must be large enough to immerse the entire sample and so be able to extract the phenolic from the entire sample. Insufficient solvent volume results in lower extraction yield due to uneven solvent distribution into the plant matrix, so not all of the phenolics in CPH can be extracted. In contrast, the excessive solvent volume requires much more time and energy to heat the solvent up to processing temperature in order to extract the phenolic (Ibrahim and Zaini, 2018) and may also lead to higher operating costs (Kaderides et al., 2019). This study compared extraction under MAE at 60 °C and CSE at 70 °C with various S/F ratios (20:1, 30:1, 35:1, 40:1, and 50:1 mL/g) to evaluate the impact of solvent volume on extraction yields. The results (Figure 5.5) showed that, in general, the TPC and AOA yields in both MAE and CSE increased as the S/F ratio increased from 20:1 to 50:1 (mL/g).

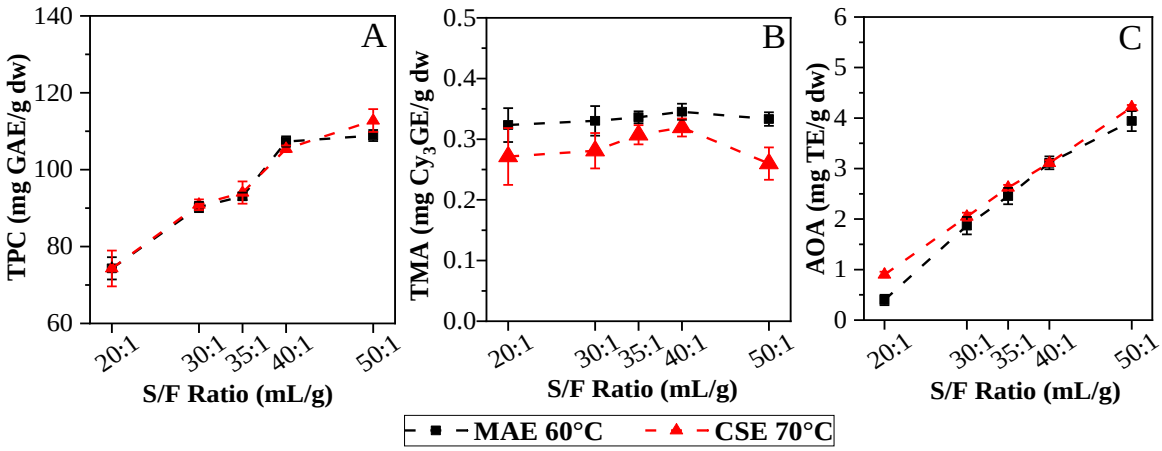


Figure 5. 5 Effect of solvent-to-feed (S/F) ratio on TPC (A), TMA (B), AOA (C) yields (CPH with particle size ≤38 micron, 50% (v/v) ethanol/water, temperatures 60 °C for MAE and 70 °C for CSE, 5 min), mean ± S.D. (n= 9, triplicate extraction and triplicate analysis)

The antioxidant activity proportionally increased with the phenolic content (Pearson's r = 0.955; p_{value} < 0.001), in which the phenolic compounds were extracted more at a high solvent volume. In contrast, the S/F ratio did not have effects on anthocyanin yield, either in MAE or CSE (p_{value} = 0.031); rather, the TMA of the extract was relatively constant and ranged from 0.26 to 0.35 mg Cy_3GE/g dw. Meanwhile, the TMA value in CSE with an S/F ratio of 50:1 mL/g experienced a decrease, as Pham et al. (2019) reported that increasing solvent loading could decrease the extracted anthocyanin. Each plant material has specific characteristics in its interaction, and so does the absorption of materials by the solvent. Thus, cells swelled and burst simultaneously when absorption capacity reached its maximum. As a result, adding an amount of solvent exceeding the maximum plant cells' capacity led to the captivity of anthocyanin within the cells (Pham et al., 2019). Comparing MAE at 60 °C and CSE at 70 °C, both had a similar amount of TPC, TMA and AOA; this proved that CSE required a higher temperature to reach the same yield as MAE when using the same concentration and ratio of solvent. The results, therefore, suggested the S/F ratio of 40:1 mL/g as the appropriate ratio to achieve a high extraction yield after taking into account less energy consumption and observing the TPC of the S/F ratio of 40:1 mL/g had no

noticeable difference from 50:1 mL/g. Besides, the use of large solvent volumes would require much more energy and longer extraction times.

In several studies, a ratio of 20:1 (mL/g) was reported to be achieved the maximum microwave extraction of phenolics from various leaves, such as dried sea buckthorn leaves (Galan et al., 2017), green tea leaves (Pan et al., 2003), and dried *Eucalyptus camaldulensis Dehn* leaves (Gharekhani et al., 2012). Furthermore, they found a similar trend to that which was reported in this study, namely that the TPC yield increased as solvent volume increased. At the same time, the phenolic extraction from fruit peels required more solvent volume loading to attain maximum yield, as reported by Kaderides et al. (2019). They extracted phenolic and antioxidant compounds from pomegranate peels using 60 mL of solvent per gram sample to reach optimum antioxidant scavenging of 94.91%. In the case of anthocyanin content, Duan et al. (2015) optimised the MAE of anthocyanin from Chinese bayberry and reported that S/F ratio of 50:1 was the optimum ratio to obtain 2.01 mg Cy_3GE/g dw. While the extraction of antioxidants from *Exotic Gordonia axillaris* fruit reached a maximum using 20:1 mL/g ratio (Li et al., 2017). These yield differences among several studies may be caused by the differences in target compounds, plant type, plant parts (leaves, seeds, fruit, peel or pod), and plant material conditions (dried, fresh, grounded or un-grounded plant). The phenolic extraction from peel, pod or skin needs more solvent loading than leaves or fruit may be due to its cell complexity.

5.2.4 Comparison of MAE and CSE on TPC yield

During microwave heating, absorbed power and bulk temperature profile were recorded by microwave equipment (Miniflow 200SS) and this data is shown in Figure 5.6. It can be seen in the graph that is Figure 5.6.A, the set temperature (60 °C) was reached for 75 seconds (heating phase) under 120 W microwave power. Then, the

microwave power was held below 10 W to maintain this processing temperature (holding phase). The cumulative energy consumed for each phase could be calculated by summing up the multiplication of absorbed power and heating time. Energy absorbed by the system during the heating and holding phases were 8.97 and 1.15 kJ, respectively, totalling 10.12 kJ. While energy consumption for CSE experiments cannot be calculated as there was no precise information on how much power was input to the system during heating time and/or holding time; the only information provided was the hotplate output power of 650 W. The temperature measurement quoted here is the bulk process temperature. Thus, the specific temperature of plant material or solvent could be hotter or cooler than bulk temperature, depending on their dielectric properties (Galan et al., 2017).

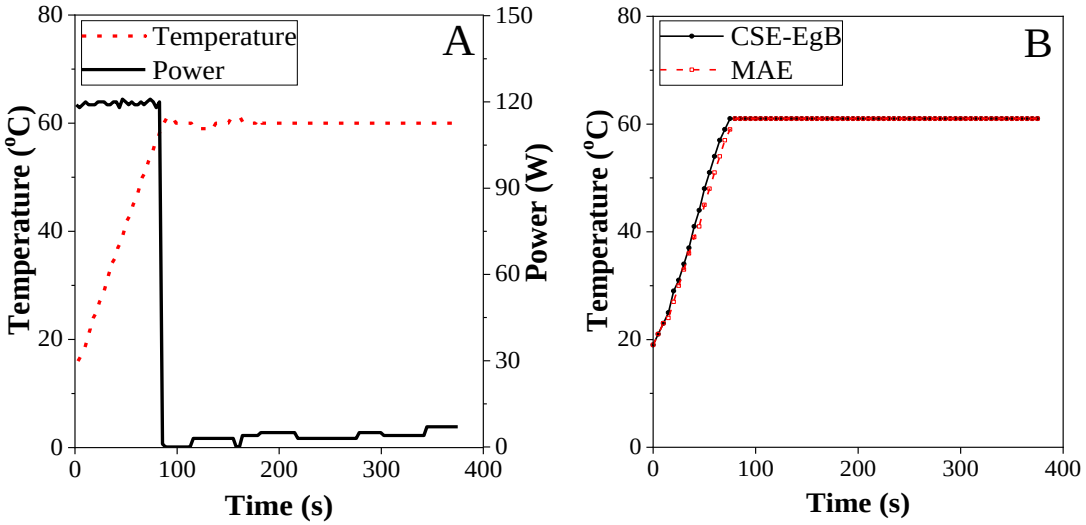


Figure 5. 6 (A) Absorbed power and temperature profiles for MAE experiment (extraction temperature 60 °C, 5 min extraction time, 50% (v/v) ethanol (40:1 mL/g), and 120 W power); (B) Bulk temperature profile for MAE (120 W) and CSE experiments at 60 °C set-point temperature

When a MAE system is set up appropriately (i.e., taking into account the penetration depth of the energy into the extraction mixture), microwaves heat volumetrically and selectively. Volumetric heating heats the mixture instantaneously throughout the entire bulk, while selective heating rapidly heats the material with a

high-loss tangent, potentially leading to higher yields at shorter extraction times. This study revealed that MAE performed volumetrically heating (Figure 4.5.A Section 4.4) and so was observed to be fourfold faster than CSE-WB (water bath) to reach the extraction temperature. This volumetric heating effect on MAE was then negated by designing the CSE experiment to have a very similar bulk heating profile as MAE (Figure 5.6.B). As a result, the extraction time for both MAE and CSE were similar at ~ 5 min, and MAE still yielded 5% higher TPC than CSE at the same temperature (60 °C), indicating that selective heating works at 60 °C, as previously reported by Galan et al. (2017).

Furthermore, when the MAE at 60 °C and CSE at 70 °C results were compared to observe the effect of ethanol concentration and S/F ratio parameters (Figures 5.3 and 5.5), it was found that TPC yields in both methods had no remarkable difference as neglecting volumetric heating. It is clear that MAE can produce a higher TPC at a lower extraction temperature (60 °C), reducing energy requirements. This could be due to the microwave selective heating effect, which leads to enhancing the TPC yield. Moreover, Figure 5.4 illustrated that introducing CPH in solvent increased the mixture's loss tangent, indicating that the CPH was selectively heated during microwave heating. Overall, high extraction yields were attained using 50% (v/v) ethanol/water (40:1 mL/g) for 5 min at 60 °C for MAE (107.3 ± 1.4 mg GAE/g dw) and 70 °C for CSE (105.4 ± 0.9 mg GAE/g dw). The result demonstrated higher TPC compared to that of CPH extract from previous studies reported using CSE by Valadez-Carmona et al. (2017) (18.9 mg GAE/ g dw), Yapo et al. (2013) (68.9 mg GAE/ g dw), Vriesmann et al. (2011) (98.0 mg GAE/g dw), Nguyen et al. (2021) (12.22 mg GAE/g dw), and using MAE by Nguyen et al. (2020) (10.97 mg GAE/g dw). This CPH extract also still higher TPC yield than phenolic extract from *Gordonia axillaris* fruit (17.7 mg GAE/g dw) (Li et

al., 2017), blackthorn flower (61.6 mg/g) (Lovrić et al., 2017), *Eucalyptus camaldulensis* Dehn leaves (76.6 mg GAE/g) (Gharekhani et al., 2012), and carob kibbles (70.1 mg GAE/g dw) (Huma et al., 2018).

5.2.5 Summary

This study demonstrated the comparison of processing parameters (extraction time and temperature, ethanol concentration, and S/F ratio) within MAE and CSE to maximise the phenolic content in CPH extracts. MAE and CSE were compared using a very similar bulk heating profile, stirrer speed, and solvent composition. The CSE experiments were conditioned using a 120 °C ethylene glycol bath (EgB) to achieve similar heating rates as MAE. It was observed that by negating the volumetric heating, the maximum yields for MAE and CSE could be obtained concurrently, which was 5 min, whereas the longer extraction time decreased the TPC, TMA or AOA yields. On the other hand, an increase in extraction temperature experienced a various trend for TPC, TMA and AOA. The TPC yield could increase when the processing temperature was raised, but conversely, the increasing temperature decreased the TMA and AOA yields. The maximum conditions for producing the highest TPC yield were using 50% (v/v) ethanol/water at 60 °C for MAE with a value of 107.3 ± 1.4 mg GAE/g dw and 70 °C for CSE with a value of 105.4± 0.9 mg GAE/g dw. The TPC yield obtained by MAE was comparable to CSE at a lower extraction temperature (60 °C) and 5% higher than that of CSE (102.6 ± 0.9 mg GAE/g dw) at 60 °C. These higher MAE yields were attributed to the microwave selective heating effect at 60 °C. However, overheating may happen at 70 °C and above (Figure 5.2), which may reduce the TPC yield. This study proposes that MAE has the potential to valorise CPH waste into valuable products through the extraction of phenolic compounds.

5.3 Comparison of MAE and CSE on maximising the antioxidant yield of extract

Section 5.3 was revised from the manuscript published in the Journal of Food and Bioproducts Processing.

Dewi, S.R., Stevens, L.A., Pearson, A.E., Ferrari, R., Irvine, D.J., Binner, E.R, 2022, Investigating the role of solvent type and microwave selective heating on the extraction of phenolic compounds from cacao (Theobroma cacao L.*) pod husk, Food and Bioprocess Processing, 134, July 2022, 210-222. https://doi.org/10.1016/j.fbp.2022.05.011*.

In Section 5.2.1, it has been shown that 50 °C was the maximum temperature to extract the antioxidant compounds, either in MAE or CSE. The highest antioxidant activity (AOA) was 3.36±0.02 mg TE/g dw for MAE and 3.44 ± 0.04 mg TE/g dw for CSE. Next, maximising the extraction yield based on the extracts' functionality (antioxidant activity) was investigated.

5.3.1 Effect of ethanol concentration

In Section 5.2.2, the influence of ethanol concentration on TPC yield has been discussed. Thus, in this section, the effect of ethanol concentration (0 to 100% (v/v) ethanol) upon maximising the extracts' total functionality (i.e., antioxidant activity) is reported. Following the conclusions from Section 5.2.1, 50 °C and 5 min were selected as the processing temperature and extraction time in the initial attempts to maximise the antioxidant yield because both conditions produced high anthocyanin and antioxidant yields. The comparison of bioactive extraction using various ethanol concentrations (0 to 100% (v/v) ethanol/water) through MAE and CSE revealed that ethanol concentration had remarkable impact on the extraction yields ($p_{value} < 0.001$), as shown in Figure 5.7.

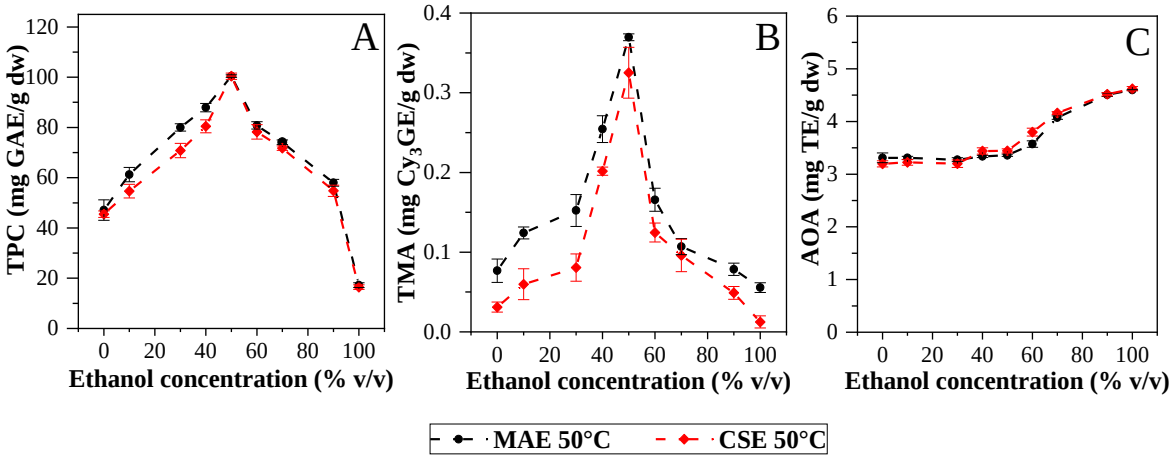


Figure 5. 7 Effect of ethanol concentration on TPC (A), TMA (B), AOA (C) yields (CPH with particle size ≤38 micron, S/F ratio 40:1 mL/g, 50 °C, 5 min), mean ± S.D. (n= 9, triplicate extraction and triplicate analysis)

Table 5. 1 Hansen Solubility Parameter (HSP) of solvent and gallic acid at 50 °C

Ethanol/Water (%v/v)	HSP of Solvent (Mpa$^{1/2}$)				HSP of Gallic acid (phenolic compound exemplar standard)				Ra
	δd	δp	δh	δt	δd	δp	δh	δt	
0	18.5	13	25.4	34.0	21.2	8.7	21.9	31.7	7.7
10	16.9	9.0	19.1	27.0	21.2	8.7	21.9	31.7	9.1
20	17.1	9.4	19.8	27.8	21.2	8.7	21.9	31.7	8.6
30	17.2	9.9	20.5	28.5	21.2	8.7	21.9	31.7	8.1
40	17.4	10.3	21.2	29.3	21.2	8.7	21.9	31.7	7.8
50	17.6	10.8	21.9	30.1	21.2	8.7	21.9	31.7	7.5
60	17.8	11.2	22.6	30.9	21.2	8.7	21.9	31.7	7.3
70	18.0	11.7	23.3	31.6	21.2	8.7	21.9	31.7	7.3
80	18.1	12.1	24.0	32.4	21.2	8.7	21.9	31.7	7.3
90	18.3	12.6	24.7	33.2	21.2	8.7	21.9	31.7	7.5
100	16.7	8.5	18.4	26.3	21.2	8.7	21.9	31.7	9.7

The TPC and TMA yields in both MAE and CSE experiments presented a similar trend (Pearson's r = 0.823; p_{value} < 0.001). At ethanol lower than 50% (v/v), the TPC and TMA of extracts increased with increasing ethanol concentration because of increase in their solubility, whereas at above 50% (v/v), they significantly dropped by increasing ethanol concentration because their solubilities in solvent gradually decreased. Both TPC and TMA yields showed a clear maximum at 50% (v/v) ethanol/water solvent, being 100.4 ± 0.5 mg GAE/g dw and 0.37 ± 0.0 mg Cy_3GE/g dw, respectively. Table 5.1 presents that hydrogen bonding part (δ_h) of gallic acid (as

phenolic standard compound) was similar to the part of 50% (v/v) ethanol/water (21.9 $MPA^{1/2}$); it means that this solvent had similar solubility to phenolics which would then easily dissolve the phenolics by hydrogen bonding.

By comparing MAE to CSE, the antioxidant activity was stable from 0 to 50% (v/v) ethanol/water at about 3.3 mg TE/g dw, then raised to 4.6 ± 0.01 mg TE/g dw at 100% ethanol. This finding was correlated to previous results (Figures 4.4 and 5.3), which demonstrated that the antioxidant activity of extract in absolute ethanol (100% (v/v) was higher than in 50% (v/v) ethanol/water. It was proposed that these observations may result from the alcohol solvent potentially interacting with phenolic compounds, leading to deprotonation, which could increase the ability of phenolics to scavenge radicals and hence act as an antioxidant (Saito et al., 2004). However, this differential behaviour had not been reported previously in the literature; rather, for example, the AOA behaviour was closely linked with TPC in different ethanol concentrations, in which both TPC and AOA peaked at 60% ethanol before declining to 100% ethanol (Chew et al., 2011b). Thus, it had been expected to see the AOA correlate with TPC and TMA yields in this study too. In light of this unexpected result, it was hypothesised that one or more highly potent antioxidants could have been extracted at high ethanol concentrations. In order to prove this hypothesis, an HPLC analysis was then performed to determine the individual compounds present in the extracts, which will be explained in Section 5.3.3. Another reason might be that the behaviour of reaction between phenolics and Folin reagent in TPC analysis differs from that of the reaction between phenolics and DPPH in AOA assay, leading to different trend between two sets of results. Future study should, therefore, look into other antioxidant analysis methods, such as ABTS and/or FRAP, to validate the extract's

antioxidant activity. Finally, because 50% (v/v) ethanol/water extracted the most phenolic and anthocyanin compounds, it was used for next investigation.

5.3.2 Effect of solvent-to-feed (S/F) ratio

In this work, the effect of solvent-to-feed (S/F) ratio on antioxidant activity was investigated. Several S/F ratios (20:1, 30:1, 40:1, 50:1 mL/g) of 50% (v/v) ethanol/water were used to extract the CPH at 50 °C for 5 min by using MAE and CSE. The results (Figure 5.8) showed that the increment solvent volume could increase the TPC yields and its functionality (antioxidant activity). This means that S/F ratio has significant effect on the extraction yields ($p_{value} < 0.001$).

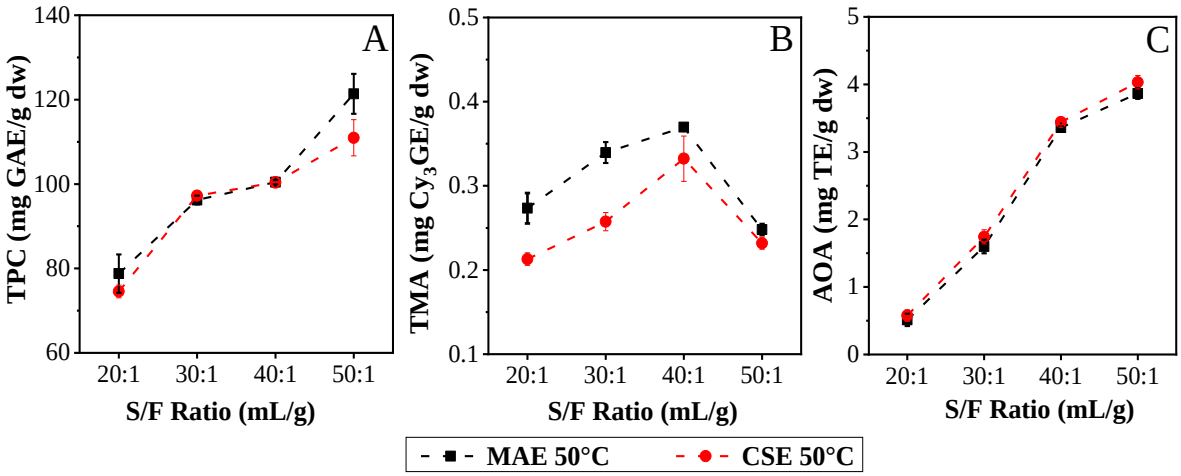


Figure 5. 8 Effect of solvent-to-feed (S/F) ratio on TPC (A), TMA (B), AOA (C) yields (CPH with particle size ≤38 micron, 50% (v/v) ethanol/water, 50 °C, 5 min), mean ± S.D. (n = 9, triplicate extraction and triplicate analysis)

In general, the extraction yields (TPC, TMA, AOA) increased with increasing S/F ratio from 20:1 to 50:1 mL/g, but in contrast, TMA decreased dramatically at the S/F ratio of 50:1 mL/g. According to Kaderides et al. (2019), a higher S/F ratio led to a more significant concentration gradient between plant material and solvent. It also caused plant material to swell excessively, improving the contact surface area between solvent and plant material. As a result, it yielded a higher extraction yield. However, a large solvent volume loading would require longer extraction time and more energy. Furthermore, the excess solvent loading could decrease the extracted anthocyanin

because the anthocyanin is retained in the cells (Pham et al., 2019). Keep in mind the consumption of less time, energy and cost, also observing the AOA with S/F ratio of 40:1 mL/g (3.44 ± 0.04 mg TE/g dw) had no significant difference from 50:1 mL/g (3.86 ± 0.1 mg TE/g dw) as well as the highest TMA reached at S/F ratio 40:1 mL/g. Thus, the S/F ratio of 40:1 mL/g was proposed as the appropriate ratio to attain high antioxidant and anthocyanin yields.

Comparing the extraction yields for MAE and CSE under the same processing parameters (time, temperature, solvent), both showed similar trends, the same amounts of phenolic content (TPC) and antioxidant activity (AOA). However, the MAE revealed a higher anthocyanin content than CSE. Then, when these findings were compared to previous results in Figure 5.5 which the TMA for MAE at 60 °C and CSE at 70 °C have a similar amount, this means that microwave heating has only an effect on anthocyanin extraction at low temperature (50 °C).

5.3.3 Identification of phenolic compounds in CPH extract

Individual phenolics in the CPH extract were determined using high-performance liquid chromatography (HPLC) based on comparisons of the retention time of standard solutions, i.e., gallic acid, (+)-catechin, (-)-epicatechin, p-coumaric acid, and quercetin. Figure 5.9 shows the chromatogram of CPH extract in three different solvents: deionised water, 50% (v/v) ethanol/water and 100% (v/v) ethanol, either by MAE or CSE experiments. The HPLC chromatogram confirmed that, in general, gallic acid, catechin, (-)-epicatechin, quercetin, and p-coumaric acid were all present in the CPH extracts. This finding was supported by Valadez-Carmona et al. (2017), who reported the presence of gallic acid, catechin, (-)-epicatechin, coumaric acid, quercetin, and protocatechuic acid in CPH extract. In another report, the phenolic compounds of gallic acid, theobromine, theophylline, (+)-catechin, (-)-epicatechin, (-)-epigallocatechin, and

(-)-epigallocatechin gallate were also found in CPH extract (Nguyen et al., 2021). In this work, catechin was not detected in the alcoholic extract, while p-coumaric acid was found in all extracts, but their concentrations were lower than the standard.

Table 5.2 presents the concentrations of individual phenolic compounds in aqueous and ethanolic CPH extracts from both MAE and CSE processing, whereas the calibration curves for calculating their concentrations of individual phenolics are shown in Figure 3.14. 100% (v/v) ethanol solvent could only extract a small amount of gallic acid and epicatechin, while the quercetin was confirmed to be a close amount to that in the aqueous extract. The highest concentration was found in the 50% (v/v) ethanol/water extract, followed by CPH aqueous (100% (v/v) water) extract and alcoholic extract (100% (v/v) ethanol), with MAE yielding higher phenolics than CSE. These results are in agreement with the TPC yield shown in Figure 5.7, Section 5.3.1.

To explain why the antioxidant activity was observed to be different from the TPC value, where AOA was highest in 100% (v/v) ethanol whilst in this media, the TPC levels were the lowest, thus the AOA of each of the individual phenolic was assessed. Figure 5.10 indicates the antioxidant activity of the identified individual phenolics following order: quercetin > epicatechin ≈ catechin ≈ gallic acid > p-coumaric acid.

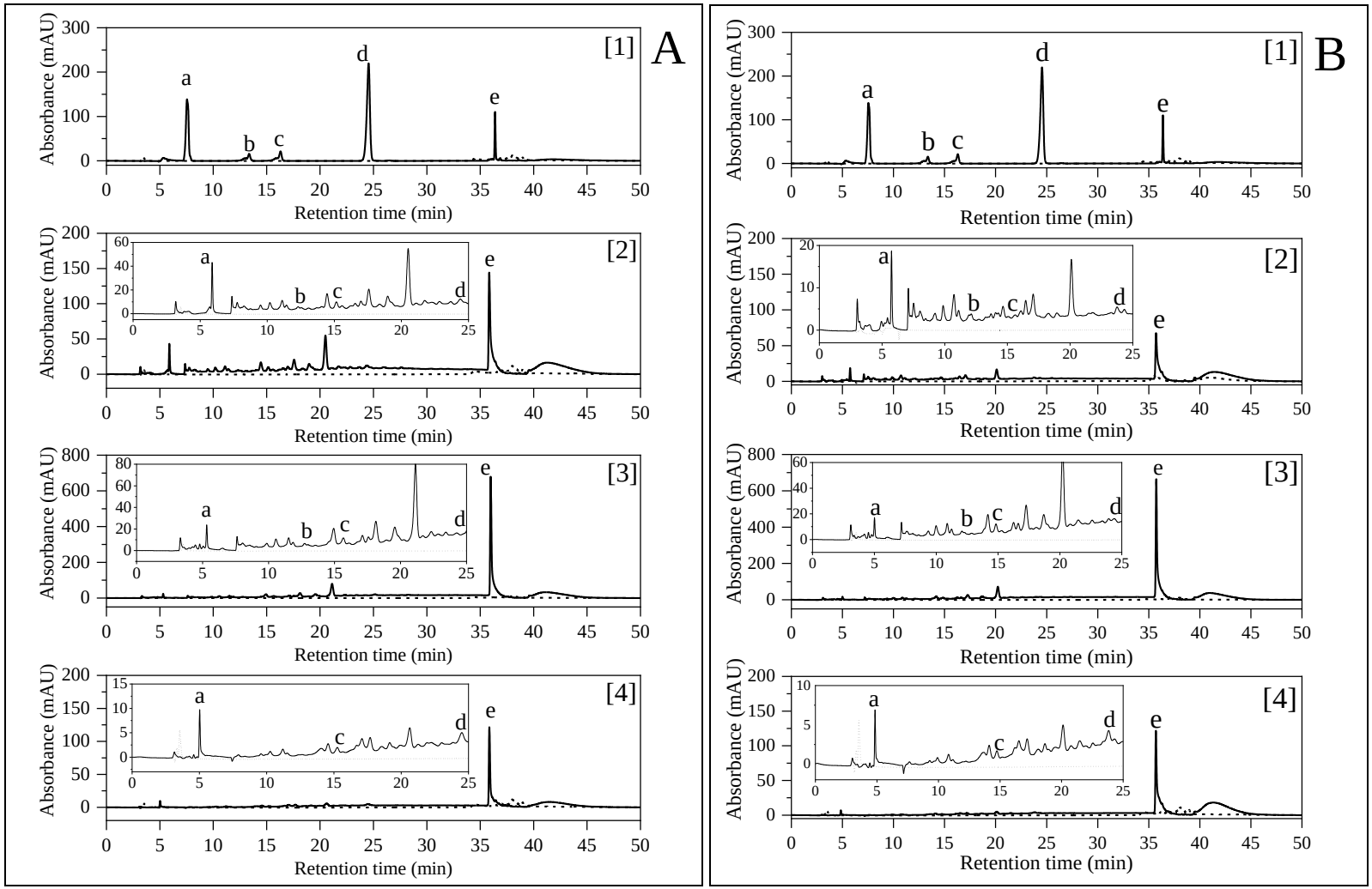


Figure 5. 9 HPLC chromatograms of phenolic compounds at 280 nm: [1] standard compounds; [2] CPH extracts in deionised water; [3] CPH extracts in 50% (v/v) ethanol/water; [4] CPH extracts in 100% (v/v/) ethanol by MAE (A) and CSE (B) at 50 °C for 5 min: (a) gallic acid; (b) catechin; (c) (-)-epicatechin; (d) p-coumaric acid; (e) quercetin; the dash line (----------) is baseline solvent.

Table 5. 2 HPLC phenolics profile of CPH extract in different solvent (µg/g dry CPH)

Compound name	Retention time (min)	MAE (A) (µg/g dry CPH)			CSE (B) (µg/g dry CPH)		
		Deionised water [2]	50% (v/v) ethanol/water [3]	100% (v/v) ethanol [4]	Deionised water [2]	50% (v/v) ethanol/water [3]	100% (v/v) ethanol [4]
Gallic acid	7.54	35.9 ± 6.9	76.4 ± 1.6	16.2 ± 1.9	28.0 ± 7.6	29.2 ± 1.7	nd
Catechin	13.36	27.1 ± 7.9	112.4 ± 1.7	0	22.1 ± 0.8	35.68 ± 1.69	0
(-)-Epicatechin	16.30	48.2 ± 5.6	332.0 ± 15.3	16.0 ± 2.5	155.9 ± 48.3	273.1 ± 8.8	87.1 ± 23.5
p-Coumaric acid	24.55	nd	nd	nd	nd	nd	nd
Quercetin	36.38	3,696.7 ± 242.1	15,505.5 ± 253.5	3,085.4 ± 426.6	1,915.7 ± 120	15,219.0 ± 583.2	2,806.4 ± 714.9
Total		3,807.9	16,026.3	3,117.6	2,121.80	15,557.0	2,893.4
TPC*		47,120	100,430	17,240	45,480	100,390	16,460

Mean ± S.D (n= 3, triplicate extraction)
nd: not defined (below limitation)
*TPC, analysed by Folin-ciocalteau method

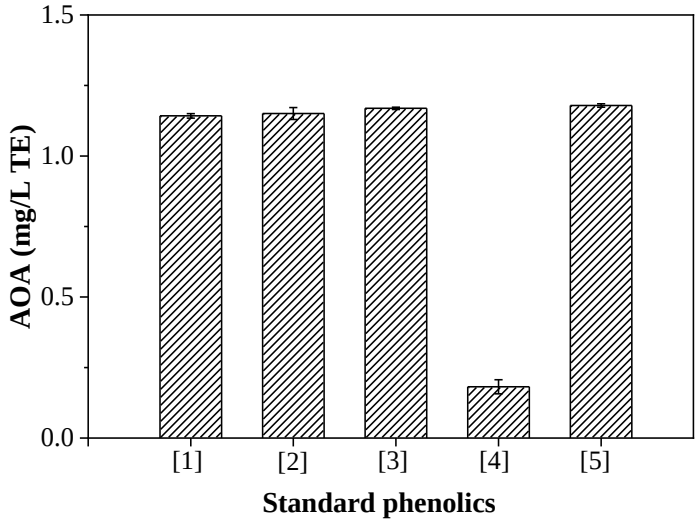


Figure 5. 10 Antioxidant activity (AOA) of standard compounds based on DPPH method: [1] gallic acid; [2] catechin; [3] epicatechin; [4] p-coumaric acid; [5] quercetin, mean ± S.D. (n= 3, triplicate analysis)

From a review of this data, if quercetin were more efficiently extracted in 100% (v/v) ethanol, this would be expected to lead to an increase in AOA of up to 4.6 mg TE/g dw, even though the total phenolic yield was very low. However, the HPLC results showed that conversely, the quercetin concentration in 100% (v/v) ethanol was also noted to be lower than in 50% (v/v) ethanol/water. Moreover, the extract in water and 50% (v/v) ethanol/water represented similar antioxidant activity, around 3.3 mg TE/g dw, even though the TPC in both extracts were extremely different. These findings indicate that CPH contains other unidentified phenolic compounds that have high potential as antioxidants, yet the anthocyanin compounds were not identified. Likewise, HPLC has not explained the increase in antioxidant activity with increasing ethanol concentration. Therefore, further analysis using more sensitive instruments, such as LCMS, is required to identify all individual phenolic peaks in CPH extract, particularly those responsible for antioxidant activity. Another possible reason to explain a high antioxidant activity in 100% (v/v) ethanol is due to the protic solvent effect. As also shown in the previous finding illustrated in Figures 4.4 and 5.3, AOA in 100% (v/v) ethanol was higher than that of 50% (v/v) ethanol/water or water because of the protic solvent effect. This is when protic solvents, such as ethanol, enhance the radical scavenging activity because they can attach to the antiradical/ antioxidant compounds, which successively deprotonate the antioxidant which then more efficiently scavenge radicals or oxidants (Saito et al., 2004). In addition, other antioxidant analysis methods, such as ABTS and/or FRAP, should be tested as comparison for determining the antioxidant activities of CPH extracts.

5.3.4 Comparison of MAE and CSE on AOA yield

In previous discussions (Section 5.2.1), it was reported that the antioxidant yield could be maximised by conducting either MAE or CSE extraction at a relatively low

temperature of 50 °C for 5 min. When comparing MAE to CSE, both methods have been found to produce no significant difference (p_{value} = 0.972) in AOA yield. During extraction at 50-70 °C for 1 to 30 min, 5 min was found as the maximum time to extract the antioxidant compounds because the extended time at elevated temperature was shown to decrease the AOA yield. Moreover, AOA decreased by increasing temperature, so 50 °C was chosen to evaluate other extraction parameters in order to further optimise the antioxidant content of the materials extracted.

With regard to ethanol concentration, a comparison between MAE and CSE at 50 °C indicated that both methods had a similar yield. The AOA yield showed a constant value from 0 to 50% (v/v) ethanol and then increased to reach the maximum at 100% (v/v) ethanol. Meanwhile, the TPC and TMA yields obtained by MAE presented higher yields than CSE when the extractant was below 50% (v/v) ethanol/water and then delivered the same yields above 50% (v/v). This means that at low ethanol concentration and 50 °C, microwave heating influenced the extraction process to maximise the phenolic and anthocyanin but was observed to produce no change in the antioxidant activity of the extract. Rather, the increase in the extracts' antioxidant activity might be attributed to the presence of protic solvent effect. Meanwhile, the comparison of MAE and CSE using the same S/F ratio at 50 °C revealed no differences in extraction yields, except at TMA. MAE extracted a higher TMA yield than CSE, but the TMA yield at an S/F ratio of 50:1 mL/g in MAE being similar to CSE.

According to Figure 5.9 and Table 5.2, there was no difference in chromatogram peaks of MAE and CSE extracts in terms of the chemical entities that are contained in the mixture. However, the individual peaks' intensities (peak area) were higher in MAE than in CSE in the case of the peak, which corresponds to the TPC yield. In extraction with 100% (v/v) ethanol, the CSE method was observed to gain higher yields of

epicatechin than MAE, but lower gallic acid. The maximum extraction yields were reached by MAE and CSE at 50 °C for 5 min using 50% (v/v) ethanol/water with S/F ratio of 40:1 mL/g. MAE could produce a TPC yield of 100.43 ± 0.5 mg GAE/g dw with functionality: 0.37 ± 0.0 mg Cy_3GE/g dw of TMA and 3.36 ± 0.02 mg TE/g dw of AOA (~30.2 μM TE/g; 75.4% scavenging); while the maximum yields on CSE were 100.39 ± 1.28 mg GAE/g dw of TPC with TMA yield of 0.33 ± 0.03 mg Cy_3GE/g dw and antioxidant activity of 3.44 ± 0.04 mg TE/g dw ((~31.8 μM TE/g 75% scavenging). The result from this study showed higher antioxidant yield compared with that of ethanolic CPH extract from previous studies reported using CSE by Martínez et al. (2012) (21.4 μM TE/g), although operated in shorter extraction time (5 min) and lower extraction temperature (50 °C). However, it was still lower than CPH extracts yields that reported by Yapo et al. (2013) (85.4% scavenging), Karim et al. (2014a) (77.6% scavenging), Valadez-Carmona et al. (2017) (70.8 μM TE/g), Teboukeu et al. (2018) (97.56% scavenging), and Nguyen et al. (2020) (5.8 mg TE/g dw). Antioxidant activity of CPH extract was also lower than pomegranate peels extract which had activity of 94.9% radical scavenging (Kaderides et al., 2019). In terms of anthocyanin yield, although the CPH extract is relatively low compared to purple corn cob (Lao and Giusti, 2018) and blueberries (Brito et al., 2014; Yuan et al., 2020) biomass, it is still higher than that in Jamun fruit pulp extract (Maran et al., 2015). The discrepancy in extraction yield between this study and others can be attributed to the following factors: (1) different CPH clones; (2) various extraction solvents that may extract different compounds; (3) different extraction methods and/or processing conditions.

5.3.5 Summary

In order to maximise the extracts' antioxidant activity, MAE was compared with CSE at 50 °C and 5 min using the same bulk heating profile and stirrer speed (1200

rpm). In both methods, the maximum TPC and TMA yields were obtained using 50% (v/v) ethanol/water with solvent-to-feed (S/F) ratio of 40:1 mL/g, while the maximum antioxidant activity was achieved when high ethanol concentration (100% (v/v) ethanol) and solvent loading (50:1 mL/g) were adopted. In conclusion, at 50 °C, by using the same extraction time, ethanol concentration and S/F ratio, MAE was found to present the same maximum point of TPC, TMA, and AOA yields as were achieved by CSE. Thus, it can be seen that no difference delivered by the heating methods. However, HPLC could not explain the high antioxidant activity in alcoholic extract at the lowest point of phenolics (TPC). Hence, further analysis using more sensitive instruments, such as LCMS, is required to identify all individual phenolic peaks in CPH extract, particularly those responsible for antioxidant activity. Additionally, it may also be necessary to assess the extracts' antioxidant activity using other techniques (such as ABTS and/or FRAP).

Microwave heating did result in an increased anthocyanin content at low ethanol concentration (0 to 40% (v/v) ethanol/water) and solvent loading (20:1 to 40:1 mL/g). Whereas the increased antioxidant activity was proposed to be a result of the influence of the protic solvent effect. Therefore, to gain maximum extract yield with high functionality (antioxidant activity), extraction of CPH could be conducted by using either MAE or CSE, with the decision about which to use coming from considering the energy used and cost for scaling-up equipment that is planned to be used. If the extraction is focused on maximising the anthocyanin yield, the extraction method and extraction parameters (extraction time, ethanol concentration, S/F ratio) need to be considered because they significantly affect the TMA yield. This study suggests that MAE provides benefits in this case because it offers extraction at low temperature (50 °C) with shorter extraction time and uses a GRAS 'green' solvent.

This work can be used as input data for future engineering design in CPH valorisation. The use of "greener" solvents such as 50% (v/v) ethanol/water will be preferable for industry; in addition, operating in a shorter extraction time (5 min) and lower extraction temperature (50 °C) will benefit the industry. However, prior to scaling up the process, those processing parameters must be further identified based on techno-economic considerations.

5.4 Potential for scaling-up process

Section 5.4 is part of a manuscript prepared for Journal Biomass and Bioenergy

Dewi, S.R, Stevens, L.A, Ferrari, R., Irvine, D.J, Binner, E.R., Extraction of phenolic-based antioxidants from cacao pod husk (CPH): implications for scaling-up process and potential applications of CPH solid residue

5.4.1 Comparison of MAE and CSE at maximum conditions

According to the findings of maximum processing parameters to obtain maximum antioxidants: extraction time of 5 min, the temperature of 50 °C, and solvent of 50% (v/v) ethanol/water with a ratio of 40:1 mL/g, experiments for different CPH particle size were then re-assessed. Figure 5.11 shows the extraction yields for both MAE and CSE methods at those maximum processing parameters.

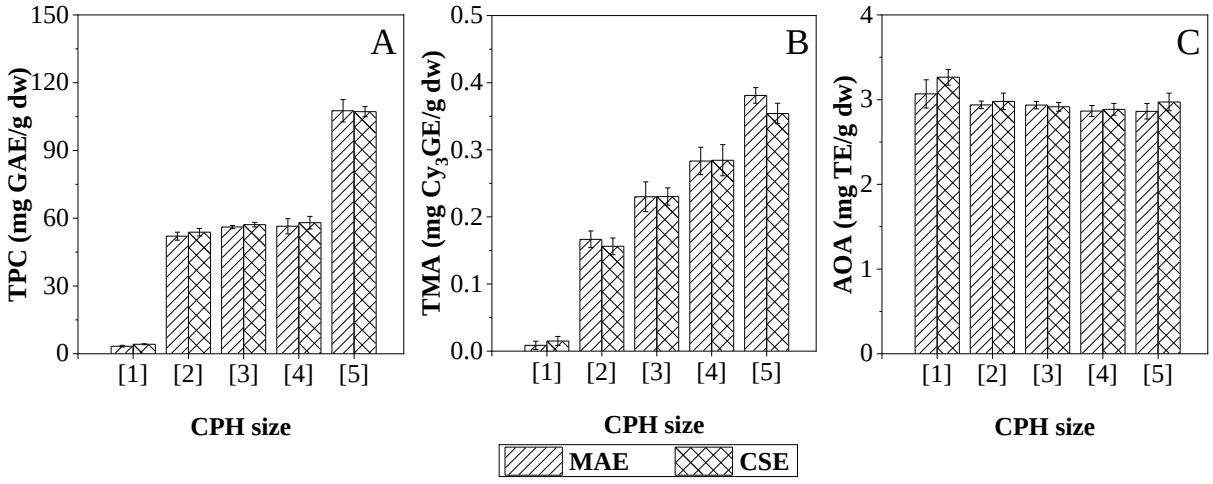


Figure 5. 11 Comparison of MAE and CSE on TPC (A), TMA (B), AOA (C) yields of dry CPH (50% (v/v) ethanol/water, S/F ratio of 40:1 mL/g, 50 °C, 5 min): CPH with particle size [1] 0.5x0.5 cm (without grinding); [2] 125-150 micron; [3] 63-90 micron; [4] 38-63 micron; [5] ≤38 micron

There are two key research findings; firstly, there is no noticeable difference in TPC, TMA and AOA of both MAE and CSE methods which confirmed that no selective heating effect was involved in the extraction under microwave heating at 50 °C. Thus, the extraction methods could be selected for the scaling-up process based on energy and equipment size requirements. Secondly, the results (Figure 5.11) also presented that size reduction significantly impacted the bioactive yields (TPC and TMA). The TMA yield increased with the increasing material surface area (the smallest particle size), while an increase in TPC due to size reduction occurred only for CPH ≤38 micron, up to 45%. Meanwhile, the AOA did not affect by different heating methods or size reduction; the AOA in the CPH was about ~3.0 mg TE/g dw. Therefore, material size reduction should be included in the process flowsheet in large-scale production.

5.4.2 Effect size of various extraction parameters

The effects of the extraction parameters on maximising the extraction yields (TPC, TMA, AOA) were determined using analysis of variance (ANOVA), and its summary is shown in Table 5.3. The TPC, TMA, and AOA yields are generally significant affected ($p_{value} < 0.001$) by almost all extraction parameters including sample pretreatments (drying and size reduction), solvent properties (solvent type, ethanol concentration, and S/F ratio), and processing parameters (extraction method, extraction time and temperature). Only the extraction / heating method which has no discernible impact on AOA yield ($p_{value} = 0.589$). ANOVA data can be used to compare the effects of various extraction parameters and identify the most influential parameter on the extraction yields. The most influential factor is determined by the parameters with the highest effect size (as indicated by a high F-statistic or eta square (η^2) value). According to Table 5.3, solvent properties (solvent type ($\eta^2 = 0.99$) and ethanol concentration (($\eta^2 > 0.92$)) and sample pretreatments (size reduction and drying) are the most influential

factors in extraction of phenolic antioxidants from CPH waste. Size reduction has a high influence ($\eta^2 > 0.94$) on phenolic and anthocyanin extraction, whereas drying gives high effect ($\eta^2 = 0.993$) on antioxidant yield. Therefore, when scaling up the extraction process, those most influential parameters must be considered in order to maximise extraction yield while minimising operational cost.

Table 5. 3 Effect size measures (p_{value}, F-statistic, η^2) of each parameter for total phenolic content (TPC), total monomeric anthocyanin (TMA), and antioxidant activity (AOA)

Extraction parameter	Total Phenolic Content			Total Monomeric Anthocyanin			Antioxidant Activity		
	p_{value}	F	η^2	p_{value}	F	η^2	p_{value}	F	η^2
Drying	< 0.001	319.97	0.580	< 0.001	171.8	0.507	< 0.001	15214	0.993
Solvent type	< 0.001	3014.6	0.997	< 0.001	161.5	0.994	< 0.001	893.55	0.99
Extraction method	< 0.001	99.209	0.903	< 0.001	59.23	0.847	0.589	0.65	0.057
Size reduction	< 0.001	14114	0.999	< 0.001	152.9	0.935	< 0.001	14.211	0.571
Extraction time	< 0.001	176.68	0.33	< 0.001	556	0.629	< 0.001	151.29	0.212
Extraction temperature	< 0.001	255.42	0.239	< 0.001	323.7	0.183	< 0.001	860.93	0.604
Ethanol concentration	< 0.001	2550.7	0.982	< 0.001	932.5	0.924	< 0.001	2317.1	0.982
Solvent-to-feed (S/F) ratio	< 0.001	561.12	0.927	< 0.001	265.8	0.692	< 0.001	6358.5	0.995

5.4.3 Influence of dielectric properties and penetration depth

To scale up a microwave process, information on dielectric properties is required to (a) aid in the design of the electromagnetic equipment and (b) understand whether and when the CPH is likely to heat selectively, potentially providing processing advantages over conventional heating. Figure 5.12.A shows the loss tangent and penetration depth of 50% (v/v) ethanol/water from 20 to 70 °C with and without CPH, which could indicate the mixture's dielectric properties during the extraction process. Loss tangent (tan δ) is the ratio of the dielectric loss to constant and is used to indicate the material's ability to absorb and convert electromagnetic energy into heat (Ibrahim and Zaini, 2018). In Figure 5.12.A, the loss tangent is lower in solvent and CPH

mixtures up to 40 °C, hits and reaches the same level as solvent at 50 °C, and goes higher at and above 60 °C, which indicates that the CPH mixture will be selectively heated above 50 °C. This statement is supported by data in Figure 5.1 that showed the TPC yield of MAE was higher than CSE at 60°C due to the selective heating effect. This indicates that unique microwave enhanced mass transfer effects (for example, Temperature-Induced Diffusion) (Taqi et al., 2020) may occur if the processing temperature is above 50 ºC. It may also exacerbate the thermal degradation of the target extracts (as the CPH is heated above the processing temperature), although the degree of selective heating is only likely to be the order of a few degrees, so the effect may be negligible. Figure 5.12.B represents the loss tangent of ethanol/water mixture from 0 to 100% (v/v); the ability of solvent and solvent-CPH mixture to be heated by microwaves increased with the increase of ethanol concentration. However, comparing those increments and the trend of extraction yields on MAE and CSE (Figures 5.3.A-B and 5.7.A-B) shows no correlation between dielectric properties and extraction yields.

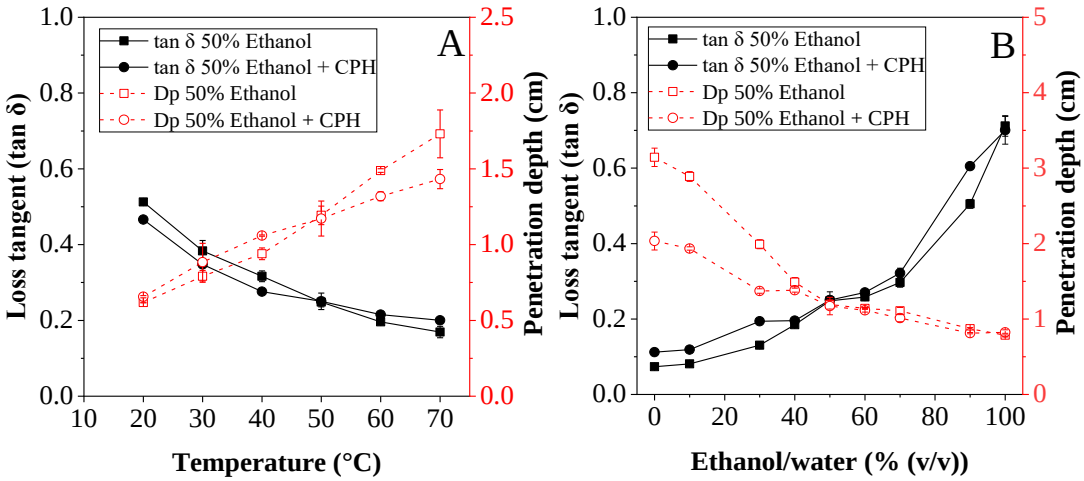


Figure 5. 12 Loss tangent and penetration depth of solvent with and without CPH at 2.45 GHz: (A) 50% (v/v) ethanol/water at different temperatures; (B) Ethanol/water mixture at 50 °C

Moreover, the information about the penetration depths is also important to understand how microwave heating can go inside the materials. It can aid in assessing

the heating uniformity and designing (scaling up) the electromagnetic heating instrument (Ibrahim and Zaini, 2018). The penetration depth data could be used to select a flow diameter; the use of a flow diameter larger than the penetration depth could acquire notable heating heterogeneity across the radial direction. For instance, a flow diameter of 10 mm was chosen for the potato pulp mixture since the penetration depth ranged from 13 to 16 mm at 20 – 90 °C (Arrutia et al., 2020). The penetration depth (Dp) is inversely proportional to the dielectric properties (loss factor) of material (Equation 1.3). If the penetration depth is smaller than its material thickness, the material will only be heated at the surface, while the remaining part will be heated by conduction (Ibrahim and Zaini, 2018). Figure 5.12. A showed the penetration depth of microwaves into CPH mixtures increased from 5 to 20 mm over the temperature ranging between 20 and 70 °C. The penetration depth of CPH mixtures was higher than that of the solvent at below 50 °C and became lower than that of the solvent above 50 °C. At the maximum yield (50 °C), the penetration depth of microwaves was about 12 mm, whereas the diameter of the extraction flask was 39 mm. The penetration depth will be problematic in the scaling-up process. Therefore, the sample was stirred during the process to make the extraction more effective.

5.4.4 Implications for engineering design and scale-up

Scaling up the microwave extraction should take into account the processing parameters under selective heating to maximise the extraction yields and calculate which operating processes offer economic attractiveness. Several main points have been reported to consider for MAE scale-up: (1) operating under selective heating's temperature; (2) optimising power delivery to plant materials based on studies of the selective heating effect in enhancing yield; (3) understanding the design's penetration depth limitation; (4) understanding the theoretical energy calculation in the system. In

a scale-up system, the energy consumption would be determined by solvent loading, solvent-to-feed ratio, and target of biomass and extract (Galan et al., 2017). Works have found that microwave selective heating could affect the extraction yield above 50 °C, and overheating might occur at 70 °C. Hence, for scaling up the process, extraction is recommended to operate at 50 – 60 °C, especially for phenolic production, so the potential benefits of microwave heating will be maximised. While the information about the mixture's penetration depth will help design the microwave extractor to maximise the power delivery. Arrutia et al.(2020) suggested using a lower frequency for scaling up the microwave extraction process because it would give two advantages: (1) larger penetration depth because of the longer wavelength and (2) a larger single-mode applicator. On the other hand, the influence of each processing parameter on the extraction yields will be used as input data for designing the process flowsheet and calculating its energy consumption and techno-economic analysis. In this work, size reduction, solvent requirements (solvent type, concentration, solvent-to-feed ratio), extraction temperature and processing time were the important parameters to maximise the extraction yields. In contrast, the extract separation and purity processes (filtration, evaporation, and drying) are essential for estimating the energy required for large-scale production.

In this laboratory-scale study, conventional heating (CSE) could be designed to achieve a fast-heating rate like microwave heating (MAE) by using an ethylene-glycol bath. This condition will increase design complexity for scaling up the process because CSE will require a large contact area (extractor) and will increase the capital cost. Conversely, scaling up the CSE procedure without accelerating the heating rate using an ethylene-glycol bath will reduce the capital cost but may increase the operating cost on energy consumption due to longer processing time. The scaling up MAE process

will also be expensive in capital cost due to the large capital investment for microwave generators. However, the investment of large production capacity (for example, 64.6 t/year) for MAE would be more economic beneficial than conventional since MAE produced higher extraction yield and required less feedstock, smaller equipment size, and lower heating energy due to shorter extraction time than conventional. The capital costs for MAE and CSE in the extraction of 64.6 t/year bio-flocculants from okra were 7.63 and 7.98 million dollars, respectively (Lee et al., 2018). Therefore, it would be suggested to scale up the MAE process rather than CSE (using an ethylene glycol bath); additionally, MAE would have low operational costs. On the other hand, to address the shortcomings of MAE in terms of penetration depth limitation, designing a microwave extractor with a stirring system or a long tubular reactor would be recommended.

The scalable MAE process has been successfully developed for extracting pectin from potato pulp using a continuous-flow microwave system with a single-mode cavity, as seen in Figure 5.13.

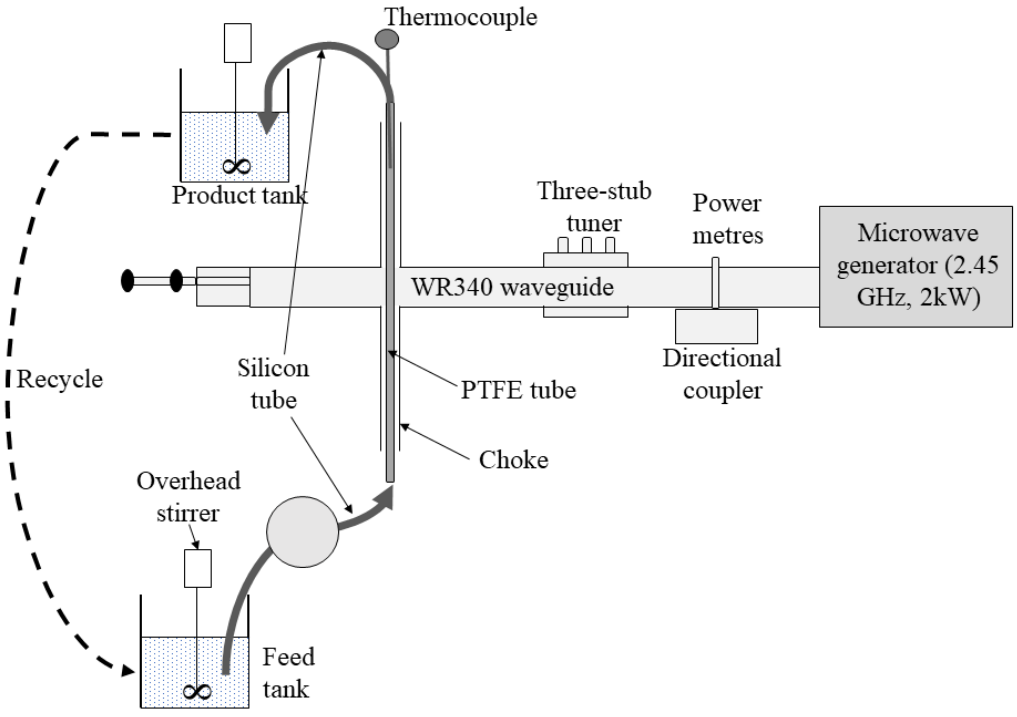


Figure 5. 13 Continuous-flow microwave rig assembly for pectin extraction, redrawn from (Arrutia et al., 2020)

A WR340 waveguide was used to transmit the microwave power from the microwave generator (Sairem, France, 2.45 GHz, 2 kW). A directional coupler connected to a power sensor (Agilent, USA) for measuring frequency as well as forward and reflected power, while to increase the delivery of microwave power, a three-stub tuner and sliding short circuit were used for impedance matching. The absorbed power (forward power – reflected power) will pass across the single-mode cavity in the PTFE tube, which was connected to the silicon tube at the inlet and outlet system. Circular chokes were used to maintain the microwave power flux at or lower the maximum permitted leakage level (5 mW/cm^2). The mixture from the feed tank was pumped through the microwave system and temperature was measured using a K-type thermocouple inserted at the choke's outlet. This experimental finding has shown that, with a feed flow rate of 250 mL/min, the microwave system had good temperature control of ±2.5 °C and quickly reached a stable temperature setting. The maximum yield (40-45 % Y_{GaIA}) was achieved for the residence time of less than one second in the microwave cavity, which was more than twofold than that of the batch (Arrutia et al., 2020).

CHAPTER 6: RESULTS AND DISCUSSION – COMPARISON OF BIOACTIVE COMPOUNDS IN EPICARP, MESOCARP, AND ENDOCARP LAYERS OF CPH

Chapter 6 is prepared as manuscript for International Journal of Food Science and Technology

Dewi, S.R, Ferrari, R., Stevens, L.A, Irvine, D.J, Binner, E.R., A comparative study of bioactive compounds in epicarp, mesocarp, and endocarp of cacao (Theobroma cacao L.*) pod husk and their antioxidant activities.*

6.1 Introduction

CPH contains phenolic compounds which have antioxidant activity (Karim et al., 2014b; Nguyen et al., 2021; Valadez-Carmona et al., 2017), and the applications of antioxidants from CPH extract were shown in palm olein to prevent lipid oxidation (Teboukeu et al., 2018) or in skin treatment for anti-wrinkle, skin whitening, and soap (Abdul Karim et al., 2016; Gyedu-Akoto et al., 2015; Karim et al., 2014b). CPH itself consists of three layers: epicarp (outer), mesocarp (middle), and endocarp (inner). The endocarp is a soft tissue protecting cacao beans in a well-lubricated inner chamber which rich in pectic compounds; the mesocarp is a hard-composite structure to cover cacao beans in place, which contains fibre; and the epicarp is the outermost layer with yellow colour (when ripe) which is enriched with lignin and pigment compounds. The soluble and insoluble proanthocyanidins (pigment) in epicarp were about 170 and 8 mg/g dw, respectively (Campos-Vega et al., 2018). The percentage of each CPH layer is presented in Figure 2.1; it showed that the mesocarp was the largest part (51-62% by cacao fruit mass), followed by the epicarp and endocarp layers. The epicarp was also reported to have the highest total ash, Ca, K, P, and other inorganic elements, while the mesocarp contained crude fibre and cellulose (Sobamiwa and Longe, 1994). Even though the phenolic compounds have been reported to be present in CPH, however,

there is no report on the phenolic content in each CPH layer. Therefore, this study will, for the first time, provide a comparison of phenolics content, including anthocyanins, in epicarp, mesocarp, and endocarp to understand the relative distribution across CPH layers.

The distribution of phenolic compounds and their antioxidant activities within the fruit tissue or layer was influenced by the variety and ripening stage. Investigation of the different concentrations of phenolic compounds in apple tissues (epicarp, mesocarp, endocarp) during ripening revealed that phenolic acids and flavonoids in epicarp and endocarp decreased with ripening. The phenolic compounds were concentrated in the epicarp tissue of apples, and the flavonols and anthocyanins were only found in the epicarp layer (Alberti et al., 2017). Another group (Aghofack-Nguemezi and Schwab, 2015) also studied the distribution of flavonoids in tomato fruit tissue during maturation and ripening. The flavonoids of caffeic-acid-hexose and caffeoylquinic acid have increased in all tissues (epicarp, mesocarp and endocarp) during ripening, which the level of caffeoylquinic acid in epicarp being the highest, followed by endocarp and mesocarp. According to references, different layers of fruit or plant will have different concentrations of bioactive compounds. Thus, the distribution of phenolic compounds, including anthocyanins, in the CPH layer is interesting to study.

Several studies on CPH layers were reported previously; Sobamiwa and Longe (1994) investigated each CPH layer in terms of proximate components for animal feed in a broiler chick diet. On mesocarp diets, chick gizzard weight increased, while the epicarp was the most limiting part of the CPH. The epicarp components, primarily lignin and pectin, were inhibiting the CPH utilisation as animal feed. Another study investigated the effect of processing parameters (milling time and rotational speed) on grinding the cocoa pod endocarp (CPE), and they provided valuable insight in the

processing of CPE as a functional fibre source and colouring agent for further application in confectionery products (Grob et al., 2021). However, there is no report on the extraction of each CPH layer, especially for phenolic extraction. Previous research only focused on determining phenolic content in the whole CPH, where the phenolic compounds may be distributed in all CPH layers. To the best of our knowledge, no study has reported the distribution of phenolic compounds in three CPH layers. Hence, this work was intended to study the comparison of phenolic compounds, anthocyanins, and antioxidant activity of three separable CPH layers: epicarp, mesocarp, and endocarp. The effect of heating methods (MAE and CSE) and different CPH particle sizes will also be compared under similar conditions (maximum condition obtained from previous work – Chapters 4 and 5).

6.2 Preliminary extraction of bioactive compounds from each layer of CPH

The CPH waste accounted for 76-86% of the fresh cacao fruit's weight; an average weight for each fresh CPH was approximately 553.7 ± 24 g, with the proportion of mesocarp layer (69.58 ± 4.04 %) being higher than epicarp (17.23 ± 1.71 %) and endocarp (13.20 ± 4.11 %) layers, as presented in Table 3.2 and Figure 6.2. As a preliminary study, bioactive compounds from epicarp, mesocarp and endocarp of CPH were firstly extracted using 50% (v/v) ethanol/water at 60 °C for 5 min under microwave heating (MAE). The phenolic content was determined as an illustration of bioactive compounds in three different CPH layers. The results showed that phenolic compounds were found in all CPH layers (epicarp, mesocarp, endocarp); the appearance of phenolic extracts and their bioactive contents from each layer are shown in Figures 6.1 and 6.2, respectively. Generally, extract from the endocarp was darker than other layers, but the TPC yields showed that the epicarp had the highest phenolic

contents instead of others, that was 155.6 ± 6.5 mg GAE/g dw. So, there is no correlation between colour density and TPC value. Figure 6.2 represented that the mesocarp was the highest part of CPH, followed by the epicarp and endocarp, but the phenolic compound was found to be the highest in epicarp layer. Even though it is known that phenolic compounds are mostly stored in epicarp layer, the distribution of bioactive compounds in every layer of CPH still needs to be addressed. Therefore, the next sections discuss the comparison of bioactive compounds (phenolics and anthocyanin) and their antioxidant activity among three CPH layers: epicarp, mesocarp, and endocarp and understand the effect of different particle sizes and extraction methods.

Figure 6. 1 Extracts from each layer of CPH

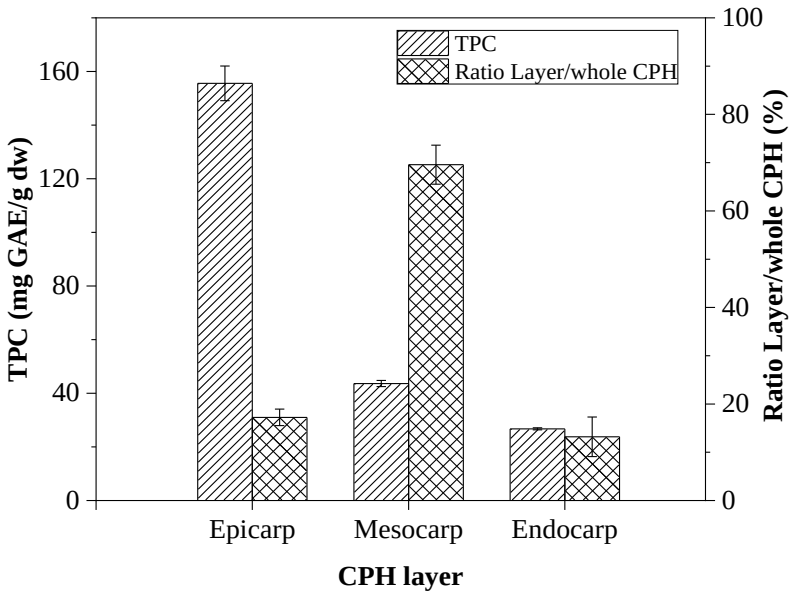


Figure 6. 2 Total phenolic content (TPC) from each CPH layer (MAE method, CPH with particle size ≤38 micron, 50% (v/v) ethanol/water, S/F ratio of 40:1 mL/g, 60 °C, 5 min), mean ± S.D. (n= 9, triplicate extraction and triplicate analysis

6.3 Effect of particle size on extraction yields of CPH layer

In this section, understanding the distribution of the bioactive compound among three CPH layers was studied by investigating the phenolic and anthocyanin contents as well as antioxidant activity of extracts. Even though Alberti et al. (2017) stated that flavonols and anthocyanins were only present in the epicarp layer of apple fruit, the statement also needs to be proven in each CPH layer by determining the phenolic and anthocyanin contents. Extracts were produced through microwave heating (MAE) and conventional heating (CSE) at the best condition previously discussed: 50% (v/v) ethanol/water (40:1 mL/g), 50 °C, and 5 min. The effect of particle size on each CPH layer on the extraction yields was investigated; each CPH layer was prepared into several particle sizes: 0.5x0.5 cm (without grinding), 125-150 micron, 63-90 micron, 38-63 micron and ≤ 38 micron.

Table 6.1 shows size reduction in each CPH layer significantly increased the surface area, micropore, mesopore and total pore volumes of material. Although the surface area of mesocarp was higher than epicarp and endocarp at particle size of 125-150 micron, size reduction to ≤ 38 micron could produce a similar surface area and micropore volume among three layers, at around 2.0 m^2/g and 0.6 mm^3/g, respectively. The average pore diameter of each CPH layer (20-30 nm) from Table 6.1 proves that CPH layers (epicarp, mesocarp, endocarp) are predominately mesoporous and macroporous materials. The data coincide with the adsorption-desorption isotherm graphs shown in Figures 6.3 – 6.5. Data for all CPH layers (epicarp, mesocarp, endocarp) illustrated Type IV isotherm (Figure 3.2.A), as expected for mesoporous material. A “knee” point at 0.01-0.06 relative pressure (P/Po) was not sharp, indicating limited micropore filling and multi-layer adsorption inside the mesopores at high

pressure. These phenomena were similar to the whole CPH behaviour explained in Section 4.5.

Table 6. 1 BET surface area and pore volumes of each CPH layer with different particle size

CPH layer with 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 (4V/A) (nm)
EPICARP					
125-150 micron	0.54 ± 0.07	0.16 ± 0.02	1.13 ± 0.23	3.90 ± 0.57	28.88 ± 3.53
63-90 micron	0.54 ± 0.03	0.16 ± 0.01	1.06 ± 0.23	3.65 ± 0.48	27.25 ± 1.43
38-63 micron	1.48 ± 0.08	0.43 ± 0.09	3.39 ± 1.58	9.54 ± 0.40	25.26 ± 4.67
≤ 38 micron	1.99 ± 0.05	0.65 ± 0.09	4.24 ± 0.95	13.04 ± 0.35	26.16 ± 0.04
MESOCARP					
125-150 micron	0.78 ± 0.07	0.21 ± 0.02	1.54 ± 0.22	4.61 ± 0.57	23.63 ± 3.53
63-90 micron	0.99 ± 0.03	0.29 ± 0.01	1.89 ± 0.16	5.13 ± 0.48	20.78 ± 1.43
38-63 micron	1.46 ± 0.27	0.42 ± 0.07	3.44 ± 1.36	9.48 ± 3.57	25.52 ± 5.14
≤ 38 micron	2.08 ± 0.40	0.63 ± 0.01	5.10 ± 0.31	14.50 ± 1.28	29.70 ± 0.78
ENDOCARP					
125-150 micron	0.45 ± 0.07	0.14 ± 0.02	0.95 ± 0.22	3.21 ± 0.57	28.40 ± 3.53
63-90 micron	0.55 ± 0.03	0.16 ± 0.01	1.10 ± 0.16	3.58 ± 0.48	26.12 ± 1.43
38-63 micron	1.33 ± 0.31	0.38 ± 0.09	2.68 ± 0.90	7.8 ± 2.79	23.30 ± 2.91
≤ 38 micron	2.00 ± 0.11	0.61 ± 0.12	4.30 ± 1.03	12.55 ± 1.36	25.12 ± 1.36

Mean ± SD (n= 2, duplicate analysis)

The hysteresis loops in epicarp, mesocarp and endocarp were close to Type H4 behaviour (Figure 3.2.B), meaning these CPH layers were micro-mesoporous carbons (Thommes et al., 2015). However, these hysteresis loops in CPH layers experienced different behaviour from the whole CPH. In whole CPH, hysteresis loops followed Type H4 behaviour for all particle sizes. In contrast, in CPH layers (epicarp, mesocarp, endocarp), size reduction caused changes in hysteresis phenomenon. In epicarp layer, all particle sizes showed hysteresis loops, with the smaller particle size the larger hysteresis loop. While in mesocarp and endocarp, there were no hysteresis loops between the particle size of 125-150 micron and 38-63 micron; hysteresis only occurred at CPH layers with particle size ≤38 micron.

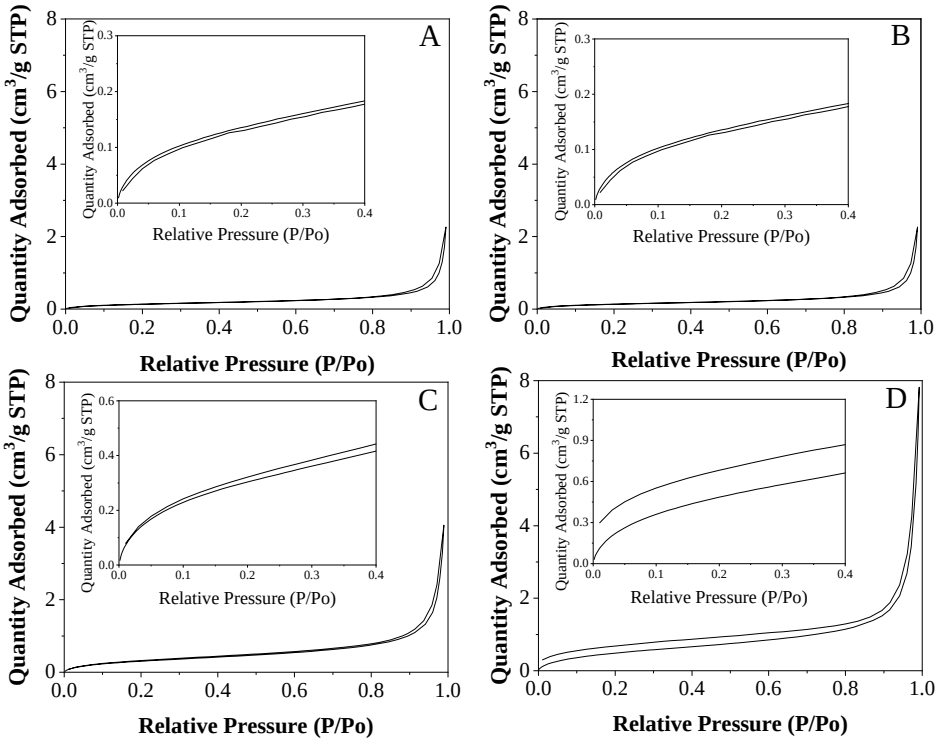


Figure 6. 3 BET Isotherm graph of CPH epicarp layer: (A) particle size 125-150 micron; (B) particle size 63-90 micron; (C) particle size 38-63 micron; (D) particle size ≤38 micron

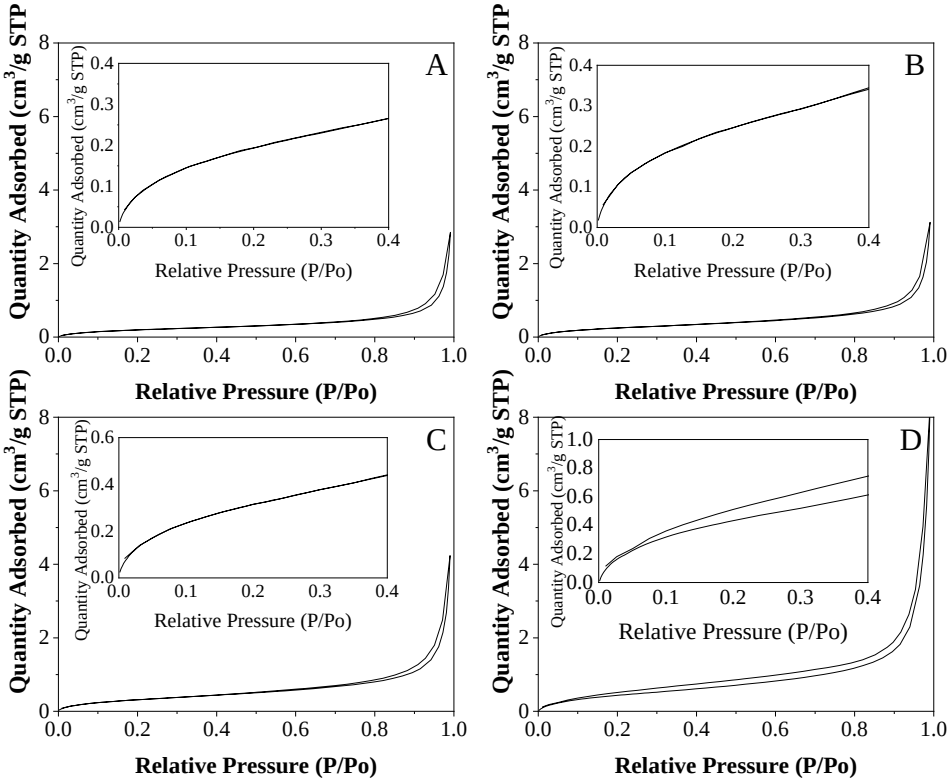


Figure 6. 4 BET Isotherm graph of CPH mesocarp layer: (A) particle size 125-150 micron; (B) particle size 63-90 micron; (C) particle size 38-63 micron; (D) particle size ≤38 micron

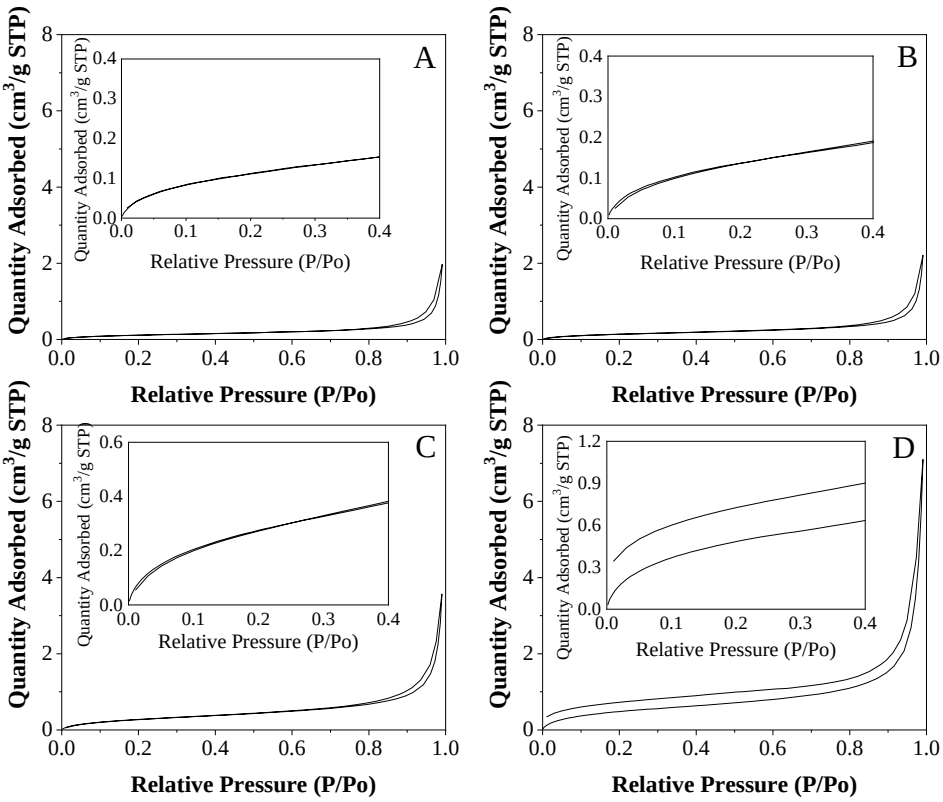


Figure 6. 5 BET Isotherm graph of CPH endocarp layer: (A) particle size 125-150 micron; (B) particle size 63-90 micron; (C) particle size 38-63 micron; (D) particle size ≤38 micron

As previously discussed in Section 4.5, size reduction can improve the surface area of plant material and enhance extraction yields. Figure 6.6 proved that size reduction significantly affected the extraction yields ($p_{value} < 0.001$). By using both MAE and CSE methods, the smaller particle size of CPH layers, the higher surface area of material and the higher phenolic and anthocyanin yields in extracts, either in epicarp, mesocarp, or endocarp. Size reduction on CPH layers from 150- to 38 micron has improved the surface area and pore volumes about three to four times; therefore, it increased the TPC yields by about 46% from 123.6 to 180.7 mg GAE/g dw in epicarp extract and up to 65% in mesocarp and endocarp extracts. It was conceivable that size reduction could increase the number of pores (where nitrogen sits) and would then increase the pore volume, allowing for an increase in surface area. This phenomenon was also found in coal (Zou and Rezaee, 2016) and shales sample (Lyu et al., 2021),

whose BET surface area and pore volume increased as the decreasing of particle size. Thus, it can be seen if the contact surface area improvement can help ease the extraction process and enhance the extraction yields. The phenolic (TPC) and anthocyanin (TMA) contents in all layers were found to be the highest in the smallest particle size (≤38 micron). The TPC yields were 180.7 ± 2.8 mg GAE/g dw, 84.0 ± 5.5 mg GAE/g dw, and 45.4 ± 2.0 mg GAE/g dw in epicarp, mesocarp, and endocarp layers, respectively. While the TMA yields were 0.46 ± 0.02 mg Cy_3GE/g dw in epicarp, 0.34 ± 0.02 mg Cy_3GE/g dw in mesocarp and 0.16 ± 0.01 mg Cy_3GE/g dw in endocarp. Meanwhile, increasing TPC and TMA yields did not increase their antioxidant activity. Size reduction significantly decreased the antioxidant activities of extract in epicarp and mesocarp layers; this may be because of the degradation of antioxidant compounds during grinding. In contrast, in endocarp extract, reduction in particle size has no significant effect on antioxidant activities of extracts. This might be because the antioxidant activity in endocarp extract was maximum at about ±3.7 mg TE/ g dw.

6.4 Comparison of bioactive compounds in each CPH layer

The comparison of TPC, TMA and AOA yields in epicarp, mesocarp and endocarp extracts is presented in Figure 6.6. In general, the epicarp layer has the highest total phenolic (TPC) and anthocyanin (TMA) contents, followed by mesocarp and endocarp layers, either by MAE or CSE. There are no previous reports about the total phenolic contents in the CPH layer. However, other studies reported a high phenolic compound in epicarp compared to the mesocarp and endocarp. Osorio-Esquivel et al. (2011) reported a total phenolic content in *O. joconostle* fruit of 19.9 mg GAE g dw in epicarp, 17.0 mg GAE/g dw in mesocarp and 17.28 mg GAE/g dw in endocarp. Alberti et al. (2017) also stated that phenolic compounds in the apple fruit's epicarp were higher

than in its mesocarp or endocarp. Those reports support these findings that phenolic compounds in CPH are most concentrated in epicarp layer.

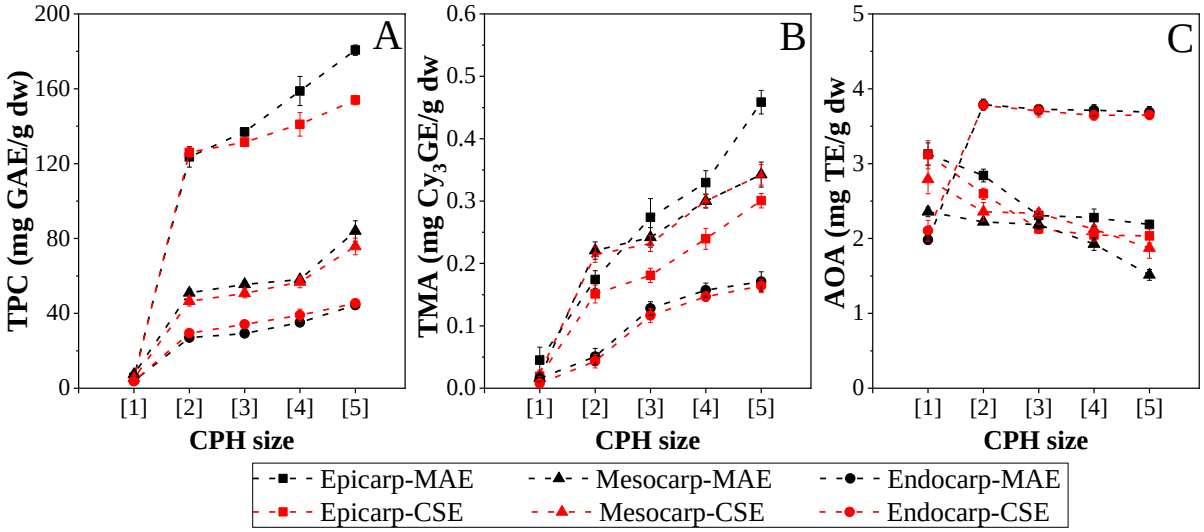


Figure 6. 6 Comparison of TPC (A), TMA (B), and AOA (C) yields in epicarp (■), mesocarp (▲), endocarp (●) obtained by MAE (black line) and CSE (red line) (50% (v/v) ethanol/water, S/F ratio of 40:1 mL/g, 50 °C, 5 min) from CPH layers with different particle size: [1] 0.5x0.5 cm (without grinding); [2] 125-150 micron; [3] 63-90 micron; [4] 38-63 micron; [5] ≤38 micron, mean ± S.D. (n= 9, triplicate extraction and triplicate analysis)

In general, TPC, TMA and AOA yields among three CPH layers were significant different (p_{value} < 0.001). The TPC value in epicarp extracts had more than twice the mesocarp's yield, but TMA yield in both extracts were close (p_{value} = 0.011). In contrast, the antioxidant activity of extract in endocarp layer was found to be maximum, around ±3.7 mg TE/g dw, although the TPC and TMA were the lowest values. This means that phenolic compounds are concentrated in epicarp layer, while anthocyanins are concentrated in both epicarp and mesocarp layers. However, phenolic compounds extracted from the epicarp layer may have low antioxidant activity compared to phenolic compounds in endocarp extract with high antioxidant activity. Similar yields were also reported by Nour et al. (2014), who analysed the walnut layer and found that the antioxidant activity of endocarp extract was significantly higher than in epicarp, although the phenolic contents in both extracts were pretty similar. It is possible that

antioxidant activity of extracts may be related to other types of phenolic compounds or other bioactive compounds. Hence, the comparison of individual phenolics in each layer extract will be shown by HPLC to prove that statement.

HPLC chromatograms (Figure 6.7) illustrate that epicarp, mesocarp and endocarp contained gallic acid, catechin, epicatechin, quercetin and p-coumaric acid, which concentration of individual phenolic compounds in each CPH layer was different. Figure 5.10 has compared the antioxidant activities of individual phenolics in CPH extract in the following order: quercetin > epicatechin ≈ catechin ≈ gallic acid > p-coumaric acid. While Figure 6.6 showed a high AOA of endocarp extract, so it can be first expected that endocarp may contain higher quercetin than other layers. However, Figure 6.7 and Table 6.2 confirmed that quercetin concentration in the epicarp was almost twice as much as in the mesocarp and ninefold higher than in the endocarp. Epicatechin was also found maximum in epicarp; it was 10-15 times higher than in mesocarp and endocarp. In contrast, catechin and gallic acid concentrations were the highest in endocarp layer. In conclusion, the endocarp layer contained high gallic acid and catechin but low in quercetin. While the epicarp layer contained high quercetin, catechin and epicatechin, whereas mesocarp only had high epicatechin and quercetin. These findings indicate that all CPH layers are rich in phenolic compounds that have the potential as antioxidants.

The higher antioxidant activity in endocarp extract, however, could not be explained by HPLC using the identified individual compounds (gallic acid, quercetin, catechin, epicatechin, p-coumaric acid). Another possibility why AOA in endocarp extract was the highest could have been due to the presence of other different compounds (both phenolics and non-phenolics) with high antioxidant activity that had not yet been identified or detected. Therefore, more sensitive instruments, such as

LCMS, are required for further analysis to identify all individual peaks in CPH extract, especially those responsible for antioxidant activity, and then determine their relative antioxidant activity. This analysis is also expected to detect anthocyanin compounds that have not previously been identified on HPLC chromatograms.

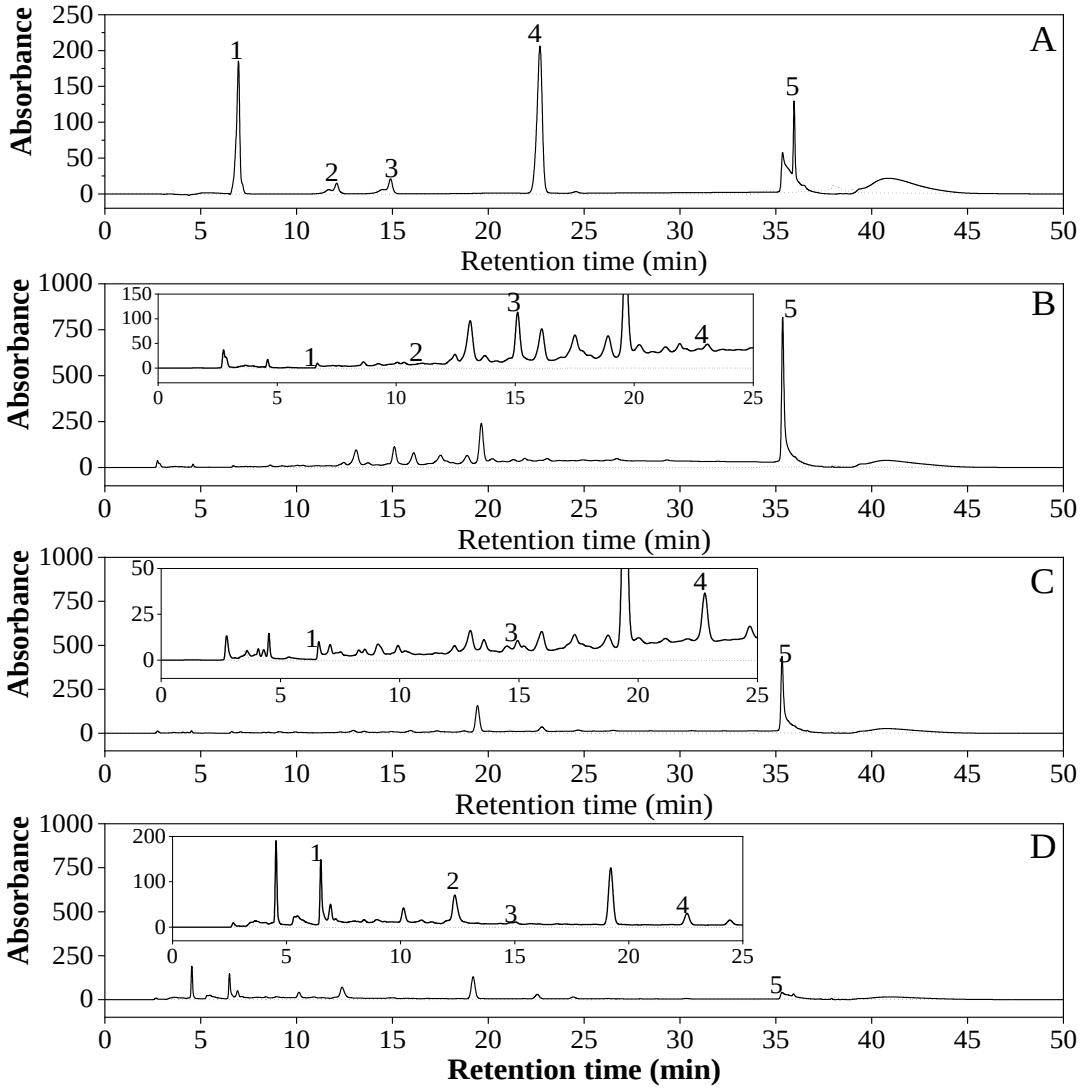


Figure 6. 7 HPLC chromatograms of phenolic compounds at 280 nm in CPH layers: (A) Standard compounds; (B) Epicarp extract; (C) Mesocarp extract; (D) Endocarp extract: [1] Gallic acid; [2] Catechin; [3] (-)-Epicatechin; [4] p-Coumaric acid, [5] Quercetin; the dash line (---------) is blank solvent

Table 6. 2 HPLC phenolics profile of CPH layers extracts (μg/g dry CPH)

Compound name	Phenolic concentration (μg/g dry CPH)					
	MAE			CSE		
	Epicarp	Mesocarp	Endocarp	Epicarp	Mesocarp	Endocarp
Gallic acid	22.9 ± 0.9	15.4 ± 2.9	140.6 ± 58.9	20.1 ± 1.8	14.0 ± 2.2	165.9 ± 3.8
Catechin	905.1 ± 11.8	nd	3671.6 ± 89.5	716.7 ± 17.9	257.04 ± 6.4	3363.1 ± 47.4
Epicatechin	3249.3 ± 334.1	238.9 ± 58.3	183.1 ± 57.7	2935.4 ± 180.8	313.1 ± 31.2	200.2 ± 11.5
p-Coumaric acid	nd	88.7 ± 107.6	98.6 ± 96.5	67.7 ± 8.8	nd	23.8 ± 4.1
Quercetin	17999.4±364.7	10646.6±1628.5	2216.4±241.8	13597.5±241.8	10305.5±993.3	1090.4±101.4
Total	22176.7	10989.5	6310.4	17337.56	10889.6	4843.3
TPC*	180,659	84,015	44,405	153,986	75,757	45,381

Mean ± S.D (n= 3, triplicate extraction); nd: not defined (below limitation); *TPC, analysed by Folin-ciocalteau method

6.5 Comparison of MAE and CSE on extraction yields

The comparison of MAE and CSE on extraction yields of epicarp, mesocarp and endocarp is presented in Figure 6.6, and the results showed that there is a significant different effect between MAE and CSE methods on the TPC, TMA ($p_{value} < 0.001$), and AOA yields ($p_{value} = 0.010$). In the epicarp layer, MAE had a higher TPC yield than CSE method (Figure 6.6.A), especially for epicarp with particle sizes of 38-63 and ≤38 micron. Microwave heating could increase the TPC up to 17% compared to CSE, and the maximum TPC was obtained from epicarp with the smallest particle size (≤38 micron), 180.7 ± 2.8 mg GAE/g dw. These results coincided with the previous findings on particle size effect discussed in Sections 4.5 and 5.4.1, and were also supported by Nguyen et al. (2020) who reported increasing phenolic extracted from CPH by MAE up to 29% over CSE.

Moreover, the TMA yield of epicarp increased up to 53% from 0.30 ± 0.01 to 0.46 ± 0.02 mg Cy_3GE/g dw by microwave heating (Figure 6.6.B). As previously discussed, the selective heating effect on MAE leads to increase the phenolics and anthocyanin yields. The AOA of epicarp extracts (Figure 6.6.C) on MAE was slightly

higher than in CSE methods ($p_{value} < 0.001$) and both decreased with the decrease in particle size. In contrast, the AOA of endocarp extract performed similar between MAE and CSE method ($p_{value} = 1.0$). Size reduction by grinding could increase the surface area of biomass and enhance the contact surface between biomass and solvent. Still, at the same time, the thermolabile antioxidant compounds could be degraded due to heat during the grinding process (Brewer et al., 2014).

A combination of size reduction and microwave heating could significantly increase the phenolic and anthocyanin yields. Size reduction can enlarge the material's surface area and contact surface with solvent. Thus, it could accelerate the mass transfer of extracted compounds from the material. In addition, if the thickness of biomass is thicker than its penetration depth, the plant material will be heated only at the surface, and the rest is heated by conduction (Ibrahim and Zaini, 2018). Hence, size reduction could help to reduce the material thickness and increase the penetration depth of heating process. The penetration depth of the mixture of solvent with CPH (≤38 micron) was about 1.2 ± 0.1 cm; this means that the microwave penetration depth in the mixture was higher than the biomass thickness, implying that microwave heating could be effective. Size reduction from 150- to 38 micron on epicarp layer could enhance the TPC yield by 46% from 123.6 ± 5.5 to 180.7 ± 2.8 mg GAE/g dw for MAE but only increased 22% from 126.0 ± 2.8 to 154.0 ± 2.5 mg GAE/g dw in CSE experiment.

On the other hand, the extraction yields in other CPH layers (mesocarp and endocarp) (Figure 6.6) were not conclusively different in TMA or AOA ($p_{value} = 1$) between MAE and CSE methods, except the AOA of mesocarp layer ($p_{value} < 0.001$). This implies that microwave selective heating in mesocarp and endocarp layers does not really work as well as in epicarp layers. This might be because of the different locations of phenolics within plant cells of CPH layers or different chemical and

physical characteristics between epicarp and other layers. Differences in material characteristics may lead to different interactions between material and microwave energy or solvent, resulting in differences in extraction yields. Campos-Vega et al. (2018); and Sobamiwa and Longe (1994) claimed that mesocarp and endocarp layers contained high fibre and pectic compounds. Thus, it is hypothesised that phenolic compounds in those layers may be linked to fibre or other compounds, reducing the interaction between phenolics and solvent. As a result, less phenolic compounds are extracted into the solvent.

The HPLC phenolics profiles (Figure 6.7 and Table 6.2) also confirm that individual phenolics detected in MAE were higher than in CSE. For example, catechin and quercetin in epicarp and endocarp extracts achieved by MAE showed higher content than CSE. However, some phenolics were not detected in the extract; under microwave heating, catechin and p-coumaric acid were not found in mesocarp and epicarp, respectively. Additionally, p-coumaric was not found in mesocarp extract by CSE. The extract may contain these compounds, but their concentrations were too low to be detected. In epicarp layer, the individual phenolics detected in MAE were higher than in CSE, which was correlated with the TPC results in Figure 6.6.A. In addition, catechin and quercetin in endocarp extract were higher in MAE than CSE. However, in both MAE and CSE methods, antioxidant activity experienced similar yields, although MAE produced higher TPC and individual phenolics concentration. This suggests that microwave heating could reduce the antioxidant activity of extract because some antioxidant compounds, such as anthocyanin, are thermally sensitive. Anthocyanin compounds have been reported to begin degrading at 41 °C (Yu et al., 2016).

In contrast, the mesocarp extract showed that MAE yield was similar to CSE, as correlated with the TPC and AOA yields. Hence, these HPLC results cannot explain

the similar antioxidant activity between MAE and CSE in epicarp and endocarp layers. This may be because some peaks were not analysed or some individual phenolics with high antioxidant activities were not detected by HPLC. Therefore, another more sensitive instrument, such as LCMS, still needs to be used to determine all individual phenolics in the extract.

6.6 Summary

CPH consists of three layers: epicarp, mesocarp, and endocarp. Mesocarp was the highest part (51-62 %) of CPH fruit, followed by epicarp and endocarp. The results confirm that every layer in CPH contained phenolic and anthocyanin compounds with antioxidant activity. HPLC chromatograms confirm that all CPH layers contained gallic acid, catechin, epicatechin, quercetin and p-coumaric acid. The phenolic compounds were concentrated in the epicarp layer, which was only about 13-14% of CPH fruit mass, while anthocyanins were concentrated in both epicarp and mesocarp layers. The TPC and TMA yields found maximum in the epicarp layer, 180.7 mg GAE/g dw and 0.46 mg Cy_3GE/g dw, respectively. However, the antioxidant activity of extract from epicarp was lower than that of the endocarp layer. The highest antioxidant activity (3.71 mg TE/g dw) was found in the endocarp layer. The endocarp layer is easily oxidised by exposure to air at room conditions. Endocarp contains lower phenolic content, but it has high antioxidant activity. This may be because phenolics extracted from endocarp have higher antioxidant activity than phenolics from other layers. HPLC cannot confirm this statement, thus, a more sensitive instrument, such as LCMS, could be used to prove antioxidant activity of all individual phenolic compounds in extracts.

Size reduction and extraction methods significantly affected the TPC and TMA yields ($p_{value} < 0.001$), but they tended to decrease the AOA yield ($p_{value} < 0.001$). Size reduction can improve the surface area and help to break the plant cell; thus, it can

increase the mass transfer and enhance the extraction yields. However, at the same time, the grinding process can degrade the phenolic compounds in material due to heat. For example, gallic acid and catechin started to degrade at 60 °C (Volf et al., 2014), while anthocyanin could degrade at 41 °C (Yu et al., 2016). Microwave heating enhanced the TPC and TMA yields by up to 17%, and 53%, respectively, in the extraction of epicarp layer. The increment in the extraction yields was attributed to the selective heating effect on microwave heating.

These findings are useful information for understanding the location of phenolic compounds in the CPH layer. Phenolic compounds are presented in all CPH layers but are most concentrated in the epicarp and mesocarp layers. Extraction of epicarp and mesocarp layers (±77 % of total CPH mass) could recover up to 88 % of the phenolic compounds from CPH (Figure 6.2). While the endocarp layer has been reported to be rich in protein and pectin, which will be more useful for animal food. However, considering the preparation of the CPH layer is time- and energy-consuming, processing the extraction using the whole CPH may be preferable for scale-up process.

CHAPTER 7: RESULTS AND DISCUSSION – CHARACTERISATION OF CPH SOLID RESIDUE AND IDENTIFY ITS POTENTIAL APPLICATION

Chapter 7 will be modified as part of a manuscript prepared for Journal Biomass and Bioenergy

Dewi, S.R, Stevens, L.A, Ferrari, R., Irvine, D.J, Binner, E.R., Extraction of phenolic-based antioxidants from cacao pod husk (CPH): implications for scaling-up process and potential applications of CPH solid residue

7.1 Introduction

CPH has been reported to contain lignocellulosic compounds (Daud et al., 2013; Marsiglia et al., 2016; Nazir et al., 2016; Shet et al., 2018), which are valuable in the production of useful materials, such as activated carbon (Cruz et al., 2012; Pua et al., 2013; Rachmat et al., 2018; Tsai and Huang, 2018), bioenergy (Syamsiro et al., 2012), bio-oil, and biochar (Adjin-Tetteh et al., 2018; Tsai et al., 2020). In addition, minerals in CPH have potential for soap making, fertiliser, and catalysts (Lu et al., 2018). There are some studies about the potential applications of CPH; Adjin-Tetteh et al. (2018) have reported the pyrolysis of CPH at 550 – 600 °C with a heating rate of 400 °C/min to produce bio-oil, biochar and non-condensable gas. Biochar is a combustible solid that can be burned to generate heat energy, while bio-oil is an emulsion of water and broad range of oxygenated organic compounds including organic acids, aldehydes, alcohols, phenols, carbohydrates, and lignin-derived oligomers. Bio-oil and biochar typically have heating value of about 22 MJ/kg dry basis and 18 MJ/kg, respectively. Non-condensable gas (syngas), on the other hand, is primarily a mixture H_2 and CO, but also typically contains CH_4, CO_2, H_2O, and several low-molecular-weight volatile organic compounds; heating value of non-condensable gas is relatively low (~6 MJ/kg) (Laird et al., 2009). The CPH with characteristics: 61.73 % volatile matter, 10.96 %

fixed carbon, and high ash content of 16.24% produced 58%wt of bio-oil, 30%wt biochar and 12%wt of non-condensable gas; the bio-oil was rich in a valuable acid, 9,12-octadecadienoic acid (Adjin-Tetteh et al., 2018). The high amount of volatile matter is a good indication of combustion, gasification, and pyrolysis. Tsai et al. (2020) also produced microporous biochar from CPH at 400 – 800 °C and effectively applied it as an adsorbent for methylene blue with a maximum capacity of 13.18 mg/g. They then suggested using CPH-based biochar as an adsorbent to remove cationic compounds from an aqueous solution.

The carbonaceous material, such as CPH, can also be converted into activated carbon, which is widely used as an adsorbent in product purification or waste treatment. Tsai and Huang (2018) have successfully produced the activated carbons from CPH and acid-leaching CPH with a BET surface area ranging from 289.5 to 637.7 m^2/g. Activated carbon is a valuable material used as an adsorbent for waste treatment. For example, the use of activated carbon from CPH for removing methylene blue from an aqueous solution was reported by Pua et al. (2013). The CPH-based activated carbon performed with an adsorption capacity of 263.9 mg/g, which means that each milligram of activated carbon can remove 263 mg of methylene blue. This capacity was higher than wheat shells (21.5 mg/g) but lower than activated carbon from coconut husk (434.8 mg/g). CPH-activated carbon could also remove arsenic (As) up to 80% in less than one hour (Cruz et al., 2012). In another study, CPH-based activated carbon was successfully applied as an adsorbent for decreasing free fatty acid in waste cooking oil. Activated carbon with a surface area of 9.6 m^2/g could reduce the free fatty acid by 86.7% (Rachmat et al., 2018).

During the extraction process, CPH solid residue will be generated. The CPH solid residue is predicted to contain the same potential compounds as CPH, which can

be used as a bioenergy feedstock, activated carbon precursor or other products. However, each process will affect different characteristics of solid material. For instance, the microstructure of CPH changed after drying due to the migration of moisture from cells, which led to shrinkage and structural collapse (Valadez-Carmona et al., 2017). Convective heating in hot air drying resulted in sudden evaporation of superficial water, triggering cellular collapse due to internal cell pressure. As a result, the cells area of dried CPH increased up to twice that of fresh cells.

Several studies have been reported to convert the solid residue from the extraction process into activated carbon. Tea residue from water extraction of tea compounds has been reported to have a high potential for preparing porous activated carbon for wastewater treatment (Bai et al., 2022). Activated carbon from tea residue prepared using $ZnCl_2$ activation agent at 400 °C for 60 min possessed a high surface area and a high efficiency for waste removal: 99.9% of Hg, 74.7% of Cu, 100% of pigment and pesticide. The activation process can increase the BET surface area from 2.29 m^2/g (tea residue) to 871.03 m^2/g (activated carbon). These findings proposed that tea residue could be used for water purification, air purification, energy storage, catalysts, combustion and food industry.

It is worth mentioning that the waste after specific treatment, such as extraction or pyrolysis, might be a cheap carbon precursor as absorbents for removing the pollutants. The solid residue (biochar) from bio-oil extraction (pyrolysis) was also reported to be converted into activated carbon. The biochar from hops had high ash (26.2%) with a low surface area of 2 m^2/g but still could produce activated carbon with a large surface area of about 416 m^2/g (Bazan et al., 2016). In another study, Ren et al. (2021) evaluated coffee grounds to produce bio-oil and used its biochar as an activated carbon precursor. The coffee ground contained high volatiles (74.8%), indicating a high

potential for bio-oil production. While the biochar obtained from the pyrolysis contained only 8.8% volatile but had high fixed carbon (86.9%), which was very promising to be an activated carbon precursor. The study produced activated carbon with a high BET surface area of 1420 m^2/g and a total pore volume of 0.75 cm^3/g. The studies above proved that biomass residue left after thermochemical processes, such as extraction or pyrolysis, can be an inexpensive and promising precursor for activated carbon.

According to those references, the characterisation of CPH solid residue and analysis of its potential application is also worth researching. This chapter will focus on the characterisation of CPH material, including the surface area, thermogravimetric analysis, lignocellulosic analysis, proximate analysis, and surface imaging of CPH material before and after the extraction process. This study can be used to analyse the potential of CPH solid residue for future applications, such as for the production of bio-oil and biochar or activated carbon. The outcome could be used for consideration to prevent the production of other by-products after the extraction process (reduce negative environmental impact). The diagram of the potential of CPH solid residue is shown in Figure 7.1.

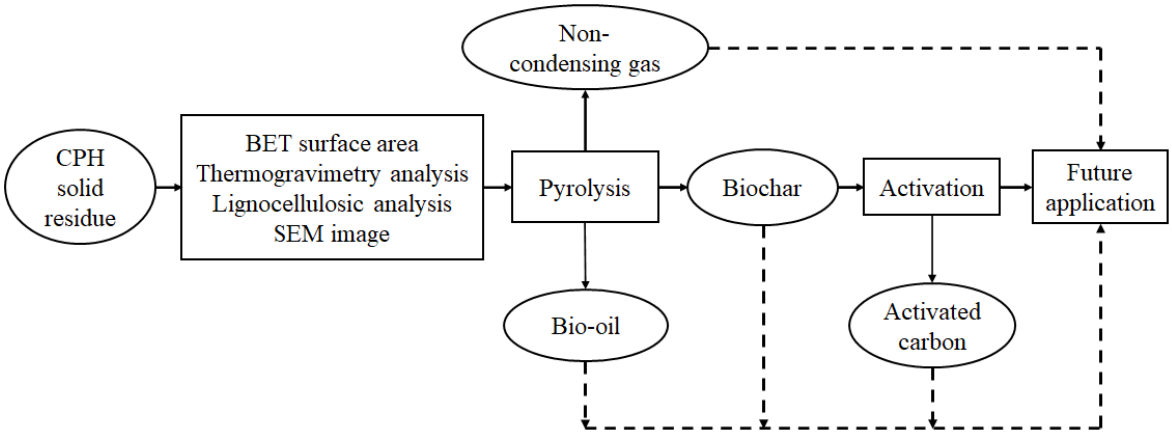


Figure 7. 1 Diagram of potential of CPH solid residue

7.2 Chemical compositions and lignocellulosic compounds

Proximate analysis can help classify a food material's nutritional value based on the nutritional parameters, such as ash content, moisture content, crude fat, total protein and carbohydrate. The proximate compositions of CPH powder (≤38 micron) before extraction are presented in Table 7.1.

Table 7. 1 Chemical composition of CPH

Composition	(Campos-Vega et al., 2018)	(Lu et al., 2018)	**This study**	
			Results	**Analysis method**
Moisture content (%)	6.4 – 14.1	NR	8.46 ± 0.89	Gravimetric
Ash content (%)	5.9 – 13.0	6.4 – 8.4	6.08 ± 0.52	TGA
Carbohydrate (%)	17.5 – 47.0	32 – 47	72.86 ± 1.73	Difference
Fat (%)	0.6 – 4.7	1.5 – 2	1.46 ± 0.06	Soxhlation
Protein (%)	2.1 – 9.1	7 – 10	9.87 ± 0.88	Kjeldahl

Mean ± S.D. (n= 2, duplicate analysis); NR: not reported

The CPH has high carbohydrate content (72.86%) but is low in crude protein (9.87%) and fat (1.46%) content. These results are in agreement with the fact that carbohydrate is a high proportion content reported by Campos-Vega et al. (2018) and Lu et al. (2018), in which the proximate content of CPH ranged from 5.9 to 13.0% w/w of ash content, 2.1 to 10% of protein, 0.6 to 4.7 % of fat, and 17.5 to 47% of carbohydrates. The high carbohydrate content shows that CPH can be used for animal feed as carbohydrate is the primary energy source. However, it should be noted that CPH also contains lignocellulosic compounds, in which the lignin may limit the utilisation of CPH in broiler diets (Sobamiwa and Longe, 1994). Lignocellulosic biomass refers to a complex macromolecular of lignocellulose with smaller amounts of extractives and ash that have potential renewable sources to valorise into valuable products. Lignocellulosic compounds are classified into lignin, cellulose, and hemicellulose, in which cellulose is the main building block and is filled with binding compounds of hemicellulose and lignin. Lignin is a complex heteropolymer of phenylpropane units and is strongly

attached to cellulose and hemicellulose, giving the plant cell wall rigidity. The lignin is accumulated in epicarp layer of CPH (Lu et al., 2018).

As previously discussed, CPH comprises three layers: epicarp, mesocarp, and endocarp; the lignocellulosic contents in the whole CPH and its three distinct layers are depicted in Table 7.2. The three distinct layers of CPH contained lignocellulosic compounds, ranging from 11 to 39%. In the whole CPH, the lignin content was lower than the cellulosic compounds (cellulose and hemicellulose), which was about 15%. The lignin and hemicellulose compounds were concentrated in epicarp and mesocarp layers; both revealed a similar composition: 19% lignin and 39% hemicellulose. While the cellulose content was high in mesocarp and endocarp layers, the composition of lignocellulosic in the endocarp layer was not much different among the three compounds. In general, the endocarp layer was lower in lignocellulosic compounds than other layers. This finding is in agreement with reports by Lu et al. (2018), in which CPH consisted of 19.7 – 26.1% of cellulose, 8.7 – 12.8% of hemicellulose, and 14 – 28% of lignin, where the lignin was concentrated in epicarp layer and cellulose in mesocarp layer.

Table 7. 2 Lignocellulosic contents in CPH

Composition	**CPH**			
	Whole husk	**Epicarp**	**Mesocarp**	**Endocarp**
Lignin (%)	15.13 ± 0.03	19.12 ± 0.50	19.70 ± 0.04	16.49 ± 0.76
Cellulose (%)	24.95 ± 0.13	11.43 ± 0.78	23.36 ± 0.37	20.20 ± 0.27
Hemicellulose (%)	24.18 ± 0.11	39.61 ± 0.49	38.80 ± 0.76	16.90 ± 0.50

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

This chapter focuses on analysing the potential of CPH solid residue left from phenolic extraction. As mentioned, lignocellulosic compounds have high potential for conversion to other products; thus, their compounds in CPH solid residue were also determined. Table 7.3 presents the lignocellulosic compounds in CPH solid residue

from MAE and CSE processes. Overall, lignocellulosic compounds increased after the extraction process, but there was no significant difference between microwave and conventional heating, except for the hemicellulose after microwave heating. It seems that heating methods (MAE vs CSE) did not affect the lignocellulosic compounds; this might be because the extraction was operated at a low temperature, 50 °C. CPH is a lignocellulosic biomass containing lignin, cellulose, hemicellulose, organic extractives, and inorganic ash (Mukherjee et al., 2022). As a result, the increase in lignocellulosic compounds of CPH solid residue could be attributed to the removal of extractive compounds released from CPH during the extraction heating. However, Wang et al. (2017) reported otherwise, that the amount of cellulose, hemicellulose and lignin did not significantly change after extraction treatments, either using solvent or ultrasound-assisted extraction.

Table 7. 3 Lignocellulosic contents in CPH solid residue

Composition	**CPH**		
	Untreated	**MAE**	**CSE**
Lignin (%)	15.13 ± 0.03	22.70 ± 1.67	23.97 ± 0.49
Cellulose (%)	24.95 ± 0.13	36.69 ± 0.27	36.30 ± 0.60
Hemicellulose (%)	24.18 ± 0.11	22.00 ± 1.37	26.11 ± 0.67

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

The cellulose content in CPH solid residue significantly improved from 25 to 37%, while the lignin became 24%, initially only 15%. Due to a high content of lignocellulosic compounds, the CPH solid residue is very promising to be valorised into other valuable products, such as bio-oil and biochar, bioethanol or activated carbon. However, it still needs to measure the proximate composition in order to understand the ash, fixed carbon and volatile contents. After all, those parameters would influence the quality of the following products. Biomass with high volatile content is promising for bio-oil production, while high fixed carbon is useful for activated carbon. Meanwhile,

the low ash content in that biomass will be advantageous in the future process, either in pyrolysis or carbonisation (Ren et al., 2021). Ash content in biomass can inhibit the combustion and reduce the calorific values (high heating value, HHV) (Mukherjee et al., 2022; Syamsiro et al., 2012); it will also hinder pore development during activation in the production of activated carbon (Tsai and Huang, 2018).

The pyrolysis of rice straw containing 36.7% cellulose, 26.8% hemicellulose and 14.5% lignin using continuous fast microwave pyrolysis at 500 °C and a feed rate of 24 g/min yielded 31.9% bio-oil, 32.4% biochar, and 35.7% gas. Meanwhile, C. *oleifera* shell with higher hemicellulose (30.7%) and lignin (36.4%) produced high non-condensable gas (44.0%) but low in bio-oil (26.5%) and biochar (29.5%) at 500 °C pyrolysis with a feed rate 100 g/min (Wang et al., 2018). Mukherjee et al. (2022) informed that lignin was a major biochar precursor, while cellulose and hemicellulose were attributed to volatile products in the pyrolysis process. Biochar from lignin (45.7%) showed higher yields than woody biomass (19%). Another study also showed that torrefaction at 300 °C for coconut husk, which has higher lignin content (43.3%), could produce higher biochar (52.2%) than rice husk (41.3%) that only contained 25.7% of lignin (Nakason et al., 2019). The researchers suggested that residual biomass with high lignin content was highly recommended for thermochemical processes such as pyrolysis. Additionally, lignocellulosic biomass has several advantages as carbon precursors: 1) renewable resources, 2) low cost, 3) no net emission of CO_2 due to low sulphur and nitrogen, and 4) environmentally friendly.

7.3 Thermogravimetric analysis

7.3.1 CPH solid residue

Thermogravimetric analysis was used to demonstrate thermal decomposition and proximate analysis of CPH powder and its solid residue. Proximate analysis was

performed using a slow temperature increase (5 °C/min) in Nitrogen (1 bar, 100 mL/min). Samples were heated to 105 °C and treated at this temperature for 30 min to measure the moisture content. Subsequently, temperature was increased to 950 °C at the same heating rate and remained heated for 30 min to get the volatile matter content. Then the gas was switched to air (1 bar, 100 mL/min) and held for another 30 min to get the fixed carbon and ash content. The amount of fixed carbon can be determined by the difference between the amount of ash, volatile matter, and original samples. The thermogravimetric and derivative thermogravimetric (TG/DTG) curves of CPH powder and its solid residue after extraction are shown in Figure 7.2.

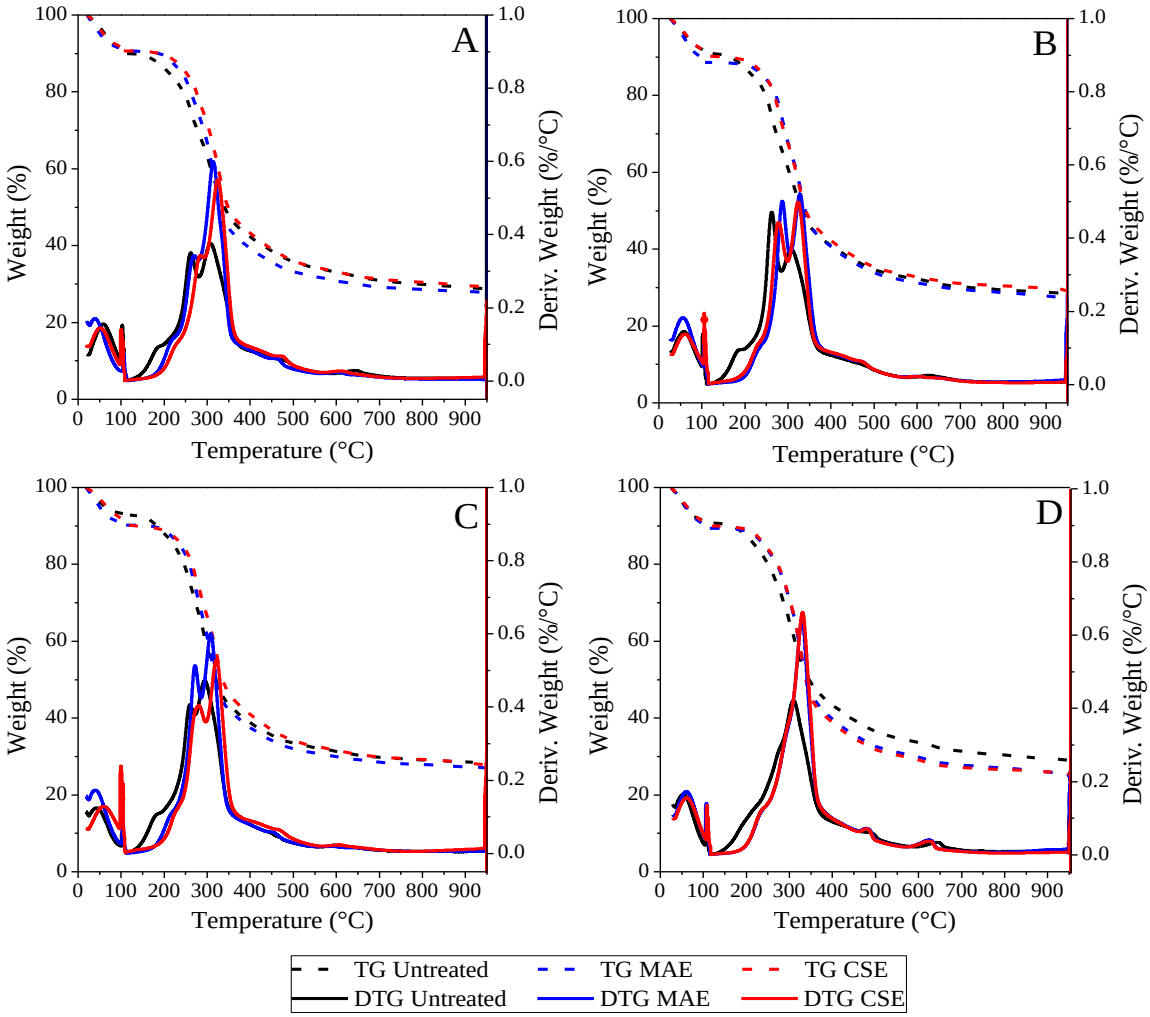


Figure 7. 2 TG and DTG curves of CPH powder and its solid residue with particle size: (A) 125-150 micron; (B) 63-90 micron; (C) 38-63 micron; (D) ≤38 micron up to 950 °C in Nitrogen

TG curves showed three steps of weight loss: less than 200 °C, 200 – 600 °C, and between 600 and 950 °C, which may correspond to loss of moisture, volatile organic matter, and inorganic carbonates (lignin), respectively. This statement is supported by Tsai and Huang (2018) findings, which showed the TG curves of CPH: weight loss at a temperature less than 200 °C due to the release of absorbed water and poorly bonded materials, and peaks at 200 – 400 °C which corresponded to volatile matter release from thermal decomposition of hemicellulose and cellulose. While Ren et al.(2021) classified the TG curves of coffee grounds into three stages: <150 °C for loss of moisture, stage II (150 – 500 °C) for weight loss due to evaporation of volatile matter, tars elimination and primary carbonisation, and stage III at 500 – 800 °C for carbonisation of remaining compounds (inorganic carbonates). The slight weight loss at temperatures less than 200 °C was related to the release of adsorbed water. In comparison, degradation of the main component in CPH was shown by extreme weight loss at 200 – 600 °C, presumably due to the thermal decomposition of hemicellulose and cellulose. Hence, for future treatment, carbonisation of CPH is suggested to be set at 600 °C or higher to prepare a carbon-rich product.

On the other view, the DTG curves of all CPH samples, unless ≤38 micron, showed two distinct peaks at approximately 250 and 300 °C, which correspond to hemicellulose and cellulose peaks, respectively. These peaks are in accordance with research findings on the decomposition of woody plant material that occurred at temperatures of 250 – 300 °C for hemicellulose, followed by cellulose at 300 – 350 °C and lignin at 300 – 500 °C (Carrier et al., 2011). While CPH with particle size ≤38 micron showed one peak around 300 °C which is related to cellulose compound, and no peak or shoulder at around 250 °C which corresponds to hemicellulose. This may

be because of overlapping cellulose and hemicellulose peaks, as shown in Table 7.3, that both compounds in CPH have the same percentage.

Furthermore, according to Figure 7.2, the TG curves for CPH >38 micron (Figure 7.2. A-C) showed a similar weight percentage before and after extraction treatment, which indicated that extraction treatments had no impact on the weight ratio of CPH samples. Otherwise, the weight percentage of CPH solid residue ≤38 micron significantly reduced compared to untreated CPH powder. In detail, these changes can be seen in the DTG curves in Figure 7.2. Peaks of hemicellulose and cellulose in CPH solid residue shifted to the right, which means that the cellulose content in solid residue increased compared to untreated CPH. However, there was no remarkable difference between microwave and conventional heating treatments. Data in Table 7.3 can confirm this statement, showing the increment of cellulose after the extraction process and no different cellulose yields between both treatment methods. Finally, it is noted that DTG curves can be used to support the lignocellulose analysis by the Chesson method discussed in Section 7.2.

Table 7. 4 Proximate contents of CPH powder and its solid residue

Whole CPH with particle size:	**Condition**	**Proximate content (%)**			
		Moisture content (MC)	**Volatile matter (VM)**	**Fixed carbon (FC)**	**Ash (A)**
125-150 micron	Untreated	8.60 ± 0.69	63.43 ± 0.35	22.99 ± 1.28	4.99 ± 0.94
	MAE	10.04 ± 0.95	63.64 ± 0.54	23.37 ± 0.44	2.95 ± 1.04
	CSE	9.47 ± 0.18	62.63 ± 0.04	24.90 ± 0.20	3.01 ± 0.34
63-90 micron	Untreated	8.62 ± 0.65	63.96 ± 1.16	22.54 ± 0.59	4.88 ± 1.10
	MAE	11.23 ± 0.18	63.51 ± 0.93	22.78 ± 0.03	2.49 ± 0.78
	CSE	10.17 ± 0.52	61.30 ± 0.05	24.77 ± 0.03	3.77 ± 0.60
38-63 micron	Untreated	7.72 ± 0.78	65.32 ± 0.02	21.06 ± 0.79	5.91 ± 0.01
	MAE	10.64 ± 1.24	64.75 ± 1.35	21.57 ± 0.95	3.04 ± 1.65
	CSE	9.62 ± 0.18	64.19 ± 0.17	23.43 ± 0.00	2.76 ± 0.02
≤38 micron	Untreated	8.64 ± 0.83	63.25 ± 0.61	22.03 ± 0.30	6.08 ± 0.52
	MAE	10.74 ± 0.22	65.38 ± 0.42	20.98 ± 0.05	2.91 ± 0.15
	CSE	9.96 ± 0.10	65.30 ± 0.47	20.94 ± 0.03	3.80 ± 0.34

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

The proximate values play an important role in biomass conversion as they determine many technical problems in thermochemical processes (such as combustion, gasification, and pyrolysis). For instance, fixed carbon (FC) and volatile matter (VM) corresponded to HHV (high heating value); meanwhile, the high moisture and ash contents in biomass can inhibit the combustion and decrease the HHV (high heating value) (Syamsiro et al., 2012). Proximate analysis results (Table 7.4) revealed that ash content in CPH significantly decreased after the extraction; otherwise, its moisture content went up. The decreasing ash contents may be because reducing the bound minerals which are usually linked to ash. Minerals may bind the phenolic compounds and they can also be removed along with the phenolic extraction, leading to a decrease in ash content. An increase in moisture content in CPH solid residue may be because there was residual solvent trapped in the surface or pores of the material. Meanwhile, fixed carbon of CPH solid residue with particle size of ≤38 micron decreased, compensating for the increased volatile matter. The increase in the volatile matter was in agreement with the data on the increment of cellulose content in CPH solid residue (Table 7.3). The reducing of fixed carbon and ash contents and increasing of the volatile matter were also reported after the extraction of eucalyptus by solvent extraction and UAE (Wang et al., 2017). Biomass with high volatile matter and low ash content was promising in terms of thermochemical conversion processes such as bio-oil production (Wang et al., 2017). On the contrary, extraction of CPH with a larger particle size (>38 micron) increased the fixed carbon but decreased volatile matter, particularly after conventional heating extraction. Due to high fixed carbon and low ash, CPH solid residue can be used as an activated carbon precursor.

Regarding the comparison of microwave (MAE) and conventional (CSE) heating, there was a notable difference in the proximate yields ($p_{value} < 0.001$). MAE showed

significant impact on moisture content and ash contents ($p_{value} < 0.001$), while CSE treatment affected moisture content ($p_{value} = 0.003$), fixed carbon and ash content ($p_{value} < 0.001$). However, the two results can be argued to be not all that different in moisture content ($p_{value} = 0.052$) and ash content ($p_{value} = 0.450$). On the other hand, particle size only showed significant effects on volatile matter and fixed carbon contents ($p_{value} < 0.001$). In general, CPH solid residue had moisture content of about 9 – 11%, volatile matter ranging from 62 to 65%, fixed carbon of 21 – 25%, and 3 – 6% ash which was very promising for the next thermo-chemical conversion step. Syamsiro et al. (2012) reported that CPH with proximate: 16.1% moisture content, 49.9% volatile matter, 20.5% fixed carbon, and 13.5% ash could produce energy (HHV) of 17.0 MJ/kg. Hence, the CPH solid residue in this study is expected to produce HHV which is equivalent to or higher than Syamsiro's as it has a higher fixed carbon content. HHV of lignocellulosic materials is a function of its lignin content, and both correlations follow the regression Equation 7.1 (Demirbas, 2002). HHV of lignocellulosic biomass depends on their lignin content; for instance, sunflower shell which had 17% lignin content has HHV of 18 MJ/kg whereas walnut shell with 52.3% of lignin content performed HHV about 21.6 MJ/kg (Acar and Ayanoglu, 2012).

$$HHV = 0.0877\ (L) + 16.4951 \qquad [7.1]$$

Where L is lignin content (%), it is estimated that the CPH solid residue in this study, which has a lignin content of around 23%, will produce HHV of 18.51 MJ/kg, which is higher than that reported by Syamsiro et al. (2012). The heating value of wood varies between 17 to 23 MJ/kg for bone-dry wood; holocellulose (cellulose and hemicelluloses) itself have HHV about 18.6 MJ/kg, whereas HHV of lignin ranges from 23.26 to 26.58 MJ/kg (Demirbas, 2010). In comparison, Central German brown coal which had high volatile matter (51.78%) and fixed carbon (30.55%) produced HHV up

to 24.23 MJ/kg (Reichel et al., 2013). According to those descriptions, the HHV of CPH is comparable to other biomass or coal, making it a promising lignocellulosic biomass for renewable energy source.

7.3.2 Solid residue of CPH layers

The solid residue of CPH layers: epicarp, mesocarp, and endocarp, were also analysed using thermogravimetric analysis. However, these results will not be confirmed with lignocellulose data (Chesson method) because, as discussed previously (Section 7.3.1), lignocellulose data has an agreement with the DTG curves. So, the prediction of lignocellulosic compounds in solid residue of CPH layers will only be explained from DTG curves. TG curves of those CPH layers can be seen in Figures 7.3 – 7.5. CPH layers had three weight-loss steps, as seen in CPH solid residue (Section 7.3.1). TG curves for all layers showed loss of moisture content and decomposition of volatile matter and inorganic carbon.

Meanwhile, the DTG curves for the epicarp, mesocarp and endocarp exhibited different patterns. The DTG curves of epicarp and mesocarp layers (Figures 7.3 and 7.4) present two distinct peaks at around 250 and 300 °C, corresponding to hemicellulose and cellulose compounds, respectively. The hemicellulose peak in epicarp layer is higher than that of cellulose which reflects that epicarp contains high hemicellulose. This is supported by lignocellulose data in Table 7.2 that the CPH epicarp had 39.6% hemicellulose and 11.4% cellulose. The data also showed that mesocarp contained higher hemicellulose (38.80 ± 0.76%) than cellulose (23.36 ± 0.37%), but the DTG graphs did not describe the same as in epicarp. This may be because the cellulose content in the mesocarp was higher than in the epicarp, so the hemicellulose only appears shoulder-like peak. After extraction treatments, those peaks shifted, indicating a change in component percentages. In epicarp solid residue, the

peaks shifted to the right, and the cellulose peak increased; in contrast, the peaks in mesocarp solid residue shifted to the left, reflecting the increasing hemicellulose composition. There was no conclusive difference in heating method between MAE and CSE in the composition of solid residue.

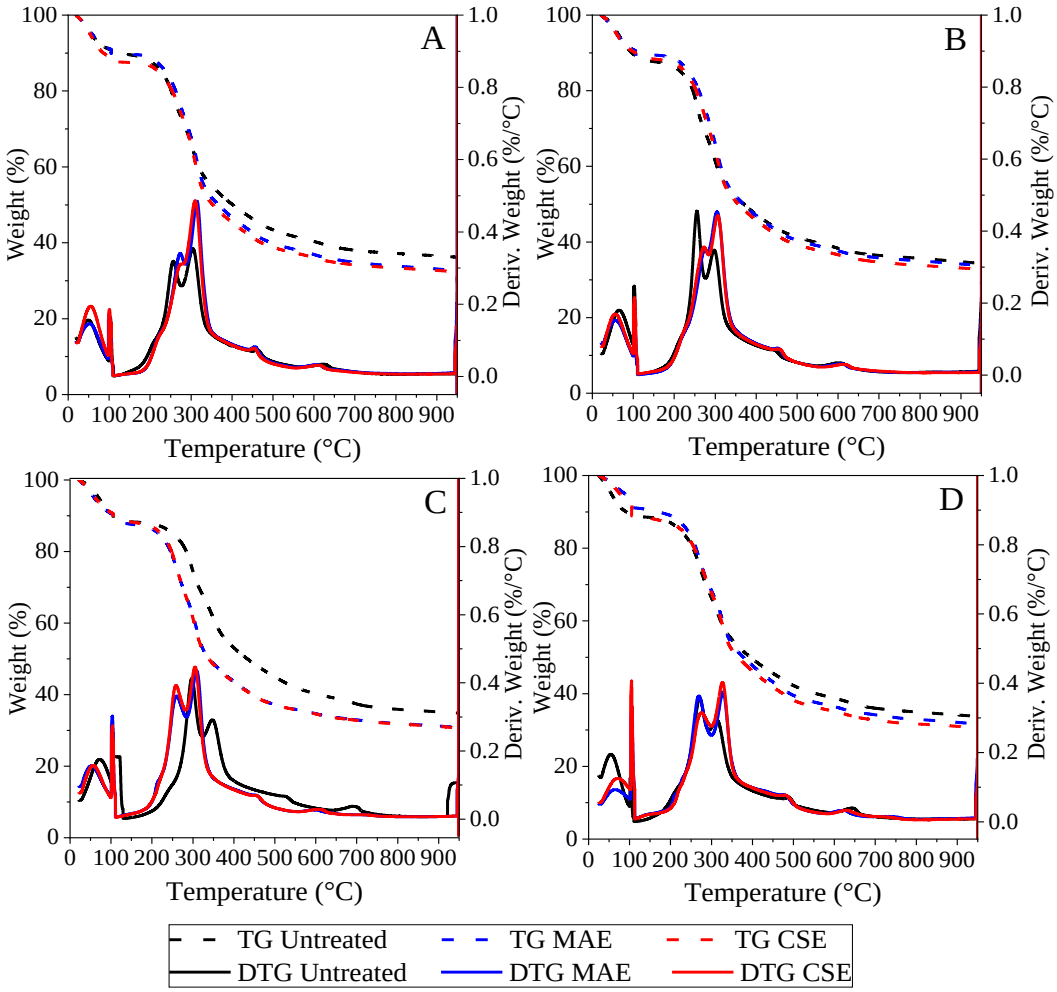


Figure 7. 3 TG and DTG curves of CPH epicarp and its solid residue with particle size: (A) 125-150 micron; (B) 63-90 micron; (C) 38-63 micron; (D) ≤38 micron up to 950 °C in Nitrogen

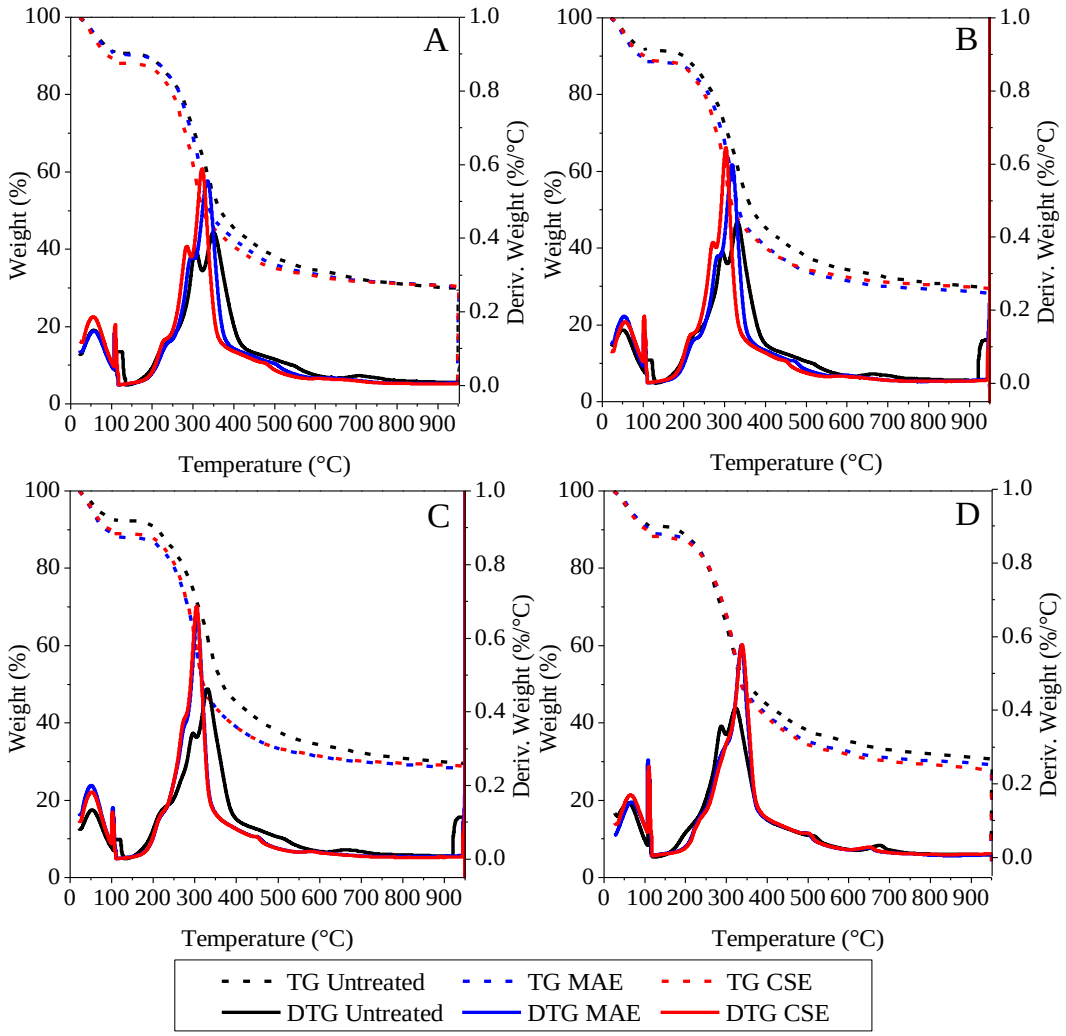


Figure 7. 4 TG and DTG curves of CPH mesocarp and its solid residue with particle size: (A) 125-150 micron; (B) 63-90 micron; (C) 38-63 micron; (D) ≤38 micron up to 950 °C in Nitrogen

Apart from other CPH layers, DTG graphs from the endocarp layer (Figure 7.5) describe a very different pattern from the two other layers. CPH endocarp layer has one sharp peak around 300 – 350 °C, which corresponds to cellulose compounds, where the hemicellulose peak is only a sloping shoulder. When comparing to data of lignocellulose content in Table 7.2, the cellulose content (20.20 ± 0.27%) in endocarp was indeed much higher than hemicellulose (16.90 ± 0.50%) and lignin (16.49 ± 0.76%) compounds. The extraction process, either MAE or CSE, caused the cellulose peak to be higher and shift to the right, meaning the increment of cellulose content.

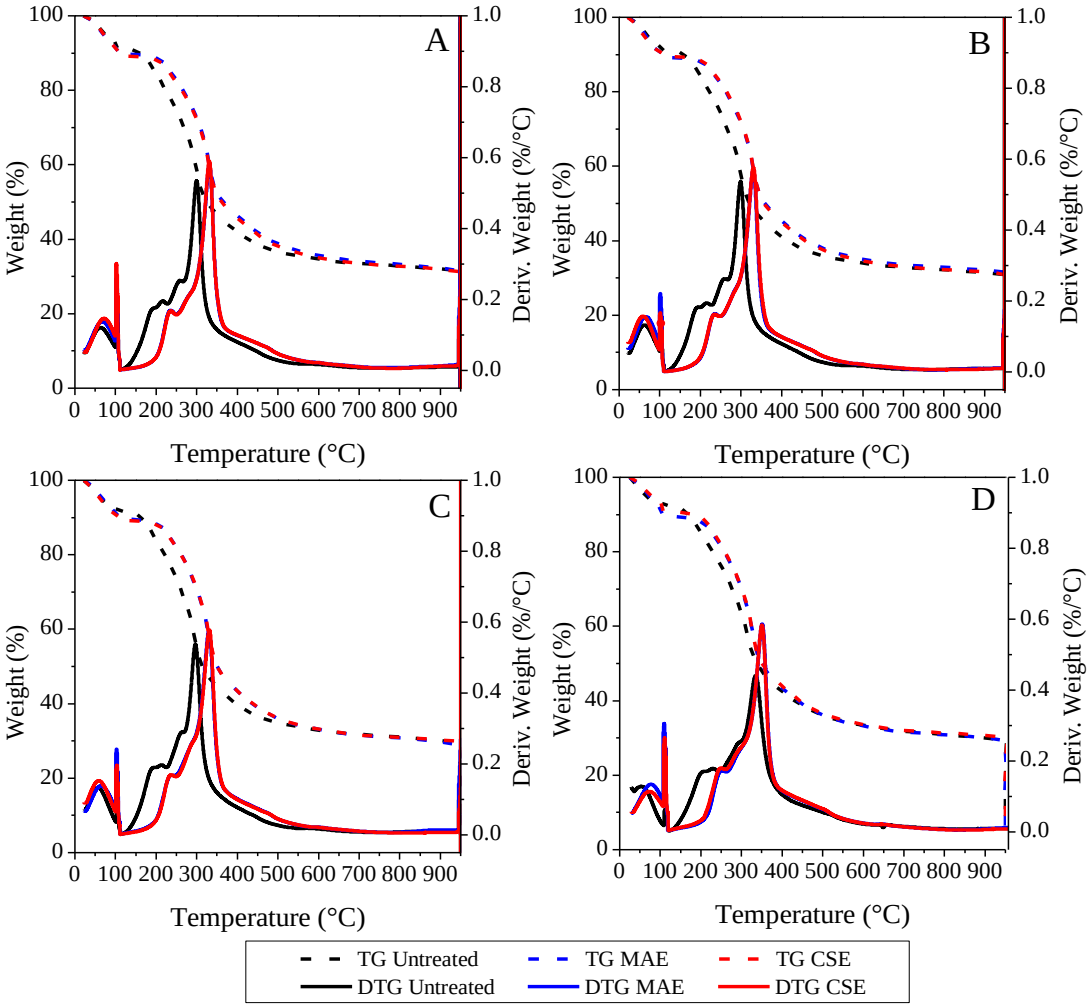


Figure 7. 5 TG and DTG curves of CPH endocarp and its solid residue with particle size: (A) 125-150 micron; (B) 63-90 micron; (C) 38-63 micron; (D) ≤38 micron up to 950 °C in Nitrogen

Proximate analysis results of CPH layers (epicarp, mesocarp, endocarp) and their solids residue are tabulated in Table 7.5. The results determined that extraction treatment has different effects on the proximate solid residue, particularly on the volatile matter (VM) and fixed carbon (FC) contents. The VM content of solids residue in the mesocarp and endocarp layers decreased after treatment, either by MAE or CSE, which resulted in rising FC content. On the contrary, the VM content in epicarp solid residue increased but reduced in FC content. These changes may be due to the different compositions of the main component in each CPH layer. Ash contents of the solids residue were lower than that of the original CPH layers. This may be because of reduced minerals that are usually linked to ash. Yet, this explanation needs to be proven by

further analysis. Otherwise, the moisture contents of the solid residue of CPH layers tended to increase after the extraction. This is probably due to the residual solvent trapped in the pores of the surface area of solid residue.

Table 7. 5 Proximate contents of CPH layers and their solid residue

CPH layer with particle size:	Condition	Proximate content (%)			
		Moisture content (MC)	Volatile matter (VM)	Fixed carbon (FC)	Ash (A)
EPICARP					
125-150 micron	Untreated	8.73 ± 2.07	55.01 ± 1.17	30.30 ± 0.95	5.96 ± 0.04
	MAE	9.63 ± 0.62	58.86 ± 0.70	27.78 ± 0.31	3.74 ± 0.39
	CSE	12.25 ± 0.08	56.14 ± 0.14	26.85 ± 0.16	4.77 ± 0.10
63-90 micron	Untreated	12.08 ± 0.00	54.67 ± 0.07	27.60 ± 0.13	5.65 ± 0.06
	MAE	10.06 ± 0.47	56.9 ± 0.3	27.03 ± 0.45	5.96 ± 0.34
	CSE	12.93 ± 2.23	55.6 ± 1.3	25.94 ± 0.98	5.46 ± 0.05
38-63 micron	Untreated	11.63 ± 0.03	53.96 ± 0.17	27.97 ± 0.10	6.44 ± 0.04
	MAE	11.38 ± 0.76	59.9 ± 2.63	23.68 ± 1.69	4.96 ± 0.17
	CSE	10.81 ± 0.76	58.9 ± 0.12	25.27 ± 1.02	4.98 ± 0.37
≤38 micron	Untreated	10.83 ± 0.72	55.86 ± 0.23	26.76 ± 0.16	6.55 ± 0.33
	MAE	9.40 ± 0.75	60.45 ± 0.32	24.93 ± 1.01	5.23 ± 0.57
	CSE	11.34 ± 0.28	58.94 ± 0.06	24.64 ± 0.40	5.09 ± 0.19
MESOCARP					
125-150 micron	Untreated	9.23 ± 0.10	61.35 ± 0.23	23.33 ± 0.24	6.09 ± 0.11
	MAE	9.62 ± 0.04	61.87 ± 0.59	25.67 ± 0.01	2.85 ± 0.64
	CSE	11.89 ± 0.09	58.45 ± 0.05	26.00 ± 0.12	3.67 ± 0.16
63-90 micron	Untreated	8.75 ± 0.25	62.05 ± 0.11	22.83 ± 0.10	6.38 ± 0.05
	MAE	11.29 ± 0.18	62.21 ± 0.78	23.79 ± 0.16	2.72 ± 0.44
	CSE	11.38 ± 0.34	59.75 ± 0.70	25.20 ± 0.42	3.67 ± 0.06
38-63 micron	Untreated	7.62 ± 0.09	63.10 ± 0.08	23.07 ± 0.26	6.22 ± 0.09
	MAE	12.35 ± 0.57	60.07 ± 1.40	24.17 ± 0.54	3.42 ± 0.29
	CSE	11.12 ± 0.14	60.68 ± 0.19	24.78 ± 0.20	3.43 ± 0.15
≤38 micron	Untreated	9.03 ± 0.01	60.39 ± 0.03	24.96 ± 0.12	5.63 ± 0.14
	MAE	11.21 ± 0.42	60.73 ± 0.16	24.71 ± 0.33	3.36 ± 0.25
	CSE	12.46 ± 1.12	61.14 ± 1.03	23.89 ± 0.09	2.51 ± 0.19
ENDOCARP					
125-150 micron	Untreated	9.29 ± 0.83	60.59 ± 0.05	25.38 ± 0.35	4.75 ± 0.44
	MAE	10.34 ± 0.22	59.22 ± 0.71	27.27 ± 0.19	3.18 ± 0.30
	CSE	9.65 ± 1.58	59.51 ± 0.51	27.71 ± 0.26	3.13 ± 0.74
63-90 micron	Untreated	8.84 ± 0.01	61.17 ± 0.06	24.49 ± 0.15	5.50 ± 0.08
	MAE	10.82 ± 0.01	58.79 ± 0.25	26.22 ± 0.02	4.17 ± 0.26
	CSE	10.36 ± 0.16	58.93 ± 0.52	25.90 ± 0.23	4.81 ± 0.44
38-63 micron	Untreated	8.21 ± 0.15	63.35 ± 0.35	23.06 ± 0.09	5.39 ± 0.29
	MAE	10.45 ± 0.18	61.39 ± 1.12	24.55 ± 0.07	3.61 ± 1.01
	CSE	10.73 ± 0.01	60.36 ± 0.51	24.69 ± 0.69	4.22 ± 0.19
≤38 micron	Untreated	7.56 ± 0.13	63.11 ± 0.13	23.58 ± 0.12	5.76 ± 0.12
	MAE	9.52 ± 1.13	61.30 ± 0.18	25.83 ± 0.66	3.35 ± 0.29
	CSE	8.84 ± 0.23	61.92 ± 0.65	25.17 ± 0.57	4.07 ± 0.16

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

7.4 Surface area

Understanding the surface area and porosity of CPH solid residue is important for development of CPH applications because they provide information on how CPH will behave in various applications. The surface area and porosity of CPH solid residue will affect its material properties, assisting in application selection. The surface area and porosity of biomass, for example, are important in determining its ability to adsorb or absorb other substances such as gases or liquids. High surface area and porosity materials can effectively adsorb gases or liquids, making them potentially useful for adsorbent application.

7.4.1 CPH solid residue

This section focuses on the surface area and pore volumes of the CPH solid residue. In contrast, the surface area and pore volume of the CPH sample (before extraction) has been discussed in previous chapters (Sections 4.5 and 6.3). Pore size is usually cited as micropore (<2 nm), mesopore (2 – 50 nm), and macropore (>50 nm) (Izadifar, 2013). Table 7.6 shows the BET surface area, pore volumes and average pore diameter of CPH solid residue removed from extraction with MAE and CSE. The N_2 gas adsorption isotherms show the presence of mesopores and micropores either in untreated CPH or in CPH solid residue. Those pore volumes significantly decreased after extraction ($p_{value} < 0.001$), where the decrement was higher after conventional heating than microwave heating ($p_{value} = 0.008$). For instance, the micropore volume in CPH solid residue ≤38 micron decreased from 0.56 to 0.39 mm^3/g after microwave heating and reduced up to 0.34 mm^3/g by conventional heating. This may be due to pores collapsing during the extraction process. SEM images in Figure 7.10.C can justify this statement by showing the rupture of CPH solid residue by conventional heating.

Table 7. 6 BET surface area and pore volumes of CPH and its solid residue

Whole CPH with particle size:	Condition	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 (4V/A) (nm)
125-150 micron	Untreated	0.77 ± 0.07	0.22 ± 0.01	1.54 ± 0.08	5.00 ± 0.53	26.12 ± 5.05
	MAE	0.44 ± 0.06	0.14 ± 0.01	1.21 ± 0.45	2.99 ± 0.86	26.75 ± 4.15
	CSE	0.44 ± 0.09	0.14 ± 0.03	0.80 ± 0.14	2.27 ± 0.33	20.98 ± 1.40
63-90 micron	Untreated	0.94 ± 0.03	0.26 ± 0.00	1.87 ± 0.10	6.20 ± 0.87	26.30 ± 2.93
	MAE	0.48 ± 0.04	0.15 ± 0.01	1.01 ± 0.13	2.66 ± 0.38	21.93 ± 1.30
	CSE	0.32 ± 0.03	0.08 ± 0.01	0.95 ± 0.25	1.81 ± 0.19	22.43 ± 0.06
38-63 micron	Untreated	1.50 ± 0.06	0.42 ± 0.05	2.63 ± 0.01	8.11 ± 0.22	21.68 ± 1.45
	MAE	0.64 ± 0.03	0.17 ± 0.01	1.06 ± 0.16	3.11 ± 0.70	19.20 ± 3.37
	CSE	0.56 ± 0.15	0.16 ± 0.02	1.06 ± 0.28	2.48 ± 0.28	20.75 ± 3.59
≤38 micron	Untreated	1.96 ± 0.25	0.56 ± 0.02	3.35 ± 0.73	9.39 ± 2.61	19.71 ± 3.95
	MAE	1.44 ± 0.01	0.39 ± 0.01	2.31 ± 0.02	6.41 ± 0.59	17.83 ± 1.73
	CSE	1.16 ± 0.02	0.34 ± 0.00	1.62 ± 0.05	4.30 ± 1.79	19.79 ± 0.55

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

However, another fact indicates that the BET surface area decreased after extraction ($p_{value} < 0.001$). The reason behind this decrease is because of the reduction of micropore and mesopore volume after extraction and the potential of pore-clogging by trapped solvent or other compounds. When micropore volume decreased from 0.56 to 0.34 mm^3/g (39%), the BET surface area also reduced from 1.96 to 1.16 m^2/g (40%), while the moisture content increased by 15% from 8.64 to 9.96%. The heating process can break the phenolic binding in plant cells, subsequently, solvent will dissolve and remove the phenolics from plant cells. However, some residual solvents or compounds may remain trapped within the pore structure even after degassing the sample under high vacuum at 90 °C. The increment of moisture content after the extraction (Table 7.4) supports this hypothesis. SEM images could describe the presence of small clumps on the surface of CPH solid residue (Figure 7.10.B), which previously appeared smooth (Figure 7.10.A). The results of this study are contrary to those reported by Izadifar (2013), that pore volumes and BET surface area of wheat dried distiller's grain increased after ultrasound pretreatment due to the disruption of plant cells, resulted in

a large hollow opening surface. Even though the pore volume of CPH solid residue decreased and its surface area became smaller, however, the CPH solid residue is still promising to be used as a carbon precursor for activated carbon or bio-oil and biochar production.

Table 7.6 shows that CPH and its solid residue are predominately mesoporous and macroporous materials with an average pore diameter of 17 – 27 nm within the mesopore range. The N_2 isotherm shown in Figure 7.6 represents that CPH and its solid residue are close to Type IV behaviour, but a slight "knee" point around P/Po 0.01-0.06 indicates limited micropore filling at higher pressure multi-layer adsorption within mesopores are observed. Above 0.95 relative pressure, macropores are evident; most adsorption is at higher relative pressure confirming the CPH and its solid residue are meso- and microporous materials. The hysteresis loops observed in the isotherms show Type H4, which correlated to micro-mesoporous carbons. In general, there is no difference pattern between CPH and its solid residue graphs. However, the quantity of N_2 adsorbed of CPH solids residue was lower than that of untreated CPH. This is attributed to the solid residue's decreasing surface area and pore volume. Tsai and Huang (2018) also reported that activated carbon from CPH experienced the shape of Type IV isotherm for mesoporous materials, which is also often encountered in microporous materials with high adsorption uptake at low relative pressure because of narrow pore width (< 2 nm) and high adsorption potential. There are also hysteresis loops presenting mesoporous structures.

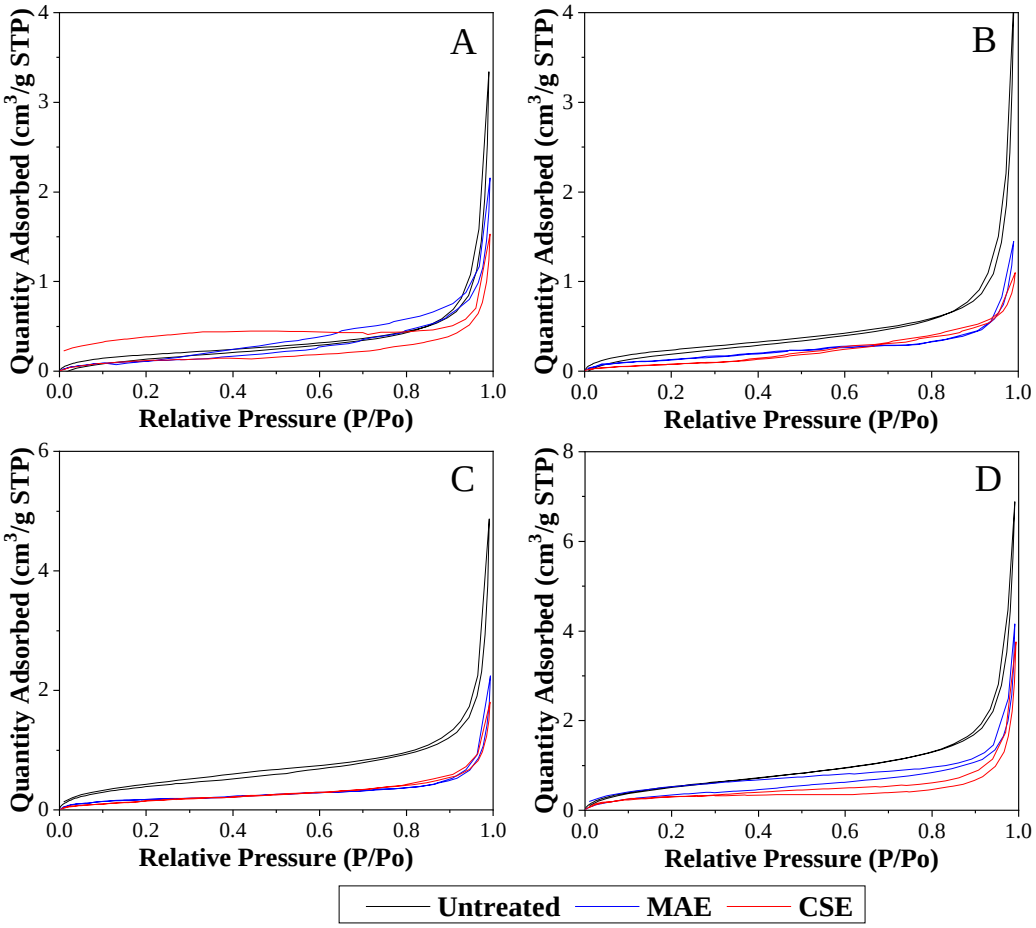


Figure 7. 6 N_2 isotherm graphs of CPH solid residue with particle size: (A) 125-150 micron; (B) 63-90 micron; (C) 38-63 micron; (D) ≤38 micron

7.4.2 Solid residue of CPH layers

The BET surface area of the solid residue of CPH layers (epicarp, mesocarp, endocarp) can be seen in Table 7.7. Generally, the BET surface area and pore volumes of the solid residue of each CPH layer decreased after extraction, as discussed in CPH solid residue (Section 7.4.1). The reason for these results is the same as in CPH solid residue. The decrease in surface area and pore volumes might be due to the collapsing of pores during extraction and trapped residual solvent or unextracted compounds on solid residue pores.

Table 7. 7 BET surface area and pore volumes of the solid residue of CPH layer

CPH layer with particle size:	Condition	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 (4V/A) (nm)
EPICARP						
125-150 micron	Untreated	0.54 ± 0.07	0.16 ± 0.02	1.13 ± 0.23	3.90 ± 0.57	28.88 ± 3.53
	MAE	0.27 ± 0.07	0.07 ± 0.02	0.57 ± 0.19	1.78 ± 0.57	26.55 ± 3.53
	CSE	0.20 ± 0.07	0.06 ± 0.02	0.43 ± 0.18	1.29 ± 0.57	26.35 ± 3.53
63-90 micron	Untreated	0.54 ± 0.03	0.16 ± 0.01	1.06 ± 0.23	3.65 ± 0.48	27.25 ± 1.43
	MAE	0.18 ± 0.03	0.06 ± 0.01	0.55 ± 0.16	1.32 ± 0.48	28.62 ± 1.43
	CSE	0.32 ± 0.03	0.08 ± 0.02	0.66 ± 016	1.85 ± 0.48	23.35 ± 1.43
38-63 micron	Untreated	1.48 ± 0.08	0.43 ± 0.09	3.39 ± 1.58	9.54 ± 0.40	25.26 ± 4.67
	MAE	0.24 ± 0.03	0.07 ± 0.03	0.58 ± 0.15	1.52 ± 0.40	24.89 ± 2.80
	CSE	0.42 ± 0.07	0.14 ± 0.03	0.58 ± 0.15	2.14 ± 0.40	20.30 ± 2.80
≤38 micron	Untreated	1.99 ± 0.05	0.65 ± 0.09	4.24 ± 0.95	13.04 ± 0.35	26.16 ± 0.04
	MAE	0.49 ± 0.03	0.13 ± 0.07	0.80 ± 0.06	2.21 ± 0.23	20.18 ± 2.08
	CSE	0.64 ± 0.07	0.17 ± 0.01	1.10 ± 0.27	3.11 ± 0.65	19.44 ± 2.08
MESOCARP						
125-150 micron	Untreated	0.78 ± 0.07	0.21 ± 0.02	1.54 ± 0.22	4.61 ± 0.57	23.63 ± 3.53
	MAE	0.22 ± 0.07	0.07 ± 0.01	0.61 ± 0.19	1.62 ± 0.57	29.60 ± 3.53
	CSE	0.22 ± 0.07	0.07 ± 0.02	0.49 ± 0.18	1.37 ± 0.57	24.55 ± 3.53
63-90 micron	Untreated	0.99 ± 0.03	0.29 ± 0.01	1.89 ± 0.16	5.13 ± 0.48	20.78 ± 1.43
	MAE	0.24 ± 0.03	0.07 ± 0.01	0.49 ± 0.16	1.46 ± 0.48	24.47 ± 1.43
	CSE	0.39 ± 0.03	0.12 ± 0.01	0.73 ± 0.16	1.81 ± 0.48	18.82 ± 1.43
38-63 micron	Untreated	1.46 ± 0.27	0.42 ± 0.07	3.44 ± 1.36	9.48 ± 3.57	25.52 ± 5.14
	MAE	0.37 ± 0.08	0.11 ± 0.03	0.63 ± 0.15	1.81 ± 0.40	19.69 ± 2.80
	CSE	0.41 ± 0.08	0.14 ± 0.03	0.67 ± 0.15	1.91 ± 0.40	18.83 ± 2.80
≤38 micron	Untreated	2.08 ± 0.40	0,63 ± 0.01	5.10 ± 0.31	14.50 ± 1.28	29.70 ± 0.78
	MAE	0.67 ± 0.09	0.18 ± 0.01	0.90 ± 0.27	3.31 ± 1.66	19.75 ± 2.08
	CSE	0.83 ± 0.09	0.22 ± 0.01	1.67 ± 0.27	3.67 ± 1.66	17.69 ± 2.08
ENDOCARP						
125-150 micron	Untreated	0.45 ± 0.07	0.14 ± 0.02	0.95 ± 0.22	3.21 ± 0.57	28.40 ± 3.53
	MAE	0.68 ± 0.07	0.19 ± 0.02	1.32 ± 0.22	3.19 ± 0.57	18.82 ± 3.53
	CSE	0.38 ± 0.07	0.11 ± 0.02	0.72 ± 0.22	2.19 ± 0.57	22.80 ± 3.53
63-90 micron	Untreated	0.55 ± 0.03	0.16 ± 0.01	1.10 ± 0.16	3.58 ± 0.48	26.12 ± 1.43
	MAE	0.41 ± 0.03	0.14 ± 0.01	0.82 ± 0.16	2.62 ± 0.48	25.32 ± 1.43
	CSE	0.50 ± 0.03	0.15 ± 0.01	0.96 ± 0.16	3.21 ± 0.48	25.49 ± 1.43
38-63 micron	Untreated	1.33 ± 0.31	0.38 ± 0.09	2.68 ± 0.90	7.8± 2.79	23.30 ± 2.91
	MAE	0.66 ± 0.08	0.17 ± 0.03	1.34 ± 0.15	4.12 ± 0.40	25.13 ± 2.80
	CSE	0.60 ± 0.08	0.17 ± 0.03	1.06 ± 0.15	3.26 ± 0.40	21.79 ± 2.80
≤38 micron	Untreated	2.00 ± 0.11	0.61 ± 0.12	4.30 ± 1.03	12.55 ±1.36	25.12 ± 1.36
	MAE	0.71 ± 0.17	0.20 ± 0.05	0.98 ± 0.10	3.50 ± 0.34	20.00 ± 2.85
	CSE	0.77 ± 0.09	0.19 ± 0.01	1.93 ± 0.27	4.25 ± 1.66	22.05 ± 2.08

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

According to Tables 7.5 and 7.7, the moisture content of endocarp solid residue (≤38 micron) increased from 7.56 to 9.52% (26%) when the micropore volume and

BET surface area decreased from 0.61 to 0.20 mm^3/g (67%) and from 2.0 to 0.71 m^2/g (65%), respectively. Figures 7.7 – 7.9 show no significant difference in isotherm graphs among solids residue of three CPH layers. As explained in CPH solid residue, the solids residue of the CPH layer also exhibited Type IV Isotherm, which was usually found on mesoporous material. Furthermore, the quantity adsorbed of solids residue was lower compared to that of the untreated CPH layer. There were also found hysteresis loops Type H4 when relative pressure (P/Po) increased to 0.2 or more, which were attributed to mesoporous carbons' behaviour.

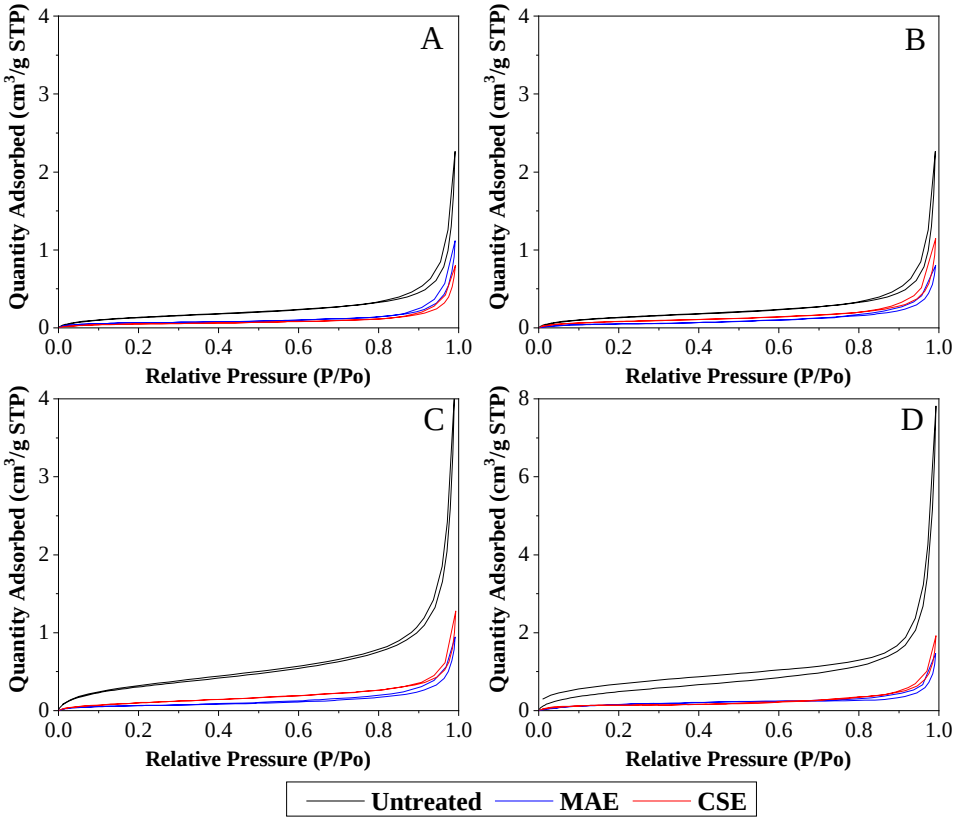


Figure 7. 7 N_2 isotherm graph of CPH solid residue of epicarp with particle size: (A) 125-150 micron; (B) 63-90 micron; (C) 38-63 micron; (D) ≤38 micron

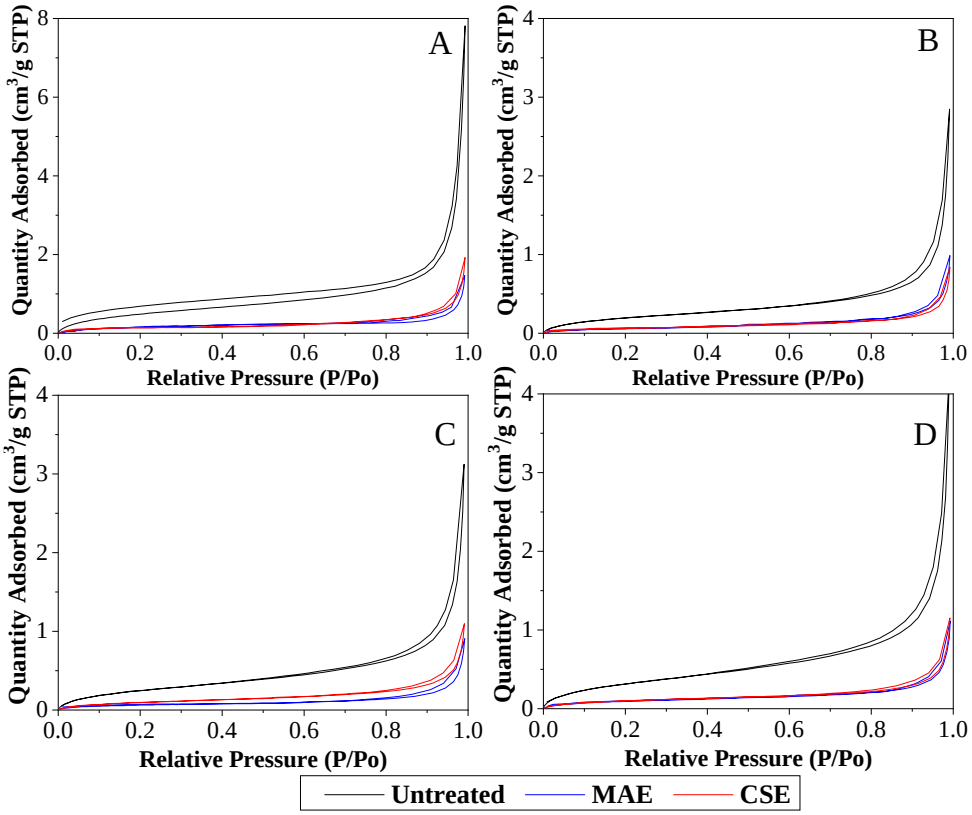


Figure 7. 8 N_2 isotherm graphs of CPH solid residue of mesocarp with particle size: (A) 125-150 micron; (B) 63-90 micron; (C) 38-63 micron; (D) ≤38 micron

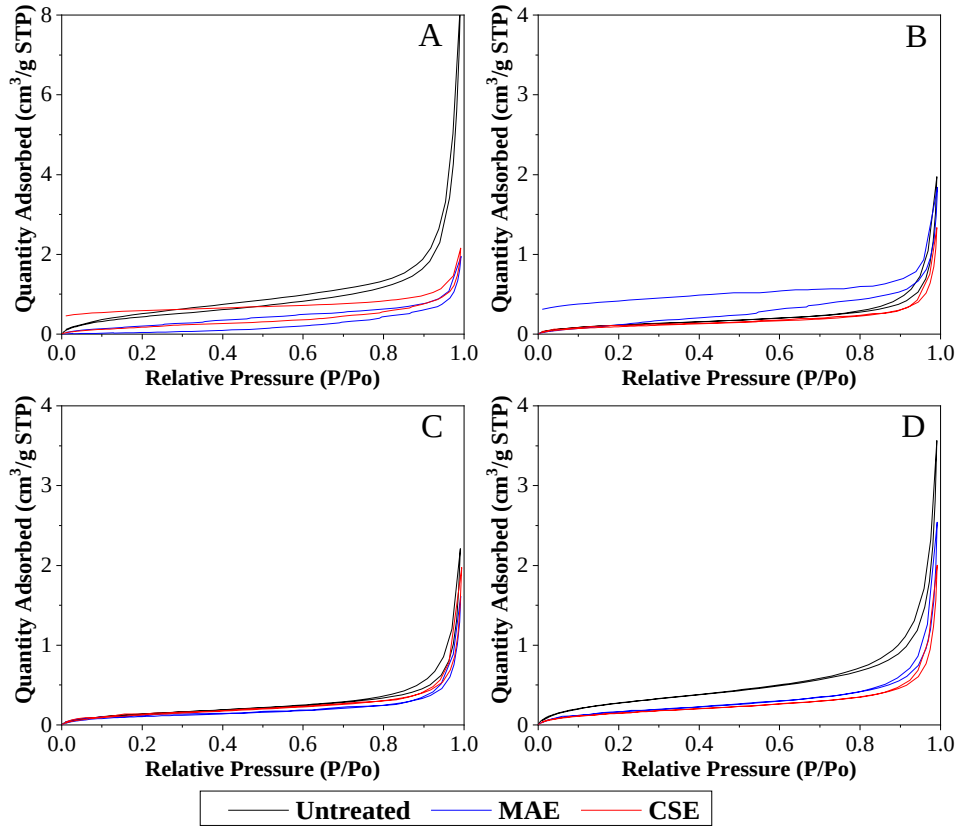


Figure 7. 9 N_2 isotherm graphs of CPH solid residue of endocarp with particle size: (A) 125-150 micron; (B) 63-90 micron; (C) 38-63 micron; (D) ≤38 micron

7.5 Microstructural analysis

The morphologies of CPH powder and its solid residue after the extraction with microwaves (Figure 7.10.B) and conventional (Figure 7.10.C) heating were investigated by scanning electron microscope (SEM).

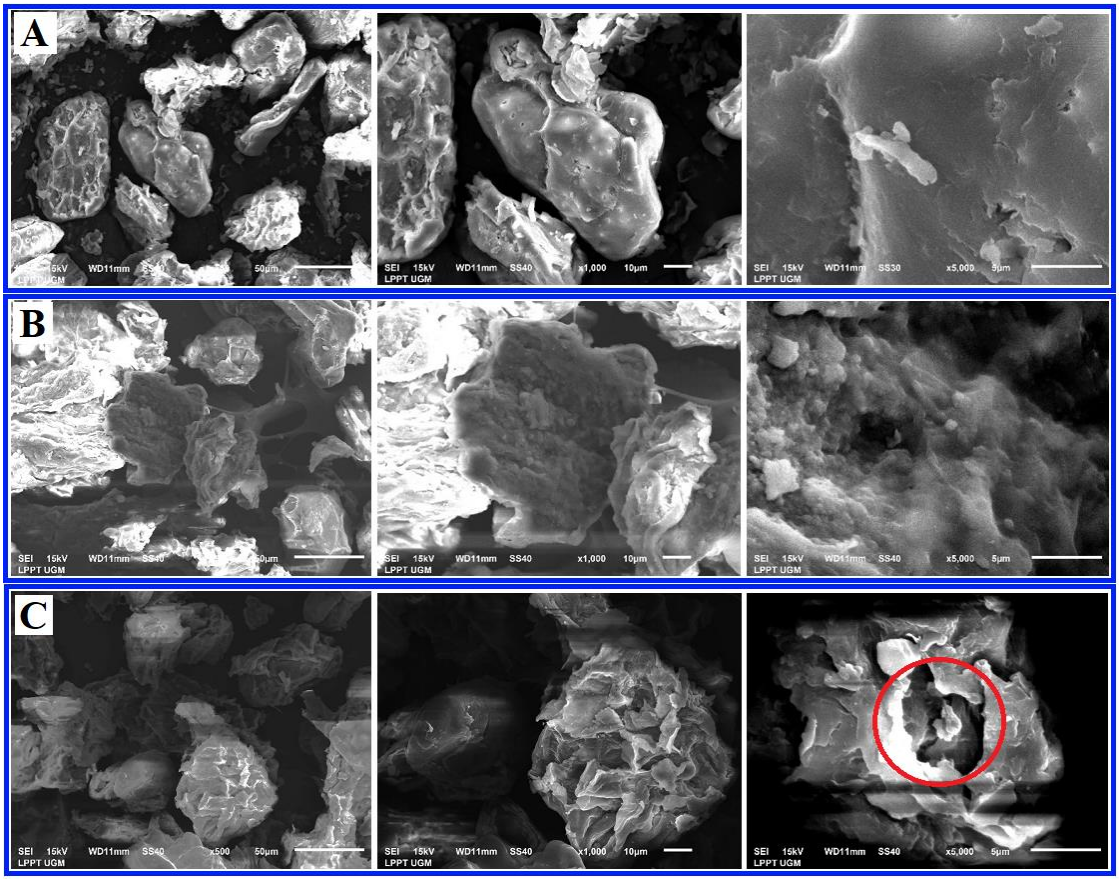


Figure 7. 10 SEM Images of CPH with particle size 38-63 micron: (A) CPH untreated; (B) CPH after MAE treatment; (C) CPH after CSE treatment (x500 – 5000 magnifications)

SEM images of untreated CPH looked like a compact flat surface with an amorphous structure, as shown in Figure 7.10.A, whereas after extraction treatments, the surface appearance of CPH solid residue became rough amorphous. This morphology can be attributed to the collapsing of pores during the extraction process and decreasing porosity, leading to a low surface area of CPH, which was only 1.5 m^2/g. Similar images were also reported by Nguyen et al. (2021), who investigated the drying effect on CPH from Vietnam. The microstructural changes in CPH after the extraction process were

associated with releasing phenolics from cells. Conventional heating caused more severe changes in the microstructure of CPH compared with microwave heating, as shown in Figures 7.10.B and C. Conventional heating could rupture the CPH cells and open some large pits or channels while collapsing smaller ones. The CPH solid residue (Figure 7.10.C) still looked rough but had a large extensive cracked area (red line).

By contrast, the CPH microstructural after microwave extraction showed almost similar to those untreated CPH, which was a thick flat surface and amorphous. This can be attributed to the BET surface area of CPH solid residue by microwave being slightly higher (0.64 m^2/g) than by conventional (0.56 m^2/g) (Table 7.6). These results agree with Valadez-Carmona et al. (2017), who reported that convective drying of CPH resulted in more severe microstructural changes than microwave drying. The researchers suggested this phenomenon might be because the sudden evaporation of superficial water during convective drying generated internal stress, resulting in cellular collapse and crust formation. Otherwise, the CPH microstructure dried by microwave heating was similar to those in fresh CPH. The microwave was absorbed by the entire sample containing moisture, resulting in pressure gradients which caused gradual drying from inside to the surface of the plant sample, thereby reducing the shrinkage and preventing the cell collapse and hard layer formation.

7.6 Potential application of CPH solid residue

According to the characterisation of CPH solid residue, the results show that CPH solid residue is beneficial for future applications due to its content, such as high lignocellulosic compounds, high volatile matter and fixed carbon and low ash and moisture contents. CPH solid residue contained approximately 23% of lignin, 37% of cellulose and 22 – 26% of hemicellulose, which can be further processed by thermochemical conversion. At the same time, cellulose itself is possibly to be

converted into bioethanol. However, bioethanol production will still release solid waste by-products, so this process is not good for valorising the CPH solid residue. Lignin is a significant precursor for biochar and activated carbon, while cellulose and hemicellulose are suitable for producing volatile products. Several thermochemical processes are profitable for lignocellulosic biomass conversions, such as pyrolysis and carbonisation (Mukherjee et al., 2022).

Pyrolysis is an endothermic degradation process of the organic matter of biomass in an unsusceptible environment (such as the absence of air or oxygen and atmospheric pressure condition), which will decompose long-chain hydrocarbon into smaller compounds. During pyrolysis, thermal degradation of biomass can be determined by char formation, depolymerisation reaction and fragmentation of lignocellulosic components. There are three types of pyrolysis processes: slow pyrolysis (300-700 °C), intermediate pyrolysis (450-550 °C) and fast pyrolysis (500-1000 °C), which generate different primary products. Slow pyrolysis will produce biochar as the main product, with bio-oil and synthesis gas as by-products. While to get primary products of bio-oil and biochar, thermal degradation can be conducted using intermediate pyrolysis. Last, fast pyrolysis will produce bio-oil as the primary product with by-products of biochar and synthetic gas (Mukherjee et al., 2022).

Biochar is an economical and eco-friendly material that is often used for soil remediation, carbon sequestration, CO_2 adsorption, wastewater treatments (removal of heavy metal, pesticides, synthetic dye or toxic pollutants), energy storage and a supercapacitor (Mukherjee et al., 2022). Bio-oil can be used for transportation fuel, heat, and power generation (Mediani et al., 2013). Non-condensable gases, mainly composed of H_2, CO_2, CO, CH_4, and C_2H_6, also have the potential for power, industrial and transportation fuel (Uddin et al., 2013). During pyrolysis, inorganic minerals may

hinder pore development in biochar (Tsai et al., 2020). The high ash and or potassium content also resulted in low carbon content and high oxygen content. However, acid leaching using 3M HCl has been reported to increase 16% of fixed carbon while removing 90% of ash content, including potassium content in CPH. The acid solution easily leached out the inorganic elements (including potassium) and trace metals that were contained in ash, so that the ash content in sample greatly reduced. The absence of inorganic elements, particularly heavy metals, will negate the emission of toxic metals during combustion. In addition, the acid-leaching could increase BET surface area of activated carbon from 289.5 to 427.8 m^2/g (Tsai and Huang, 2018). Hence, de-ashing the CPH can be a solution in technical limitations of carbonisation or pyrolysis. Furthermore, the solution from acid-leaching may be reused as a liquid fertiliser due to high content of potassium ions.

The carbonaceous material, known as "activated carbon", has an amorphous structure, high porosity, well-developed surface area, and oxygenated functional groups. Activated carbon can be prepared in two steps: carbonisation and activation. Carbonisation is thermal decomposition of raw materials in a furnace under nitrogen purge in an inert atmosphere; carbonisation is also called slow pyrolysis, which is defined as a slow heating rate (<0.01 – 2 °C/s) at moderate temperatures (200 – 300 °C) with biochar as the primary product (Mukherjee et al., 2022; Reza et al., 2020). The carbonisation process can be defined in four stages, as shown in Table 7.8. Carbon precursor obtained from carbonisation is then activated by chemical or physical activation. By chemical activation, carbon precursor is impregnated in activating agents, such as NaOH, KOH, $ZnCl_2$, and H_3PO_4. Chemical activation can be divided into three forms: basic activation, acidic activation, and neutral activation. The activating agent has various effects on activated carbon; for instance, basic activation

such as KOH and NaOH can produce activated carbon with a very high surface area, up to 2000 m^2/g. Meanwhile, biochar or carbon precursor is activated in physical treatment using oxidising gases to improve the pore structures. During physical activation, new pores are produced, and the narrow pores are further developed, enhancing the porous structure. However, this activation has relatively lower yield (Reza et al., 2020).

Table 7. 8 Stages of the carbonisation process (Reza et al., 2020)

Stage	Temp (°C)	Process
1	< 200	Initial drying to remove moisture
2	170 – 300	Pre-carbonisation phase produces some pyroligneous liquids, light tars and non-condensable gases
3	250 – 300	Producing biochar by removing the pyroligneous liquids and light tars produced in phase two
4	> 300	Enhancing the fixed carbon content of biochar by eliminating volatile and non-carbon species

According to the proximate analysis (Table 7.4), CPH solid residue had high volatile matter (62-65%) and fixed carbon contents (21-25%) but low in ash (2-4%) and moisture contents (9-10%). The biomass with these characteristics is very promising for pyrolysis and carbonisation. Both processes are preferable because they are zero-waste. Even though the surface area of CPH solid residue was too low, only 0.4 – 1.4 m^2/g, it is still possible to be processed. There is no special requirement for surface area in the pyrolysis or carbonisation process. Several studies provided evidence that pyrolysis and carbonisation could improve the surface area. The S_{BET} value of biochar increased with the increase in pyrolysis temperatures (Chen et al., 2022). For example, cellulose biochar had S_{BET} lower than 2.5 m^2/g at 400-500 °C and subsequently increased to 213 m^2/g at 600 °C and 505 m^2/g at 800 °C.

Some studies also reported the production of activated carbon from biomass with a small surface area. Melia et al. (2018) have successfully used agricultural waste

biomass (grape wastes, flax shive, flax mat, wheat straw, barley straw) with a low surface area (<10 m^2/g) for Cd removal; grape wastes with a surface area only 1.6 m^2/g could remove Cd up to 96% from the solution. They suggested that using agricultural waste biomass on a large scale is economically advantageous due to its low or zero cost. Tea residue (surface area of 2.29 m^2/g) was also reported to be converted into activated carbon (surface area of 871 m^2/g) through chemical activation. It successfully removed 100% of pigment and pesticides and 99.9% of Hg from the solution (Bai et al., 2022). Therefore, pyrolysis of CPH solid residue has very high potential because bio-oil and non-condensable gas products are advantageous for energy. In contrast, biochar products can be used for activated carbon precursors. Grand View Research reported that activated carbon was valued at USD 3.4 billion in 2021 and is predicted to reach USD 4.45 billion by 2030 (Activated Carbon Market Size & Share Report, 2030 (grandviewresearch.com)).

The solid residue of each CPH layer has the same potential as CPH solid residue, but we need to consider energy, cost and time for preparation. Preparation of CPH into three layers (epicarp, mesocarp, and endocarp) requires high energy and more time for separating, drying and grinding them. Hence, using the CPH solid residue for further processes is more beneficial.

7.7 Summary

CPH solid residue contained lignocellulosic compounds with high volatile matter and fixed carbon but low ash and moisture contents. These characteristics are very beneficial for further processing. Lignocellulosic compounds (cellulose, hemicellulose, and lignin) increased after the phenolic extraction process, but there was no conclusive difference between microwave and conventional heating treatments. These increments were confirmed by Chesson analysis, TG/DTG curves, and proximate data. TG/DTG

peaks of hemicellulose and cellulose in CPH solid residue shifted to the right after extraction, which means that the cellulose content in solid residue increased compared to untreated CPH. The volatile matter of solid residue also increased which indicated the increment of cellulose content. In addition, the extraction of phenolics from CPH increased the moisture contents but decreased the ash carbon of CPH solid residue. The increasing moisture content might be because of the residual solvent trapped, which can be confirmed by BET surface area and SEM images. The pore volume and S_{BET} of CPH solid residue were lower than untreated CPH, which may be attributed to the pore collapsing under extraction, and pore-clogging by residual solvent or other compounds. These decreases caused by conventional heating were higher than by microwave heating because conventional heating could rupture the CPH cells and open some large pits while collapsing the smaller ones. SEM images confirmed the presence of small clumps blocking the porosity (pore-clogging) (Figure 7.10.B) and cracked area caused by conventional heating (Figure 7.10.C) on the surface of CPH solid residue, which previously appeared smooth (Figure 7.10.A). Generally, CPH solid residue had S_{BET} of about 0.32 – 1.44 m^2/g and consisted of approximately 36 % of cellulose, 22-26 % of hemicellulose, and 23 % of lignin with the proximate contents: 9 – 11 % moisture content, 62 – 65 % volatile matter, 21 – 25 % fixed carbon, and 3 – 6% ash. Biomass with high lignocellulosic content, high volatile matter and fixed carbon, and low ash content and surface area are very promising in thermochemical conversion processes, such as pyrolysis. Accordingly, the CPH solid residue can potentially be converted into other valuable products, such as bio-oil, non-condensed gases and activated carbon. Even more, that process can improve the economic value and reduce the waste by-products without releasing other wastes.

CHAPTER 8: CONCLUSIONS AND FUTURE RECOMMENDATIONS

This chapter concludes the key findings in accordance with the objectives of this study. The current challenges of phenolic extraction from CPH and recommendations for future work were also presented.

8.1 Conclusions

This study aimed to comprehend how the processing parameters would maximise the extraction yields and influence the process efficiency in the valorisation of CPH through extraction of bioactive compounds, particularly phenolic-based antioxidants. The work was divided into four research objectives. Firstly, the effect of material pretreatment (drying and size reduction), solvent type, and different extraction techniques on extraction yields (total phenolic, anthocyanin, antioxidants) was investigated to understand their viabilities on the CPH valorisation process (Objective 1). According to that knowledge, the study was developed to investigate how the processing parameters (extraction time and temperature, ethanol concentration and solvent-to-feed (S/F) ratio) within MAE and CSE would maximise the extraction yield and its quality in order to understand the advantages of microwave heating over conventional (Objective 2). Those extraction parameters would be important to evaluate the energy-intensive and operational costs in designing an efficient process for CPH valorisation. On the other hand, CPH has been reported to have three layers (epicarp, mesocarp, endocarp), so extractions of bioactive compounds (phenolic, anthocyanin, antioxidant) from those three distinct layers were studied to understand the distribution of bioactive compounds within CPH layers (Objective 3). Last, the proximate analysis, lignocellulose content, chemical compositions and surface area of CPH solid residue

were characterised to analyse its potential contents for further processing (Objective 4). The schematic overview of this thesis study are elaborated in Figure 8.1 according to the research objectives.

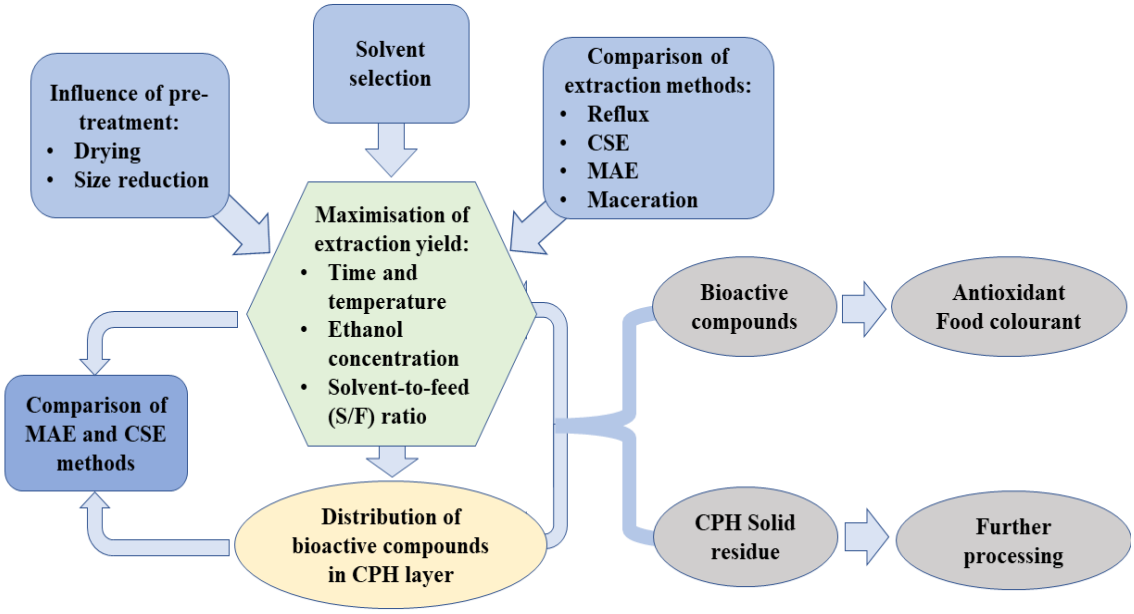


Figure 8. 1 Schematic overview of the study in the present thesis

Addressing Objective 1: *Investigation the influence of sample pretreatment (drying and size reduction), solvent type, and different extraction methods (conventional and microwave heating) on bioactive yields: total phenolic content (TPC), total monomeric anthocyanin content (TMA), and antioxidant activity (AOA)*

The work presented in this study has provided evidence that CPH is rich in phenolic and anthocyanin compounds with antioxidant activity. The research has shown that these bioactive compounds were successfully extracted from CPH using microwave and conventional heating. The research first studied how the drying and size reduction of CPH affects the yield of phenolic and anthocyanin compounds to evaluate the importance of these sample pretreatment processes in the CPH valorisation flowsheet. The CPH drying using forced air dryer at 50 °C for 24 hours would remove the moisture content in fresh CPH to extend its shelf life, but it decreased the extracts'

phenolics and antioxidants by 49% and 89%, respectively (Figure 4.2). However, considering the perishable properties of fresh CPH (86.7% moisture content – easy to rot and oxidise) and their high distribution and storage costs, the processing of dry CPH was preferable. In contrast, size reduction has been shown to improve the phenolic and anthocyanin yields but had no significant effect on the antioxidant activity (Figures 4.2 and 4.7). A reduction in particle size from 150 to 38 micron could increase twofold the bioactive compounds' yields. The study on sample pretreatment effect is useful as input data in considering energy requirements on scale-up processing or valorisation flowsheet.

This study also revealed the significance of solvent type in solvent extraction. The extraction solvent, which must have similar properties to the target compounds, determined the bioactive compounds extracted from CPH; all target compounds can, therefore, be dissolved and taken out from CPH. Prior to the experiment, the solubility of solvent and target compounds were predicted using Hansen Solubility Parameter (HSP) to select the potential extraction solvents. According to HSP value and research findings, ethanol/water mixture was found to be the most suitable solvent to extract the phenolic compounds from CPH.

On the other hand, investigation of four different extraction methods (maceration, reflux, conventional solvent extraction (CSE) and microwave-assisted extraction (MAE)) revealed that heating could improve the bioactive yields but reduce its antioxidant activity; the extract obtained by maceration (no heating) showed low phenolic and anthocyanin yields but high antioxidant activity when compared to that with the heating process (CSE, MAE). The work findings also showed that microwave heating significantly reduced the extraction time and enhanced the bioactive yields. The heating time of MAE (Figure 4.5.A) was fourfold (100 s) faster than conventional

heating (375 s), which confirmed the volumetric heating in MAE resulted in faster heating rates than in the conventional heating experiment. During volumetric heating, the mixture of plant material and the solvent was heated directly and instantaneously throughout, providing rapid heating. When the volumetric heating was negligible (designing a similar heating rate), the phenolic yield on MAE was 15% higher than conventional heating (CSE-ethylene glycol bath (EgB)) at 70 °C for 60 min (Figure 4.6), which confirmed that the increase in yields was attributed to the microwave selective heating effect. Hence, in the subsequent experiments, MAE was directly compared to CSE (using an ethylene glycol bath to reach heating temperature) by considering the effect of processing conditions on both methods to obtain the maximum yields.

The preliminary study showed that the material pretreatment and extraction solvent are the critical parameters in bioactive extraction from CPH. Therefore, selecting the most appropriate solvent and material size reduction should be done at the beginning of the extraction process to get optimal yield. These steps might also be important for any phenolic or bioactive extraction from plant material or agricultural waste.

Addressing Objective 2: *Evaluation the influence of extraction parameters: extraction time and temperature, solvent extraction, solvent-to-feed (S/F) ratio) within the microwave and conventional extraction methods to maximise the bioactive yields*

Using those preliminary findings in Objective 1, this work has further investigated how the processing parameters (time and temperature, ethanol/water concentration, and solvent-to-feed ratio) on MAE and CSE can be optimised to maximise the bioactive yield and its functionality. A direct comparison of CSE and

MAE was assessed to understand how the potential benefits of microwave selective heating promote maximising extracts' phenolic and antioxidant.

Using a similar heating rate between MAE and CSE which neglects volumetric heating in microwave, both extraction methods (MAE and CSE) found the same maximum extraction times (5 minutes) and solvent requirements (50% (v/v) ethanol/water at 40:1 mL/g). In contrast, the longer extraction time would decrease the bioactive compounds in the extract, which remained constant after 10 min. On the other hand, extraction temperature showed varying effects on the extracts' phenolic content and antioxidant activity, both on MAE and CSE. On MAE, phenolic content (TPC) increased from 50 °C and reached a maximum at 60 °C (107.3 ± 1.4 mg GAE/g dw), whereas TPC in CSE raised as temperature increased and required a higher temperature (70 °C) to gain maximum phenolics, that was 105.4 ± 0.9 mg GAE/g dw. By comparing both methods at the same temperature (60 °C) for 5 min, MAE had 5% higher TPC than CSE due to its selective heating effect. This finding confirmed that microwave selective heating occurred from 60 °C, but overheating might happen at 70 °C or higher which reduce the TPC yield. Thus, MAE is proposed to be operated at a lower temperature, using less energy. In contrast, the behaviour of anthocyanin and extracts' antioxidant activity was inversely proportional to the phenolic yields. They decreased with increasing temperature in both extraction methods and achieved a maximum of 50 °C. There was no significant effect of heating methods on antioxidant yield between MAE and CSE at 50 °C, and its activity remained constant at 3.3 mg TE/g dw. This means no selective heating effect is involved in the extraction under microwave heating at 50 °C.

The selection of extraction method and operation conditions, therefore, is determined by the primary goal while considering the necessity for equipment size,

energy, and operational cost. For instance, with regards to obtaining higher phenolic, MAE at 60 °C is recommended, while the use of either MAE or CSE at a low temperature (50 °C) is preferred for antioxidant and anthocyanin recovery, given that microwave has no selective heating effect at that temperature. The summary of recommendations extraction process to get high yields is presented in Table 8.1.

Addressing Objective 3: *Investigation of the distribution of bioactive compounds in three distinct CPH layers (epicarp, mesocarp, and endocarp) under microwave and conventional heating*

CPH can be separated into three distinct layers: epicarp, mesocarp, and endocarp. Each CPH layer was extracted based on the maximum processing conditions discussed above (Objectives 1-2). Each CPH layer contains bioactive compounds, which the phenolic and anthocyanin compounds concentrated in the epicarp layer with low antioxidant activity, whereas the endocarp layer was low in bioactive compounds, but has high antioxidant activity (Figures 6.6 – 6.7). Compared with the extraction yields of whole CPH extract (100.4 mg GAE/g dw; 3.4 mg TE/g dw), the epicarp produced 80% higher TPC yield (180.7 mg GAE/g dw) while endocarp yielded 10% higher AOA (3.7 mg TE/g dw). Extracting both epicarp and mesocarp layers (±77 % of total CPH mass) would recover up to 88 % of the phenolic compounds from CPH (Figure 6.2). However, given the high energy and time consumption for preparing CPH layers, processing the whole CPH may be more favourable because phenolic-antioxidant compounds are present in all CPH layers. Another fact showed that microwave heating could improve the phenolic yield in each CPH layer by up to 17% compared to conventional heating. Lastly, size reduction showed a significant effect on the bioactive compound yields (TPC and TMA) either for the whole CPH or each CPH layer, whilst the extraction methods (MAE and CSE) showed a noticeable effect only in the

extraction of each CPH layer. The summary of recommendations extraction process to get high yields is presented in Table 8.1.

Table 8. 1 Recommendation of extraction methods selection based on product requirements

Product requirements	**Recommended extraction methods**	**Main advantages**	**Main disadvantages**
High phenolic content	Extraction from CPH ≤38 micron using 50% (v/v) ethanol/water solvent (40:1 mL/g) by MAE at 60 °C for 5 min	• High TPC yield (107.3 mg GAE/g dw) • MAE showed 5 % higher TPC than CSE at the same temperature (60 °C)	High energy for pretreatment and difficulties for further product separation, especially for large-scale application
	Extraction from CPH ≤38 micron using 50% (v/v) ethanol/water solvent (40:1 mL/g) by CSE at 70 °C for 5 min	• High TPC yield (105.6 mg GAE/g dw) • CSE can have the same time (5 min) when volumetric heating was negated	• Need high energy (temperature) to reach a similar yield to MAE • High energy for pretreatment and difficulties for further product separation, especially for large-scale application
	Extraction from epicarp layer (≤38 micron) by MAE at 50 °C	High TPC yield (180.7 mg GAE/g dw)	Require high energy and time for sample preparation and separation, especially for scale-up application
High anthocyanin content	Extraction from CPH ≤38 micron using 50% (v/v) ethanol/water solvent (40:1 mL/g) by MAE or CSE at low temperature (50 °C) for 5 min	High TMA yield (0.33 – 0.37 mg Cy_3GE/g dw)	No selective heating effect, thus selection of extraction method and processing parameters based on energy requirements and equipment size
High antioxidant content	Extraction from CPH ≤38 micron using 100% (v/v) ethanol solvent (50:1 mL/g) by MAE or CSE at low temperature (50 °C) for 5 min	High AOA yield (4.6 mg TE/g dw)	No selective heating effect, thus selection of extraction method and processing parameters based on energy requirements and equipment size

Addressing Objective 4: *Characterisation the proximate contents and pore characteristics of CPH solid residue and identify potential applications based on its properties*

CPH solid residue was generated as new waste during the extraction process. Therefore, to maximise the processing of CPH waste as well as reduce the negative environmental impact, the CPH solid residue was characterised to understand its further potential based on the contents or properties. This study addressed the research Objective 4. According to the characterisation analysis, lignocellulosic content in CPH solid residue increased after extraction (Table 7.3), which also increased the volatile matter content (Table 7.4). Otherwise, the extraction could reduce the ash content which is advantageous because the low ash content will not be interference on further thermo-chemical processes. CPH solid residue consists of 9 – 11 % moisture content, 62 – 65 % volatile matter, 21 – 25 % fixed carbon, and 3 – 6% ash with a low surface area, which was about 0.32 – 1.44 m^2/g. The lignocellulosic content in CPH solid residue was approximately 36 % cellulose, 22-26 % hemicellulose, and 23 % lignin. According to those contents, CPH solid residue is very promising for pyrolysis reaction to being converted into other valuable products, such as bio-oil, non-condensed gases and activated carbon. Further processing of CPH solid residue will be very profitable in industries because it increases economic value, reduces new waste, and supports the zero-waste industry.

8.2 Basis of extraction design

The results presented in this study can be used as input data for preliminary engineering design and techno-economic assessment in valorisation of cocoa industry wastes. Using CPH as raw material to produce phenolic-antioxidants and convert its solid residue into

other products might improve economic value and reduce waste from the environment. The production of bioactive compounds from CPH can be proposed into five main processing steps, as presented in Figure 8.2. Techno-economic analysis could then be carried out on further preliminary designs to aid decision-making.

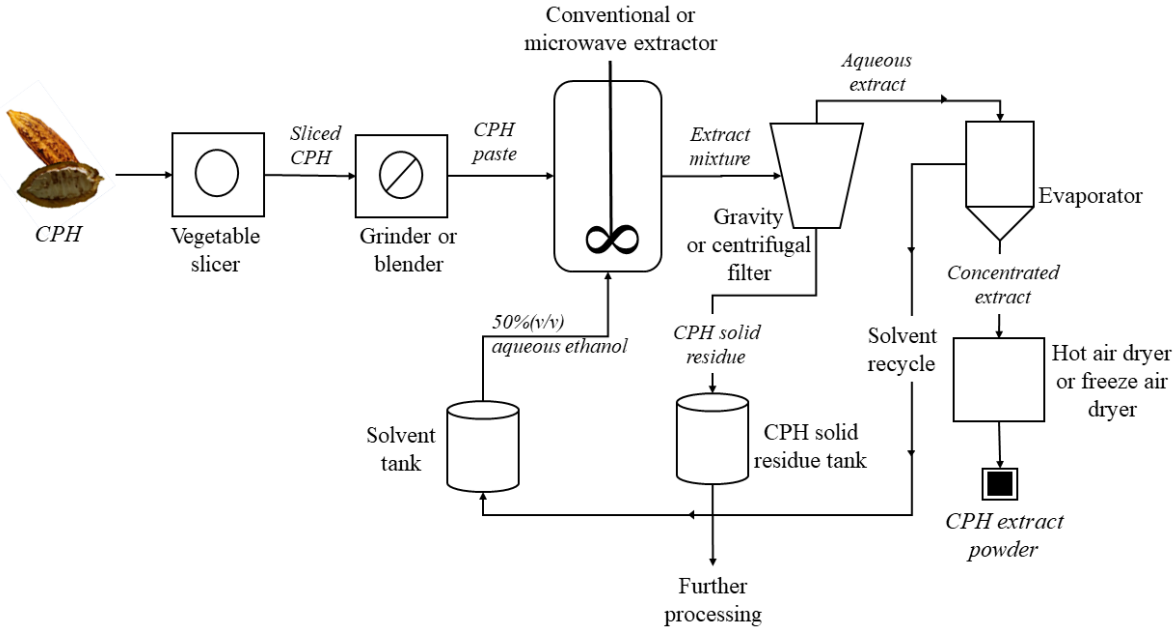


Figure 8. 2 Process flowsheet for extraction of bioactive compounds from cacao pod husk (CPH)

1. Drying and size reduction are the initial pretreatments to produce dry powder CPH. Drying can minimise CPH damage and spoilage but also decrease the bioactive yields, whereas size reduction will be required to attain significant yields of phenolics. The drying process may be eliminated in large-scale production when the processing is carried out close to the source of CPH waste. Meanwhile, the degree of size reduction in the final design will be a trade-off between grinding energy, equipment size and yield, and the data in Figures 4.7 and 5.11 can be used to feed into this calculation in the techno-economic analysis.
2. The implications of different solvents selection on the phenolics and antioxidant activity of the extract can be understood from Figures 4.4, 5.3, 5.7, and Tables 4.3 and 5.1. The 50% (v/v) ethanol/water is suggested as the most appropriate

solvent to maximise phenolic extraction. However, more work is required to gain a detailed picture of the extract composition and its antioxidant activity.

3. The extraction method will be selected based on the yield and quality of the extract, energy and solvent requirements, equipment size and other external factors (such as social and sustainability). Figure 4.6 identifies that the highest yields of TPC can be extracted using MAE (82 mg GAE/g dw), but 60 mg GAE/g dw can be achieved using maceration, which requires no heating equipment at all but will require a larger plant footprint and lots of solvent. Heating can significantly increase extraction yields, so microwave or conventional heating will be used to maximise the extraction yields. Scaling up the CSE exploiting a fast-heating rate may require a large conventional stirred tank reactor as it uses two heating baths to accelerate its heating rate. Thus, for CSE scale-up, the use of a conventional Continuously Stirred Tank Reactor (CSTR), by eliminating the benefit of a fast-heating rate, would be more advantageous. While scaling up MAE process can be carried out by designing a long tubular continuous reactor to overcome the penetration depth limitation. The study showed that microwave heating effect worked between 50 and 60 °C and may overheat at 70 °C. Hence, to maximise the potential benefits of microwave selective heating, minimise the energy used, and prevent the degradation of the bioactive compounds, temperature can be operated using low temperatures (50 – 60 °C), as discussed in Sections 5.2 and 5.3. On the other hand, information about penetration depth will help design scaling-up equipment, such as designing the microwave extractor (equipment size, stirring type) and selecting the flow diameter. The diameter of the microwave extractor would also influence the efficient penetration of microwave heating.

4. Separation is one of the important processes to separate the extract from the solid residue. Gravity or centrifugal filtration could be chosen to separate the aqueous extract from CPH solid residue. The aqueous extract is an alternative product that offers cost-saving benefits and lower production costs due to eliminating the following process (evaporation and drying). This product is suggested for on-site applications to prevent bioactive degradation due to storage time. Meanwhile, to produce extract powder, the residual solvent is removed from the mixture by evaporation, and the concentrated extract is dried by a low-temperature hot air dryer or freeze dryer. The residual solvent can be recycled and re-used as solvent extraction. Energy for filtration, evaporation and extract drying needs to be used for techno-economic analysis.

8.3 Current challenges and recommendations for future work

The bioactive compounds and their antioxidants activities of CPH extract have been demonstrated in this study. However, several findings are still unclear and require future research investigation. According to those research findings, future studies may be considered as below:

1. Developing further chromatographic analysis, such as LCMS, is needed to identify all individual bioactive (phenolics and anthocyanins) compounds in CPH extract, which HPLC has not explained. This analysis is also expected to detect the active compounds which are responsible for the antioxidant abilities in three different solvent extraction (deionised water, 50% (v/v) ethanol/water, and 100% (v/v) ethanol).
2. Determining the antioxidant activities of CPH extracts using various antioxidant assays, such as ABTS, FRAP, or CUPRAC, to support the DPPH result. Those results will validate the antioxidant yield of CPH extract.

3. Investigating the influence of processing parameters (solvent properties, extraction method, time and temperature) on the extraction of bioactive compounds from fresh CPH. The outcome will be used as input data for designing large-scale processing, particularly on-site processing.
4. Developing a further process to recycle the residual solvent and get the extract powder needs to be conducted. The extract powder should then be tested for its antioxidant activity in application to extend the shelf life of food products prior to large-scale production.
5. Develop a scaling-up process based on the processing parameters defined in this study and analyse the economy of the extraction process. Heat and mass transfer on a laboratory scale differs from a large scale. Extraction in pilot-scale equipment might be required a longer extraction time, higher stirring rate, larger solvent volume, and larger plant material size to get an effective process. Therefore, designing a large-scale microwave extractor will require further study in terms of applying the processing parameters from the laboratory scale to maximise the delivery of microwave energy into the system and ensure that selective heating can enhance the extraction yield.
6. Develop a more comprehensive biorefinery process to valorise the CPH, which not only can produce phenolic extract for antioxidant application, but also for other products, including pyrolysis products or activated carbon from CPH solid residue to prevent the discarded new waste.

The study has shown that microwave heating could decrease the extraction time (faster heating time); whereas conventional heating (CSE) could have the same heating rate as MAE when the sample is immersed in an ethylene glycol bath before moving into a water bath heating. This CSE condition would be very troublesome when applied

on a large scale. Therefore, scaling-up the microwave extractor may be more interesting. However, there are challenges to scalability of the microwave extractor, such as penetration depth limitation of microwave irradiation into the mixture that is only a few centimetres at 2.45 GHz. To deal with the obstacle, extraction should be performed with a proper stirring rate and/or using continuous-flow microwave systems, as studied by Arrutia et al. (2020).

On the other hand, in terms of the sustainability of CPH raw material, cacao plantation in Indonesia is mostly scattered in rural areas all over the islands, where the transport networks are poor. This is a challenge, especially when collecting the CPH feedstock which is high-priced in terms of time and labour as well as transport storage. This might adversely affect the overall production cost. Even more, CPH is perishable and easily oxidised. Consequently, building a large-pilot scale in only one area can be challenging. Therefore, building a smaller pilot-scale in each regional area, such as in Java, Borneo, and Sulawesi Islands, may be more profitable to reduce transportation and storage costs and prevent the degradation of bioactive compounds in CPH raw materials.

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APPENDICES

Appendix A: General data for cacao and cacao pod husk (CPH)

1. Calculation of the ratio of each part of Cacao fruit

Table A.1 Ratio of each part of the cacao fruit

CPH part	FRESH CPH							DRY CPH						
	CLONE 1		CLONE 2		CLONE 3		Average Ratio part/whole CPH (%)	CLONE 1		CLONE 2		CLONE 3		Average Ratio part/whole CPH (%)
	Mass (kg)	Ratio (%)	Mass (kg)	Ratio (%)	Mass (kg)	Ratio (%)		Mass (kg)	Ratio (%)	Mass (kg)	Ratio (%)	Mass (kg)	Ratio (%)	
Whole CPH	12.80	100	3.6	100	2.1	100	100.00 ± 0.0	1.66	100	0.44	100	0.22	100	100.00 ± 0.00
Epicarp	1.70	13.28	0.5	13.89	0.3	14.29	13.82 ± 0.51	0.23	13.94	0.08	18.14	0.05	22.73	18.27 ± 4.39
Mesocarp	7.95	62.11	1.85	51.39	1.15	54.76	56.09 ± 5.48	1.23	73.72	0.30	67.12	0.15	68.18	69.67 ± 3.54
Endocarp	1.40	10.94	0.5	13.89	0.15	7.14	10.66 ± 3.38	0.21	12.33	0.07	14.74	0.02	9.09	12.05 ± 2.83
Bean	1.75	13.67	0.7	19.44	0.5	23.81	18.98 ± 5.09							

Cacao fruit has beans ranging from 14 to 24%, which releases the CPH with about 76 – 86%. CPH has three layers with the proportions: 13 – 14% of epicarp, 51 – 62% of mesocarp, and 7 – 14% of endocarp.

2. Calculation of the ratio of each CPH layer

Table A.2 Ratio of each CPH layer

CPH part	FRESH CPH							DRY CPH						
	CLONE 1		CLONE 2		CLONE 3		Average Ratio part/whole CPH (%)	CLONE 1		CLONE 2		CLONE 3		Average Ratio part/whole CPH (%)
	Mass (kg)	Ratio (%)	Mass (kg)	Ratio (%)	Mass (kg)	Ratio (%)		Mass (kg)	Ratio (%)	Mass (kg)	Ratio (%)	Mass (kg)	Ratio (%)	
Whole CPH	11.05	100	2.85	100	1.60	100.00	100.00 ± 0.00	1.66	100	0.44	100	0.22	100	100.00 ± 0.00
Epicarp	1.70	15.38	0.5	17.54	0.30	18.75	17.23 ± 1.71	0.23	13.94	0.08	18.14	0.05	22.73	18.27 ± 4.39
Mesocarp	7.95	71.95	1.85	64.91	1.15	71.88	69.58 ± 4.04	1.23	73.72	0.30	67.12	0.15	68.18	69.67 ± 3.54
Endocarp	1.40	12.67	0.5	17.54	0.15	9.38	13.20 ± 4.11	0.21	12.33	0.07	14.74	0.02	9.09	12.05 ± 2.83

3. Calculation of moisture content measurements – an example of the determination of moisture content for fresh whole CPH using an oven

Table A.3 Moisture content measurement for fresh whole CPH

Calculation steps	Measurement 1	Measurement 2	Measurement 3
Mass of dried empty glass (g)	26.32	23.30	23.79
Mass of fresh CPH sample (g)	5.06	5.04	5.02
Total mass before drying (g)	31.38	28.34	28.81
Total mass after 1-hour drying (g)	27.58	24.59	25.11
Total mass after 2 hours drying (g)	27.42	24.36	24.85
Total mass after 3 hours drying (g)	27	24	24.42
Mass of water (g)	4.38	4.34	4.39
Moisture content (%)	**86.56**	**86.11**	**87.45**
Average moisture content (%)		**86.71 ± 0.68**	

Table A.4 Moisture content of CPH samples

Sample		MOISTURE CONTENT (%)			
		Measurement 1	**Measurement 2**	**Measurement 3**	**Average**
Fresh Whole CPH		86.56	86.11	87.45	86.71 ± 0.68
Dry Whole CPH		9.25	8.64	7.49	8.46 ± 0.89
Fresh CPH layer	Epicarp	75.00	75.67	75.67	75.44 ± 0.38
	Mesocarp	82.33	82.67	82.67	82.56 ± 0.19
	Endocarp	91.33	92.33	92.33	92.00 ± 0.58
Dry CPH layer	Epicarp	13.22	14.05	14.17	13.81 ± 0.51
	Mesocarp	14.95	15.89	15.24	15.36 ± 0.48
	Endocarp	14.14	15.00	15.15	14.76 ± 0.54

Appendix B: Calculation of extracts' qualities

1. Determination of total phenolic content (TPC) – an example data for MAE, 50% (v/v) aqueous ethanol, 50 °C, 5 min (Extraction 1)

Weight of CPH powder (sample) = 1.0068 gram

Volume of extract = 36 mL = 0.036 L

Weight of extract = 31.7653 gram

Dilution factor (DF) = 20

Measurement of UV/Vis Spectrophotometer (Extraction 1 – triplicate analysis):

Absorbance 1 = 0.650; Absorbance 2 = 0.654; Absorbance 3 = 0.656

Standard curve equation: $y = 0.0046x + 0.007$; $R^2 = 0.999$

Equation for calculation the TPC yield:

$$TPC = c \times \frac{V_{extract}}{W_{sample}}$$

Calculation the extracts' concentrations (Extraction 1 – triplicate analysis):

$$c = \frac{(A - 0.007)}{0.0046} \text{ x DF}$$

$$c_1 = \frac{(0.650 - 0.007)}{0.0046} \text{x} 20 = 2795.65 \text{ mg GAE/L}$$

$$c_2 = \frac{(0.654 - 0.007)}{0.0046} \text{x} 20 = 2813.04 \text{ mg GAE/L}$$

$$c_1 = \frac{(0.656 - 0.007)}{0.0046} \text{x} 20 = 2821.74 \text{ mg GAE/L}$$

Calculation the extracts' TPC (Extraction 1 – triplicate analysis):

$$TPC = c \text{ x } \frac{V_{extract}}{W_{sample}}$$

$$TPC_1 = 2795.65 \text{ mg GAE/L x} \frac{0.036 \text{ L}}{1.0068 \text{ g}} = 99.96 \text{ mg GAE/g dw}$$

$$TPC_2 = 2813.04 \text{ mg GAE/L x} \frac{0.036 \text{ L}}{1.0068 \text{ g}} = 100.59 \text{ mg GAE/g dw}$$

$$TPC_3 = 2821.74 \text{ mg GAE/L x} \frac{0.036 \text{ L}}{1.0068 \text{ g}} = 100.90 \text{ mg GAE/g dw}$$

Calculation of extracts' TPC (triplicate extraction, triplicate analysis) is presented in Table S.5.

Table S.5 Calculation of extracts' TPC

TPC									**Average TPC (mg GAE/g dw)**
Extraction 1			**Extraction 2**			**Extraction 3**			
1	2	3	1	2	3	1	2	3	
99.96	***100.59***	***100.90***	100.78	100.63	100.94	99.35	100.45	100.29	100.43±0.51

2. Determination of total monomeric anthocyanin (TMA) – an example data for MAE, 50% (v/v) aqueous ethanol, 50 °C, 5 min (Extraction 1)

Weight of CPH powder (sample) = 1.0068 gram

Volume of extract = 36 mL = 0.036 L

Weight of extract = 31.7653 gram

Dilution factor (DF) = 10

Measurement of UV/Vis Spectrophotometer (Extraction 1 – triplicate analysis):

Absorbance 1: A_{520nm} pH 1.0 = 0.275 A_{700nm} pH 1.0 = 0.116

A_{520nm} pH 4.5 = 0.137 A_{700nm} pH 4.5 = 0.040

Absorbance 2: A_{520nm} pH 1.0 = 0.258 A_{700nm} pH 1.0 = 0.103

A_{520nm} pH 4.5 = 0.138 A_{700nm} pH 4.5= 0.045

Absorbance 3: A_{520nm} pH 1.0 = 0.276 A_{700nm} pH 1.0 = 0.118

A_{520nm} pH 4.5 = 0.141 A_{700nm} pH 4.5 = 0.045

Equation for calculation the TMA yield:

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

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

MW = 449.2 g/mol; ε = 26900 L/cm.mol; l = 1 cm

Calculation the extracts' Absorbances (Extraction 1 – triplicate analysis):

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

$$A_1 = (0.275 - 0.116) - (0.137 - 0.040) = 0.159 - 0.097 = 0.062$$

$$A_2 = (0.258 - 0.103) - (0.138 - 0.045) = 0.155 - 0.093 = 0.062$$

$$A_3 = (0.276 - 0.118) - (0.141 - 0.045) = 0.158 - 0.096 = 0.062$$

Calculation the extracts' TMA (Extraction 1 – triplicate analysis):

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

$$TMA_1 = \frac{0.062 \times 449.2\ g/mol \times 10 \times 10^3 mg/g}{26900\ L/cm.mol \times 1\ cm} \text{ x } \frac{0.036\ L}{1.0068\ g} = 0.370\ mg\ Cy_3GE/g\ dw$$

$$TMA_2 = \frac{0.062 \times 449.2\ g/mol \times 10 \times 10^3 mg/g}{26900\ L/cm.mol \times 1\ cm} \text{ x } \frac{0.036\ L}{1.0068\ g} = 0.370\ mg\ Cy_3GE/g\ dw$$

$$TMA_3 = \frac{0.062 \times 449.2\ g/mol \times 10 \times 10^3 mg/g}{26900\ L/cm.mol \times 1\ cm} \text{ x } \frac{0.036\ L}{1.0068\ g} = 0.370\ mg\ Cy_3GE/g\ dw$$

Calculation of extracts' TMA (triplicate extraction, triplicate analysis) is presented in Table S.6.

Table S.6 Calculation of extracts' TMA

TMA									**Average TMA (mg Cy_3GE/g dw)**
Extraction 1			**Extraction 2**			**Extraction 3**			
1	2	3	1	2	3	1	2	3	
0.370	***0.370***	***0.370***	0.376	0.370	0.370	0.373	0.367	0.361	0.370±0.00

3. Determination of antioxidant activity (AOA) – an example data for MAE, 50%(v/v) aqueous ethanol, 50 °C, 5 min (Extraction 1)

Weight of CPH powder (sample) = 1.0068 gram

Volume of extract = 36 mL = 0.036 L

Weight of extract = 31.7653 gram

Measurement of UV/Vis Spectrophotometer (Extraction 1 – triplicate analysis):

Absorbance of DPPH control = 1.027

Absorbance 1 = 0.249; Absorbance 2 = 0.253; Absorbance 3 = 0.260

Standard curve equation: $y = 0.008x + 0.0231$; $R^2 = 0.996$

Equation for calculation the TPC yield:

$$A = A_{DPPHcontrol} - A_{sample}$$

$$AOA = \frac{c \text{ x } V_{extract}}{W_{sample}}$$

Calculation the extracts' concentrations (Extraction 1 – triplicate analysis):

$$c = \frac{(A_{DPPHcontrol} - A_{sample}) - 0.0231}{0.008}$$

$$c_1 = \frac{(1.027 - 0.249) - 0.0231}{0.008} = 94.36 \text{ mg TE/L}$$

$$c_2 = \frac{(1.027 - 0.253) - 0.0231}{0.008} = 93.86 \text{ mg TE/L}$$

$$c_3 = \frac{(1.027 - 0.260) - 0.0231}{0.008} = 92.99 \text{ mg TE/L}$$

Calculation the extracts' AOA (Extraction 1 – triplicate analysis):

$$AOA = c \text{ x } \frac{V_{extract}}{W_{sample}}$$

$$AOA_1 = 94.36 \text{ mg TE/L x} \frac{0.036 \text{ L}}{1.0068 \text{ g}} = 3.37 \text{ mg TE/g dw}$$

$$AOA_2 = 93.86 \text{ mg TE/L x} \frac{0.036 \text{ L}}{1.0068 \text{ g}} = 3.36 \text{ mg TE/g dw}$$

$$AOA_3 = 92.99 \text{ mg TE/L x} \frac{0.036 \text{ L}}{1.0068 \text{ g}} = 3.32 \text{ mg TE/g dw}$$

Calculation of extracts' TPC (triplicate extraction, triplicate analysis) is presented in Table S.7.

Table S.7 Calculation of extracts' AOA

AOA									**Average AOA (mg TE/g dw)**
Extraction 1			**Extraction 2**			**Extraction 3**			
1	2	3	1	2	3	1	2	3	
3.37	***3.36***	***3.32***	3.38	3.36	3.38	3.38	3.36	3.33	3.36±0.02

4. Conversion TPC value from mg GAE/g fresh weight to mg GAE/g dry weight (Chapter 4, Section 4.2)

Drying yield = 13.49 % w.b

TPC of fresh CPH extract = 29.02 mg GAE/g fresh weight

$$\text{TPC (mg GAE / g dried weight)} = \frac{100}{13.49} \text{x} 29.02 = 215.1 \text{ mg GAE / g dried weight}$$

Appendix C: Calculation of Hildebrand and Hansen Solubility Parameters

Appendix C.1: Calculation of Hildebrand Solubiity Parameter (HbSP)

HbSP value is calculated based on Equation 2.6:

$$\delta = \sqrt{\frac{\sum_i (\Delta e)_i}{\sum_i (\Delta v)_i}} \qquad [2.6]$$

Where $\sum_i (\Delta e)_i$ is the summation of cohesive energies (cal/mol), and $\sum_i (\Delta v)_i$ is the summation of molar volumes (cm^3/mol). The data of cohesive energies (Δe) and mlar volumes (Δv) are listed in Table C.1 below.

Table C.1 Atomic and Group Contributions to the Energy of Vaporisation and the Molar Volume at 25 °C (Fedors, 1974)

Atom or group	Δe_i, cal/mole	Δv_i, cm^3/mole
CH_3	1125	33.5
CH_2	1180	16.1
CH	820	−1.0
C	350	−19.2
$H_2C=$	1030	28.5
—CH=	1030	13.5
C=	1030	−5.5
HC≡	920	27.4
—C≡	1690	6.5
Phenyl*	7630	71.4
Phenylene (o, m, p)*	7630	52.4
Phenyl (trisubstituted)*	7630	33.4
Phenyl (tetrasubstituted)*	7630	14.4
Phenyl (pentasubstituted)*	7630	−4.6
Phenyl (hexasubstituted)*	7630	−23.6
Ring closure 5 or more atoms	250	16
Ring closure 3 or 4 atoms	750	18
Conjugation in ring for each double bond	400	−2.2
Halogen attached to carbon atom with double bond	−20 percent of Δe_i of halogen	4.0
CO_3 (carbonate)	4200	22.0
COOH	6600	28.5
CO_2	4300	18.0
CO	4150	10.8
CHO (aldehyde)	5100	22.3
CO_2CO_2 (oxalate)	6400	37.3
C_2O_3 (anhydride)	7300	30.0
HCOO (formate)	4300	32.5
$CONH_2$	10000	17.5
CONH	8000	9.5
CON	7050	−7.7
HCON	6600	11.3
HCONH	10500	27.0
COCl	5000	38.0
NH_2	3000	19.2
NH	2000	4.5
N	1000	−9.0
—N=	2800	5.0
CN	6100	24.0
NO_2 (aliphatic)	7000	24.0
NO_2 (aromatic)	3670	32.0
NO_3	5000	33.5
NO_2 (nitrite)	2800	33.5
SCN	4800	37.0
NCO	6800	35.0
NF_2	1830	33.1
NF	1210	24.5
O	800	3.8
OH	7120	10.0
OH (disubstituted or on adjacent C atoms)	5220	13.0
PO_4	5000	28.0
PO_3	3400	22.7
SH	3450	28.0
S	3380	12
S_2	5700	23.0
SO_3	4500	27.6
SO_4	6800	31.6
F	1000	18.0
F (disubstituted)	850	20.0
F (trisubstituted)	550	22.0
CF_2 (for perfluoro compounds)	1020	23.0
CF_3 (for perfluoro compounds)	1020	57.5

Atom or group	Δe_i, cal/mole	Δv_i, cm^3/mole
Cl	2760	24.0
Cl (disubstituted)	2300	26.0
Cl (trisubstituted)	1800	27.3
Br	3700	30.0
Br (disubstituted)	2950	31.0
Br (trisubstituted)	2550	32.4
I	4550	31.5
I (disubstituted)	4000	33.3
I (trisubstituted)	3900	37.0
B	3300	−2.0
Al	3300	−2.0
Ga	3300	−2.0
In	3300	−2.0
Tl	3300	−2.0
Si	810	0
Ge	1930	−1.5
Sn	2700	1.5
Pb	4100	2.5
P	2250	−1.0
As	3100	7.0
Sb	3900	8.9
Bi	5100	9.5
Se	4100	16.0
Te	4800	17.4
Zn	3460	2.5
Cd	4250	6.5
Hg	5450	7.5

* These values listed for convenience. The values reported for Δe_i and Δv_i can be evaluated from the appropriate entries in the table.

1. HbSP calculation of methanol solvent

Structure of methanol: $CH_3 – OH$

Functional group	Total functional group	Δe	ΔV	Σ Δe	Σ ΔV
-CH_3	1	990	34	990	34
-OH adjectent C	1	7830	8.7	7830	8.7
Total Δe or ΔV				8820	42.7
Δe/ΔV				206.56 cal/cm^3	
HbSP = $\sqrt{\Delta e/\Delta V}$				14.37 (cal/cm^3)$^{1/2}$	
HbSP	= 14.37 x 2.045			**29.4 Mpa**	

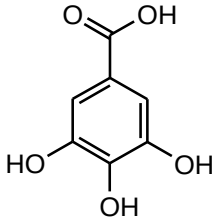
2. HbSP calculation of ethanol solvent

Structure of ethanol: $CH_3 – CH_2 – OH$

Functional group	Total functional group	Δe	ΔV	Σ Δe	Σ ΔV
-CH_3	1	990	34	990	34
-CH_2	1	1230	16.5	1230	16.5
-OH adjectent C	1	7830	8.7	7830	8.7
Total Δe or ΔV				10050	59.2
Δe/ΔV				169.76 cal/cm^3	
HbSP = $\sqrt{\Delta e/\Delta V}$				13.03 (cal/cm^3)$^{1/2}$	
HbSP	= 13.03 x 2.045			**26.6 Mpa**	

3. Calculation of HbSP of gallic acid

Structure of gallic acid:



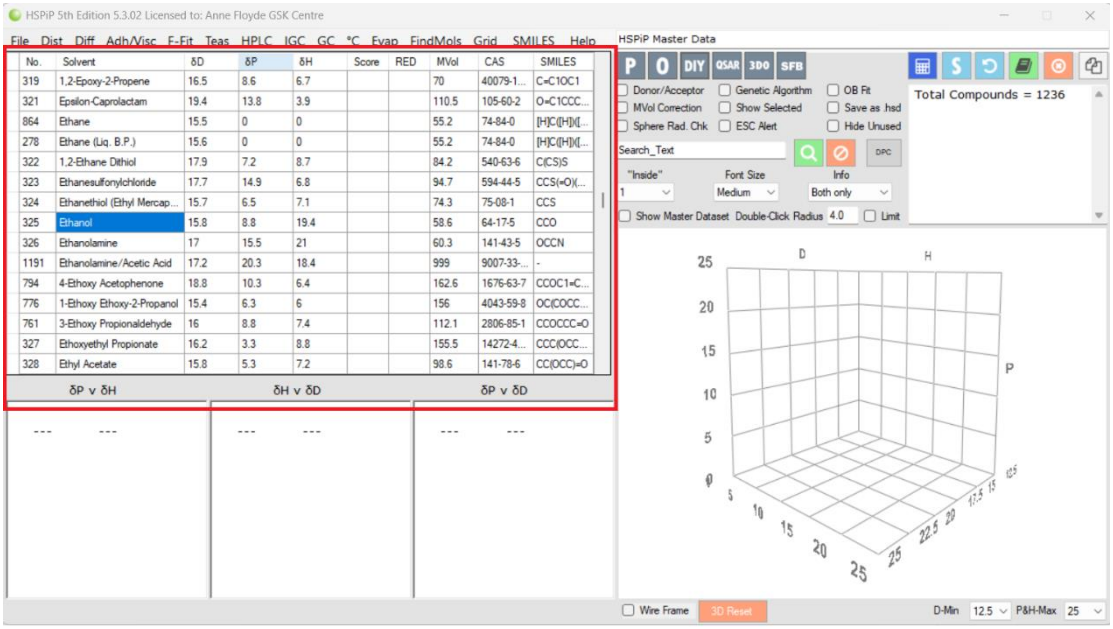
gallic acid

Functional group	Total functional group	Δe	ΔV	Σ Δe	Σ ΔV
-COOH	1	6600	28.5	6600	28.5
>C=	4	1030	-5.5	4120	-22
-CH=	2	1030	13.5	2060	27
-OH adjectent C	3	5220	13.0	15660	39
Conjugation ring	3	400	-2.2	1200	-6.6
Ring closure 5 or more	1	250	16.0	250	16
4. s	5.	6.	7.	8. 29890	9. 81.9
Δe/ΔV				364.98 cal/cm^3	
HbSP = $\sqrt{\Delta e/\Delta V}$				19.10 (cal/cm^3)$^{1/2}$	
HbSP	= 19.10 x 2.045			**39.1 Mpa**	

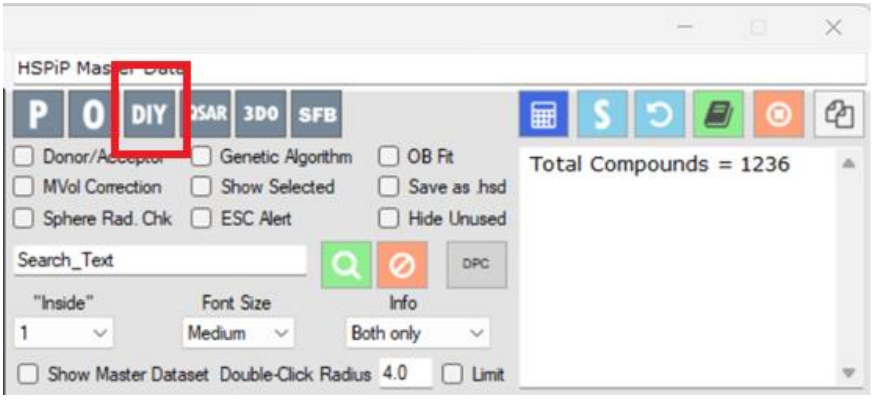
Appendix C.2: Calculation of Hnsen Solubility Parameter (HSP) using HSPiP software

HSP of each compound was calculated using HSPiP software by the following steps:

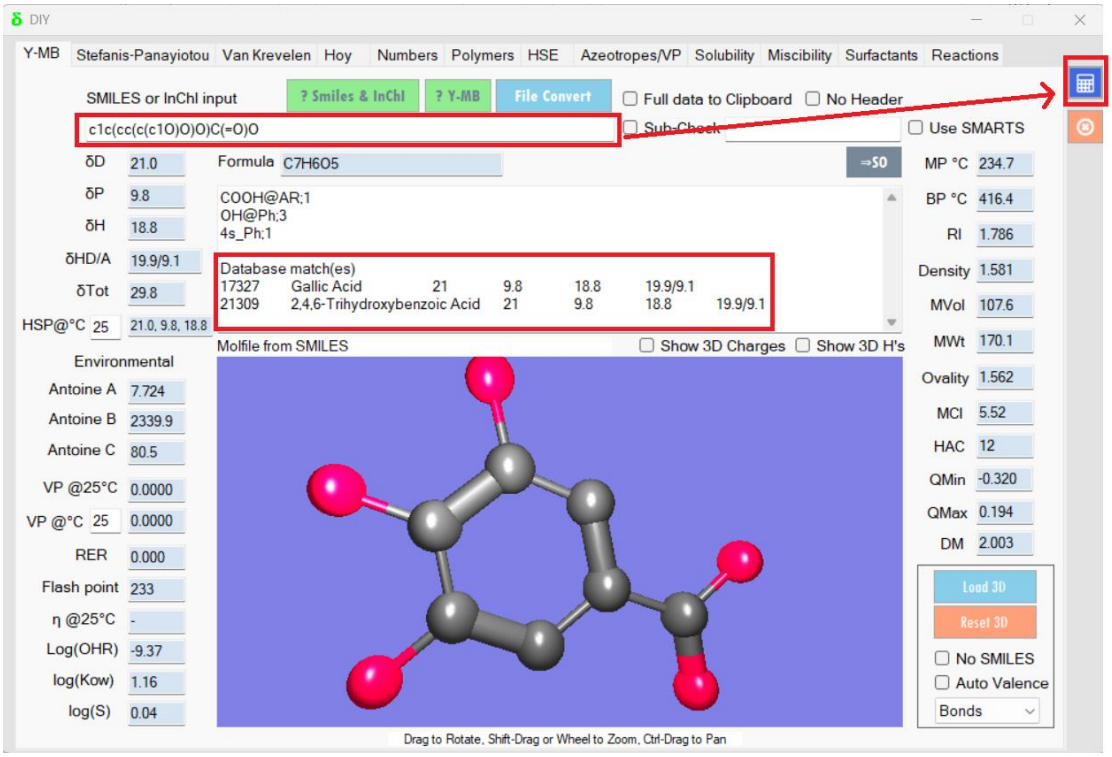
1. Open the software and find the solvent or compounds in the list (the list provides the information of general compound or solvent)



2. If the compound is not present in the list, calculate the HSP value by clicking the "DIY" and input the SMILES (Simplified Molecular Input Line Entry System) or InChl notations. SMILES or InChl notations can be found in ChemSpider.



For example, gallic acid has SMILES notation: c1c(cc(c(c1O)O)O)C(=O)O. The, input the SMILES notation and click "calculate".

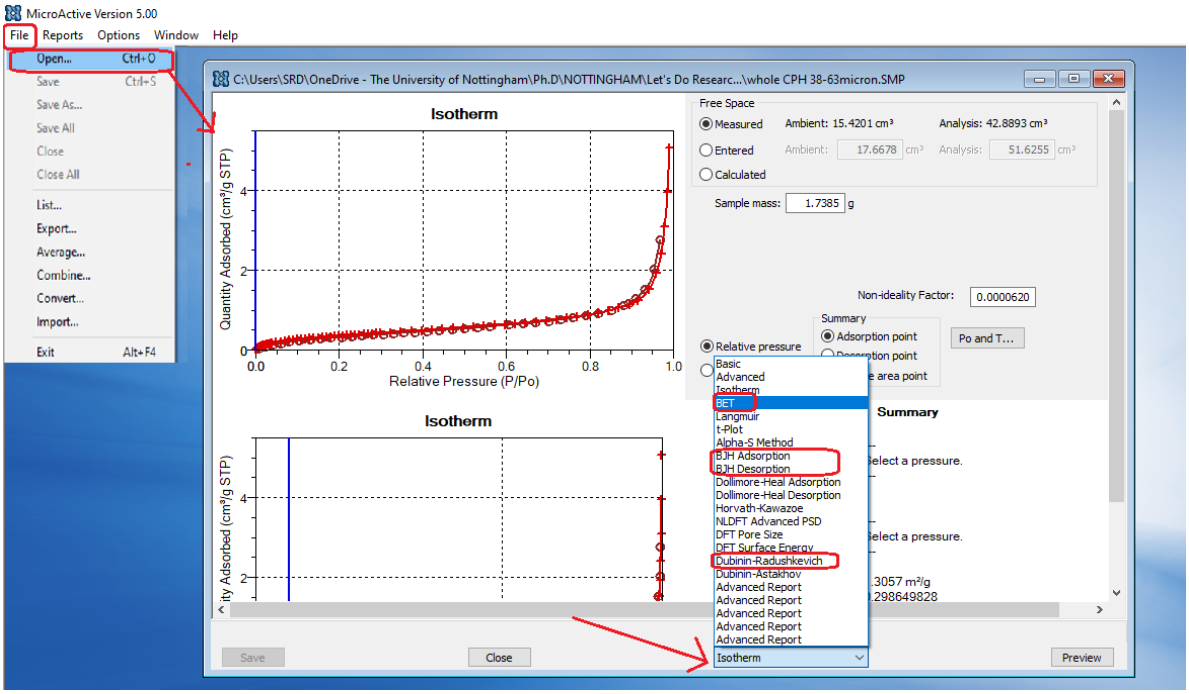


3. To calculate the HSP of each compound at different temperature, change the temperature.

Appendix D: Calculation of BET surface area and pore characteristics

Calculation of BET surface area and pore characteristics using MicroActive Software Version 5.0 – an example data for untreated CPH 38 -63 micron (Measurement 1):

1. BET Surface area: the specific surface area was measured by applying BET model in a relative pressure range of 0.05 – 0.20
2. Vmicro: micropore volume (<2 nm) was measured by Dubinin-Radeskevich (DR) model
3. Vmeso: mesopore volume (2 – 50 nm) was measured by BJH model (Harkins Jura correction – standard) at pore diameter 2 – 50 nm = Pore Volume of BJH (2 – 50 nm)
4. Vtotal: total pore volume by calculating DR and BJH models up to 220 nm = Pore Volume of BJH (2 – 400 nm) + Vmicro
5. Average pore diameter = $\frac{4xV_{total}}{BET_{SA}}$



Results from MicroActive Software:

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Full Report Set

Sample: whole CPH 38-63micron
Operator: Lee Stevens
Submitter:
Bar Code:
File: C:\Users\SRD\OneDrive - T...\whole CPH 38-63micron.SMP

Started: 29/02/2020 16:39:40
Completed: 01/03/2020 03:31:36
Report time: 18/10/2022 09:50:41
Sample mass: 1.7385 g
Analysis free space: 42.8893 cm³
Low pressure dose: None
Automatic degas: Yes

Analysis adsorptive: N2
Analysis bath temp.: -195.850 °C
Thermal correction: No
Ambient free space: 15.4201 cm³ Measured
Equilibration interval: 30 s
Sample density: 1.000 g/cm³

Summary Report

Surface Area

Single point surface area at P/Po = 0.199004083: 1.2403 m²/g

BET Surface Area: 1.4578 m²/g

Pore Volume

BJH Adsorption cumulative volume of pores between 2.0000 nm and 400.0000 nm diameter: 0.007877 cm³/g

BJH Desorption cumulative volume of pores between 2.0000 nm and 400.0000 nm diameter: 0.004407 cm³/g

Pore Size

BJH Adsorption average pore diameter (4V/A): 23.1263 nm

BJH Desorption average pore diameter (4V/A): 12.0843 nm

Dubinin-Radushkevich

Micropore surface area: 1.1095 m²/g

Monolayer capacity: 0.254898 cm³/g

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Full Report Set

Sample: whole CPH 38-63micron
Operator: Lee Stevens
Submitter:
Bar Code:
File: C:\Users\SRD\OneDrive - T...\whole CPH 38-63micron.SMP

Started:	29/02/2020 16:39:40	Analysis adsorptive:	N2
Completed:	01/03/2020 03:31:36	Analysis bath temp.:	-195.850 °C
Report time:	18/10/2022 09:55:46	Thermal correction:	No
Sample mass:	1.7385 g	Ambient free space:	15.4201 cm³ Measured
Analysis free space:	42.8893 cm³	Equilibration interval:	30 s
Low pressure dose:	None	Sample density:	1.000 g/cm³
Automatic degas:	Yes		

Summary Report

Surface Area

Single point surface area at P/Po = 0.199004083: 1.2403 m²/g

BET Surface Area: 1.4578 m²/g

Pore Volume

BJH Adsorption cumulative volume of pores between 2.0000 nm and 50.0000 nm diameter: 0.002632 cm³/g

BJH Desorption cumulative volume of pores between 2.0000 nm and 50.0000 nm diameter: 0.003112 cm³/g

Pore Size

BJH Adsorption average pore diameter (4V/A): 9.2400 nm

BJH Desorption average pore diameter (4V/A): 9.1781 nm

Dubinin-Radushkevich

Micropore surface area: 1.1095 m²/g

Monolayer capacity: 0.254898 cm³/g

Sample: whole CPH 38-63micron
Operator: Lee Stevens
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Bar Code:
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Low pressure dose: None
Automatic degas: Yes

Analysis adsorptive: N2
Analysis bath temp.: -195.850 °C
Thermal correction: No
Ambient free space: 15.4201 cm³ Measured
Equilibration interval: 30 s
Sample density: 1.000 g/cm³

Isotherm Tabular Report

Relative Pressure (P/Po)	Absolute Pressure (mmHg)	Quantity Adsorbed (cm³/g STP)	Elapsed Time (h:min)	Saturation Pressure (mmHg)
			01:21	738.326599
0.000936582	0.691454	0.0199	01:53	738.273621
0.003050021	2.251896	0.0429	02:02	738.321411
0.005540337	4.089542	0.0605	02:11	738.139587
0.007244924	5.346825	0.0701	02:17	738.009766
0.010162878	7.500092	0.0842	02:24	737.988953
0.012308172	9.082336	0.0942	02:30	737.911072
0.014973308	11.048721	0.1056	02:35	737.894470
0.017359278	12.810577	0.1148	02:40	737.967163
0.019908729	14.689754	0.1229	02:44	737.854980
0.022388235	16.523247	0.1296	02:49	738.032593
0.024862707	18.347397	0.1367	02:54	737.948486
0.027376044	20.197502	0.1439	02:58	737.780151
0.029867687	22.034111	0.1504	03:03	737.724060
0.032366036	23.880062	0.1567	03:08	737.812378
0.034833304	25.697983	0.1623	03:12	737.741760
0.037317616	27.533554	0.1685	03:17	737.816528
0.039805123	29.369125	0.1736	03:22	737.822754
0.049598503	36.593048	0.1936	03:26	737.785339
0.059765553	44.092102	0.2115	03:31	737.751099
0.069487972	51.269302	0.2269	03:35	737.815491
0.079635997	58.762131	0.2415	03:40	737.884033
0.089543830	66.058731	0.2549	03:45	737.725098
0.099500000	73.413467	0.2664	03:49	737.823792
0.109594037	80.870995	0.2771	03:53	737.914185
0.119580750	88.247536	0.2873	03:57	737.974426
0.129484604	95.556595	0.2965	04:01	737.976501
0.139331118	102.835541	0.3058	04:04	738.065857
0.149303215	110.192352	0.3152	04:08	738.044067
0.159233063	117.540863	0.3240	04:12	738.168701
0.169193957	124.895615	0.3319	04:16	738.180115
0.179150186	132.248276	0.3394	04:20	738.197815
0.189082278	139.579132	0.3480	04:23	738.192566
0.199004083	146.900650	0.3558	04:27	738.179077
0.208972195	154.254349	0.3628	04:31	738.157288
0.218875436	161.575867	0.3701	04:35	738.209229
0.228852863	168.950333	0.3774	04:38	738.248718
0.238831677	176.309235	0.3845	04:42	738.215454
0.248739720	183.629700	0.3918	04:46	738.240356
0.258654165	190.992752	0.3989	04:50	738.409729
0.268695278	198.392136	0.4061	04:53	738.353638

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Full Report Set

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Report time: 18/10/2022 09:50:41
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Automatic degas: Yes

Analysis adsorptive: N2
Analysis bath temp.: -195.850 °C
Thermal correction: No
Ambient free space: 15.4201 cm³ Measured
Equilibration interval: 30 s
Sample density: 1.000 g/cm³

Isotherm Tabular Report

Relative Pressure (P/Po)	Absolute Pressure (mmHg)	Quantity Adsorbed (cm³/g STP)	Elapsed Time (h:min)	Saturation Pressure (mmHg)
0.278564687	205.690811	0.4133	04:57	738.395142
0.288510873	213.063202	0.4200	05:01	738.492798
0.298649828	220.529037	0.4277	05:05	738.420105
0.323357040	238.797516	0.4435	05:08	738.494873
0.348206402	257.134552	0.4602	05:11	738.454407
0.373018014	275.494385	0.4770	05:14	738.555176
0.398071503	293.958069	0.4938	05:16	738.455444
0.422652279	312.155975	0.5108	05:19	738.564514
0.447575398	330.538666	0.5283	05:22	738.509460
0.472431099	348.902679	0.5460	05:25	738.526062
0.497270029	367.228271	0.5638	05:28	738.488647
0.522039338	385.555939	0.5834	05:31	738.557251
0.547041255	404.001953	0.6025	05:34	738.521912
0.571782843	422.265259	0.6229	05:37	738.506348
0.596611406	440.610596	0.6441	05:40	738.521912
0.621370576	458.964203	0.6666	05:43	738.632019
0.646441831	477.440338	0.6901	05:46	738.566589
0.671195180	495.720245	0.7148	05:49	738.563477
0.696059268	514.123718	0.7417	05:52	738.620605
0.720982282	532.518860	0.7713	05:55	738.601868
0.745772036	550.840271	0.8035	05:58	738.617493
0.770603973	569.223999	0.8386	06:01	738.672546
0.795384676	587.565186	0.8815	06:04	738.718262
0.820424625	606.041321	0.9291	06:06	738.692261
0.845091621	624.260010	0.9850	06:09	738.689148
0.869905344	642.575195	1.0552	06:12	738.672546
0.894544899	660.798035	1.1469	06:15	738.697449
0.914211853	675.382935	1.2537	06:18	738.759766
0.941326970	695.400818	1.5145	06:23	738.745239
0.959756723	709.109436	1.9257	06:29	738.842896
0.970950521	717.343567	2.4199	06:35	738.805481
0.978970519	723.327820	3.0899	06:43	738.865784
0.985004759	727.878357	3.9501	06:51	738.959229
0.989782176	731.341858	5.0808	07:01	738.891724
			07:07	738.955078
0.969460436	716.502563	2.7640	07:33	739.073547
0.954574229	705.455933	2.0325	07:40	739.026794
0.932331668	689.064575	1.5332	07:47	739.076660
0.910734002	673.062500	1.2913	07:51	739.033020
0.892732713	659.856323	1.1698	07:54	739.142090
0.881170084	651.326355	1.1098	07:57	739.160767

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Full Report Set

Sample: whole CPH 38-63micron
Operator: Lee Stevens
Submitter:
Bar Code:
File: C:\Users\SRD\OneDrive - T...\whole CPH 38-63micron.SMP

Started: 29/02/2020 16:39:40
Completed: 01/03/2020 03:31:36
Report time: 18/10/2022 09:50:41
Sample mass: 1.7385 g
Analysis free space: 42.8893 cm³
Low pressure dose: None
Automatic degas: Yes

Analysis adsorptive: N2
Analysis bath temp.: -195.850 °C
Thermal correction: No
Ambient free space: 15.4201 cm³ Measured
Equilibration interval: 30 s
Sample density: 1.000 g/cm³

Isotherm Tabular Report

Relative Pressure (P/Po)	Absolute Pressure (mmHg)	Quantity Adsorbed (cm³/g STP)	Elapsed Time (h:min)	Saturation Pressure (mmHg)
0.850815982	628.862427	0.9972	08:00	739.128601
0.820660723	606.530334	0.9172	08:03	739.075623
0.790729668	584.389282	0.8546	08:06	739.050659
0.760332729	561.878601	0.8040	08:09	738.990417
0.730571333	539.902649	0.7601	08:12	739.014282
0.700448050	517.670227	0.7223	08:15	739.055847
0.670222086	495.343414	0.6879	08:18	739.073547
0.640434305	473.278137	0.6563	08:20	738.995605
0.605817797	447.671509	0.6239	08:23	738.954041
0.560851570	414.470337	0.5834	08:26	739.001831
0.530656289	392.145508	0.5570	08:30	738.982117
0.500535906	369.917236	0.5325	08:33	739.042358
0.470564031	347.748169	0.5078	08:36	739.002869
0.440453643	325.538605	0.4854	08:39	739.098450
0.410570652	303.438049	0.4635	08:42	739.064148
0.380497516	281.224335	0.4418	08:45	739.096375
0.355436821	262.692108	0.4237	08:48	739.068359
0.330389231	244.207672	0.4060	08:51	739.151428
0.305611960	225.870667	0.3881	08:54	739.076660
0.280489613	207.304199	0.3699	08:57	739.079773
0.255458198	188.801071	0.3515	09:00	739.068359
0.230352261	170.264725	0.3331	09:03	739.149353
0.205326677	151.763672	0.3138	09:06	739.132751
0.180298474	133.264694	0.2936	09:10	739.133789
0.155278297	114.766754	0.2719	09:13	739.103638
0.130270370	96.290611	0.2476	09:16	739.159729
0.105313575	77.837318	0.2217	09:19	739.100525
0.080406305	59.426590	0.1909	09:21	739.078735
0.059899952	44.271713	0.1598	09:24	739.094299
0.040260647	29.757420	0.1225	09:28	739.119263
0.024532005	18.132484	0.0823	09:33	739.135864
0.008337487	6.162865	0.0205	09:43	739.175354

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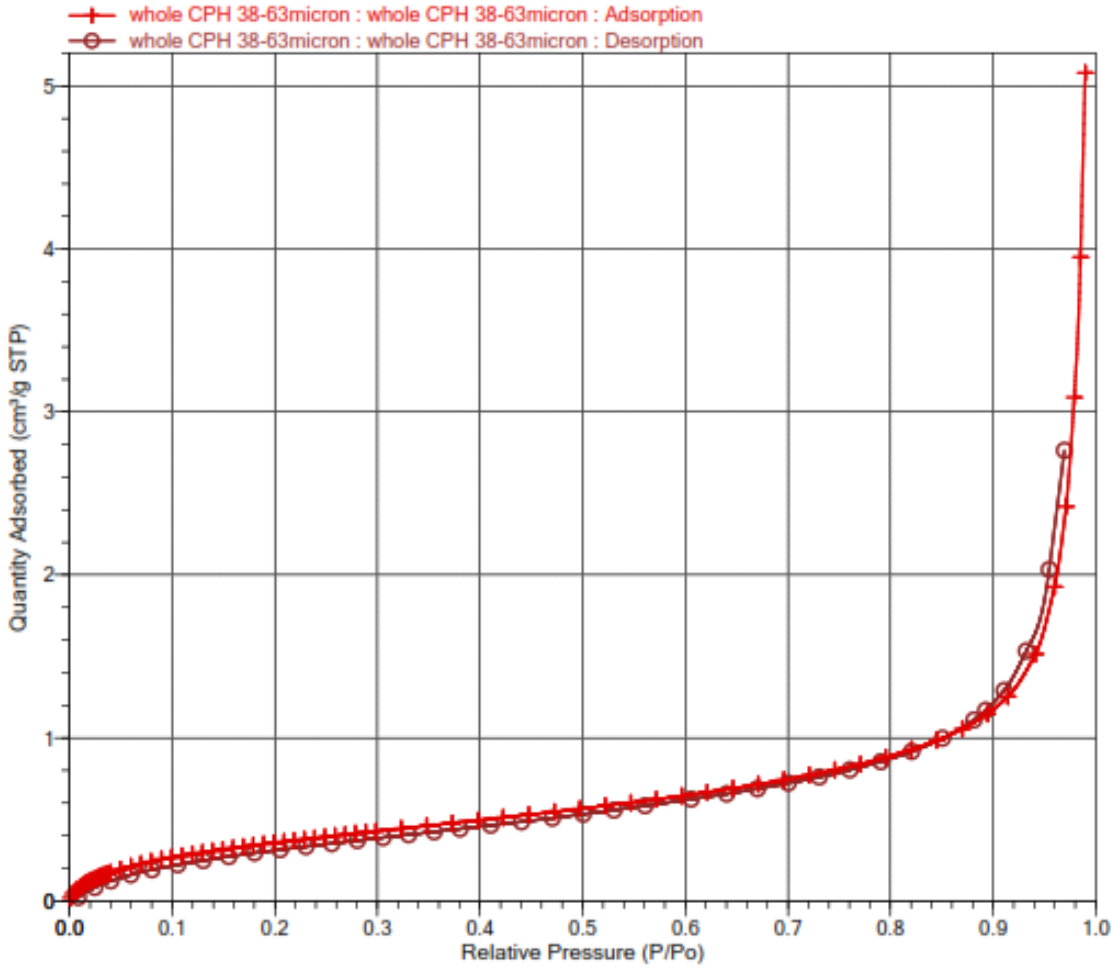
Full Report Set

Sample: whole CPH 38-63micron
Operator: Lee Stevens
Submitter:
Bar Code:
File: C:\Users\SRD\OneDrive - T...\whole CPH 38-63micron.SMP

Started:	29/02/2020 16:39:40	Analysis adsorptive:	N2
Completed:	01/03/2020 03:31:36	Analysis bath temp.:	-195.850 °C
Report time:	18/10/2022 09:50:41	Thermal correction:	No
Sample mass:	1.7385 g	Ambient free space:	15.4201 cm³ Measured
Analysis free space:	42.8893 cm³	Equilibration interval:	30 s
Low pressure dose:	None	Sample density:	1.000 g/cm³
Automatic degas:	Yes		

Isotherm Linear Plot



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Thermal correction: No
Ambient free space: 15.4201 cm³ Measured
Equilibration interval: 30 s
Sample density: 1.000 g/cm³

BET Report

BET surface area: 1.4578 ± 0.0020 m²/g
Slope: 2.855017 ± 0.004032 g/cm³ STP
Y-intercept: 0.130771 ± 0.000550 g/cm³ STP
C: 22.832197
Qm: 0.3349 cm³/g STP
Correlation coefficient: 0.9999870
Molecular cross-sectional area: 0.1620 nm²

Relative Pressure (P/Po)	Quantity Adsorbed (cm³/g STP)	1/[Q(Po/P - 1)]
0.059765553	0.2115	0.300522
0.069487972	0.2269	0.329125
0.079635997	0.2415	0.358327
0.089543830	0.2549	0.385785
0.099500000	0.2664	0.414800
0.109594037	0.2771	0.444140
0.119580750	0.2873	0.472754
0.129484604	0.2965	0.501637
0.139331118	0.3058	0.529370
0.149303215	0.3152	0.556899
0.159233063	0.3240	0.584460
0.169193957	0.3319	0.613601
0.179150186	0.3394	0.642968
0.189082278	0.3480	0.670088
0.199004083	0.3558	0.698365

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Full Report Set

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Started:	29/02/2020 16:39:40	Analysis adsorptive:	N2
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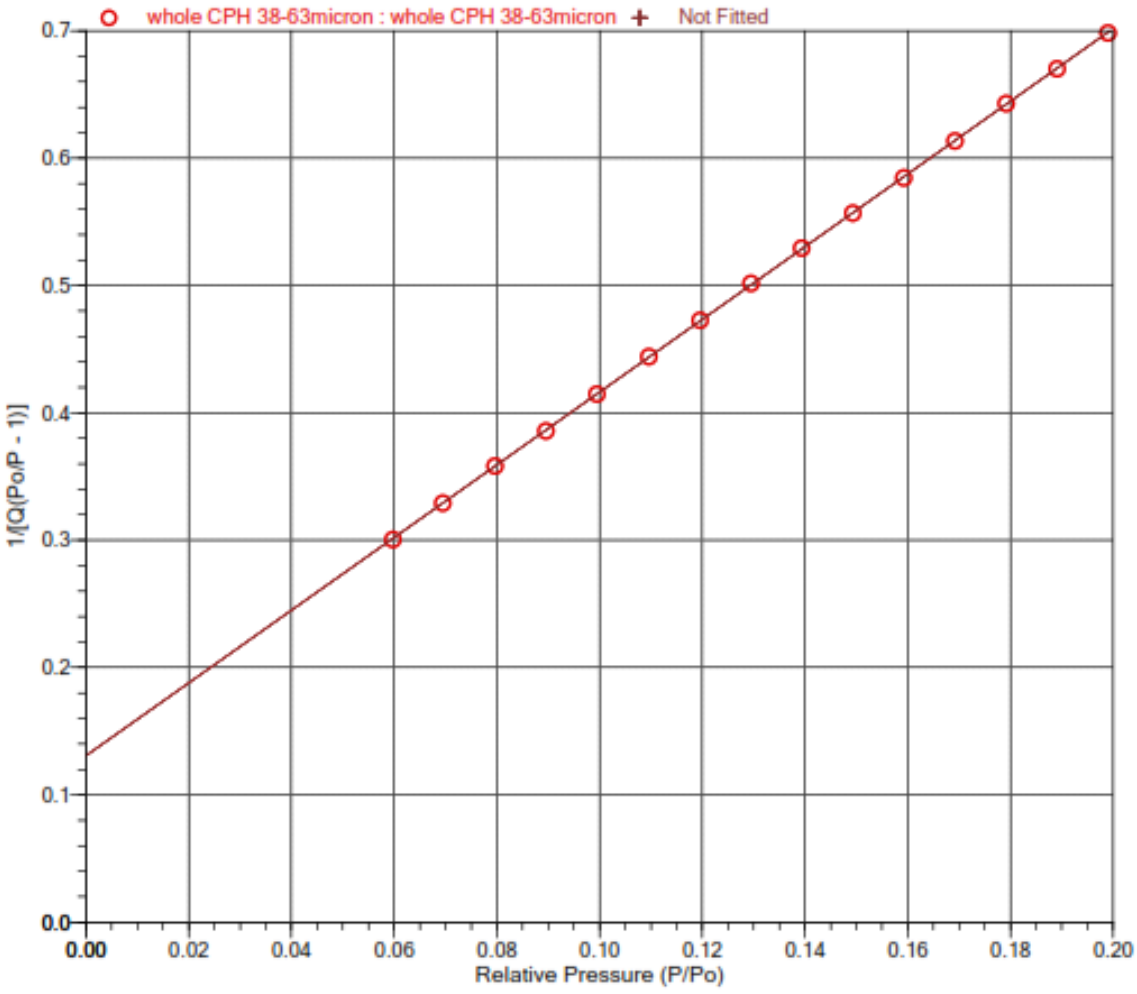
Report time:	18/10/2022 09:50:41	Thermal correction:	No
Sample mass:	1.7385 g	Ambient free space:	15.4201 cm³ Measured
Analysis free space:	42.8893 cm³	Equilibration interval:	30 s
Low pressure dose:	None	Sample density:	1.000 g/cm³
Automatic degas:	Yes		

BET Surface Area Plot



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Ambient free space: 15.4201 cm³ Measured
Equilibration interval: 30 s
Sample density: 1.000 g/cm³

BJH Adsorption Pore Distribution Report

Standard
Harkins and Jura
t = [13.99 / (0.034 - log(P/Po))] ^ 0.5

Diameter range: 2.0000 to 400.0000 nm
Adsorbate property factor: 0.95300 nm
Density conversion factor: 0.0015468
Fraction of pores open at both ends: 0.00

Pore Diameter Range (nm)	Average Diameter (nm)	Incremental Pore Volume (cm³/g)	Cumulative Pore Volume (cm³/g)	Incremental Pore Area (m²/g)	Cumulative Pore Area (m²/g)
189.4 - 129.9	148.7	0.001841	0.001841	0.050	0.050
129.9 - 93.3	105.6	0.001424	0.003265	0.054	0.103
93.3 - 68.1	76.7	0.001130	0.004396	0.059	0.162
68.1 - 49.7	56.0	0.000850	0.005245	0.061	0.223
49.7 - 34.6	39.4	0.000721	0.005966	0.073	0.296
34.6 - 24.0	27.3	0.000455	0.006421	0.067	0.363
24.0 - 19.7	21.4	0.000179	0.006600	0.033	0.396
19.7 - 16.1	17.5	0.000149	0.006749	0.034	0.430
16.1 - 13.6	14.6	0.000112	0.006861	0.031	0.461
13.6 - 11.8	12.6	0.000087	0.006948	0.028	0.489
11.8 - 10.4	11.0	0.000073	0.007021	0.027	0.515
10.4 - 9.3	9.7	0.000068	0.007089	0.028	0.543
9.3 - 8.4	8.8	0.000052	0.007141	0.024	0.567
8.4 - 7.6	7.9	0.000049	0.007190	0.025	0.592
7.6 - 7.0	7.3	0.000045	0.007235	0.025	0.616
7.0 - 6.4	6.7	0.000041	0.007275	0.024	0.641
6.4 - 6.0	6.2	0.000037	0.007312	0.024	0.665
6.0 - 5.5	5.7	0.000036	0.007348	0.025	0.690
5.5 - 5.2	5.3	0.000035	0.007383	0.026	0.716
5.2 - 4.8	5.0	0.000033	0.007416	0.026	0.742
4.8 - 4.5	4.7	0.000032	0.007448	0.028	0.770
4.5 - 4.3	4.4	0.000029	0.007477	0.027	0.797
4.3 - 4.0	4.1	0.000032	0.007510	0.031	0.828
4.0 - 3.8	3.9	0.000028	0.007537	0.028	0.856
3.8 - 3.6	3.7	0.000029	0.007566	0.031	0.887
3.6 - 3.4	3.5	0.000029	0.007595	0.033	0.920
3.4 - 3.2	3.3	0.000028	0.007623	0.034	0.955
3.2 - 3.0	3.1	0.000027	0.007650	0.035	0.990
3.0 - 2.9	2.9	0.000028	0.007679	0.039	1.028
2.9 - 2.7	2.8	0.000028	0.007707	0.040	1.069
2.7 - 2.6	2.6	0.000025	0.007732	0.038	1.107
2.6 - 2.5	2.5	0.000015	0.007747	0.024	1.131
2.5 - 2.5	2.5	0.000011	0.007758	0.018	1.149

Sample: whole CPH 38-63micron
Operator: Lee Stevens
Submitter:
Bar Code:
File: C:\Users\SRD\OneDrive - T...\whole CPH 38-63micron.SMP

Started: 29/02/2020 16:39:40
Completed: 01/03/2020 03:31:36
Report time: 18/10/2022 09:50:41
Sample mass: 1.7385 g
Analysis free space: 42.8893 cm³
Low pressure dose: None
Automatic degas: Yes

Analysis adsorptive: N2
Analysis bath temp.: -195.850 °C
Thermal correction: No
Ambient free space: 15.4201 cm³ Measured
Equilibration interval: 30 s
Sample density: 1.000 g/cm³

Pore Diameter Range (nm)	Average Diameter (nm)	Incremental Pore Volume (cm³/g)	Cumulative Pore Volume (cm³/g)	Incremental Pore Area (m²/g)	Cumulative Pore Area (m²/g)
2.5 - 2.4	2.4	0.000013	0.007772	0.022	1.171
2.4 - 2.4	2.4	0.000014	0.007785	0.023	1.193
2.4 - 2.3	2.3	0.000013	0.007798	0.022	1.215
2.3 - 2.3	2.3	0.000014	0.007812	0.024	1.239
2.3 - 2.2	2.2	0.000012	0.007824	0.022	1.261
2.2 - 2.2	2.2	0.000013	0.007837	0.024	1.286
2.2 - 2.1	2.1	0.000013	0.007851	0.025	1.311
2.1 - 2.1	2.1	0.000012	0.007862	0.022	1.333
2.1 - 2.0	2.0	0.000015	0.007877	0.029	1.362

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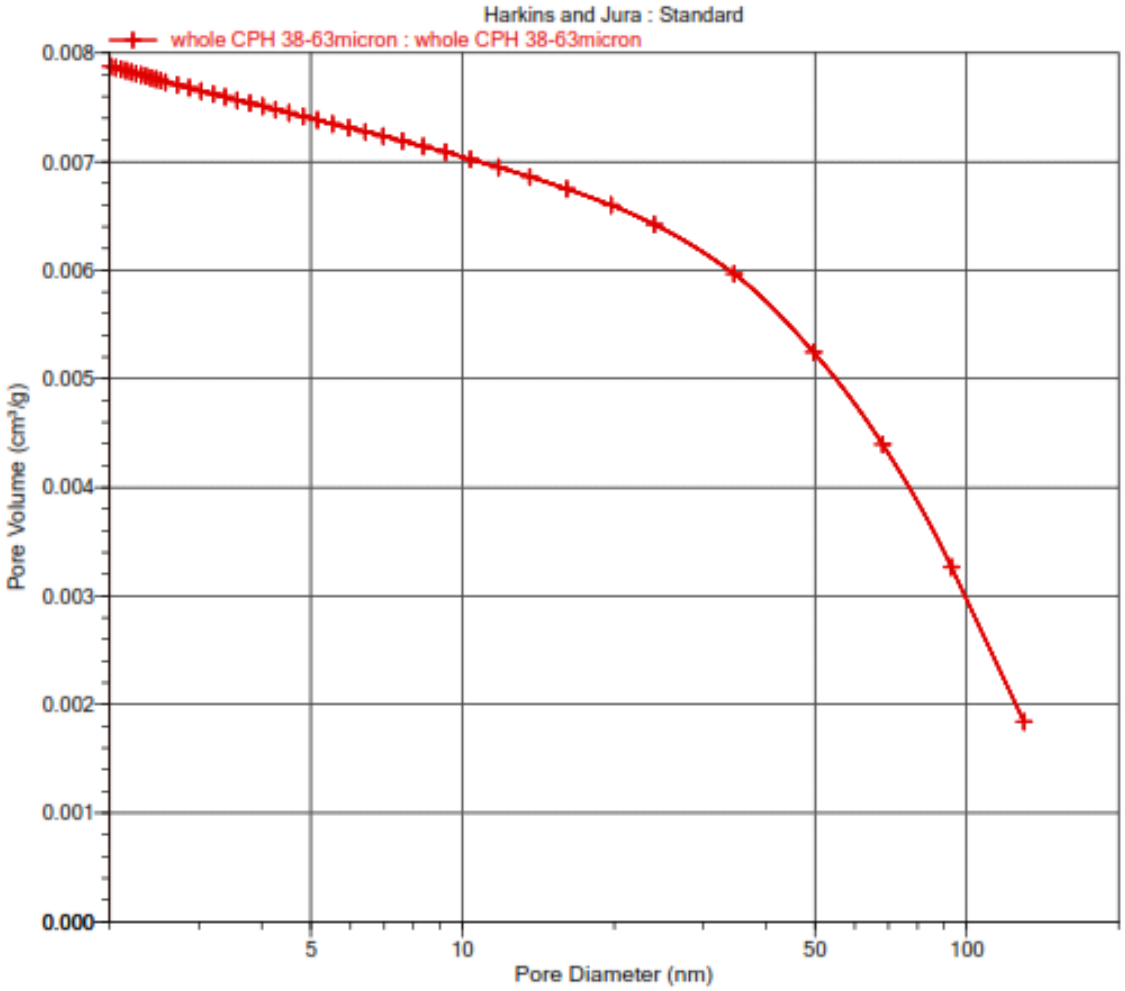
Full Report Set

Sample: whole CPH 38-63micron
Operator: Lee Stevens
Submitter:
Bar Code:
File: C:\Users\SRD\OneDrive - T...\whole CPH 38-63micron.SMP

Started: 29/02/2020 16:39:40
Completed: 01/03/2020 03:31:36
Report time: 18/10/2022 09:50:41
Sample mass: 1.7385 g
Analysis free space: 42.8893 cm³
Low pressure dose: None
Automatic degas: Yes

Analysis adsorptive: N2
Analysis bath temp.: -195.850 °C
Thermal correction: No
Ambient free space: 15.4201 cm³ Measured
Equilibration interval: 30 s
Sample density: 1.000 g/cm³

BJH Adsorption Cumulative Pore Volume (Larger)



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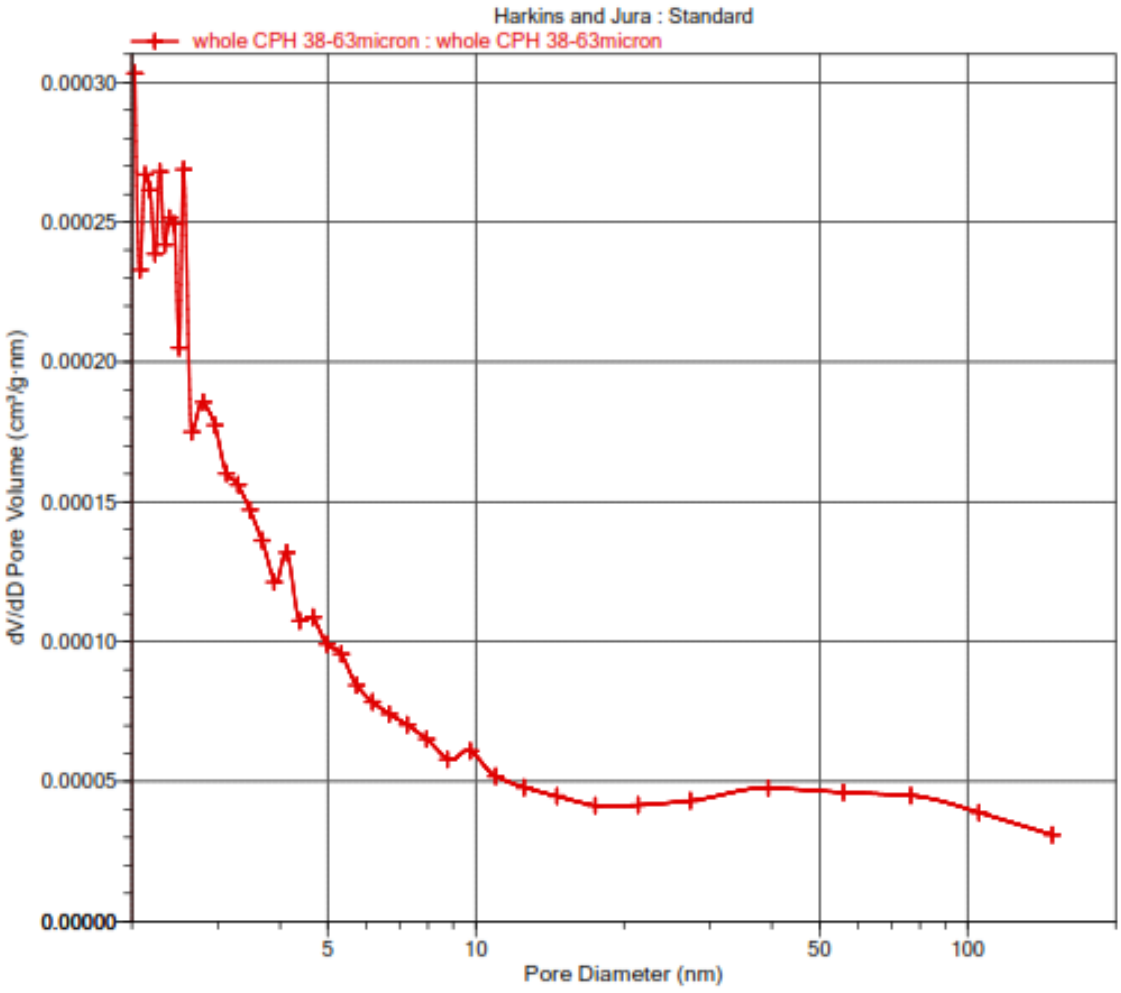
Full Report Set

Sample: whole CPH 38-63micron
Operator: Lee Stevens
Submitter:
Bar Code:
File: C:\Users\SRD\OneDrive - T...\whole CPH 38-63micron.SMP

Started: 29/02/2020 16:39:40
Completed: 01/03/2020 03:31:36
Report time: 18/10/2022 09:50:41
Sample mass: 1.7385 g
Analysis free space: 42.8893 cm³
Low pressure dose: None
Automatic degas: Yes

Analysis adsorptive: N2
Analysis bath temp.: -195.850 °C
Thermal correction: No
Ambient free space: 15.4201 cm³ Measured
Equilibration interval: 30 s
Sample density: 1.000 g/cm³

BJH Adsorption dV/dD Pore Volume



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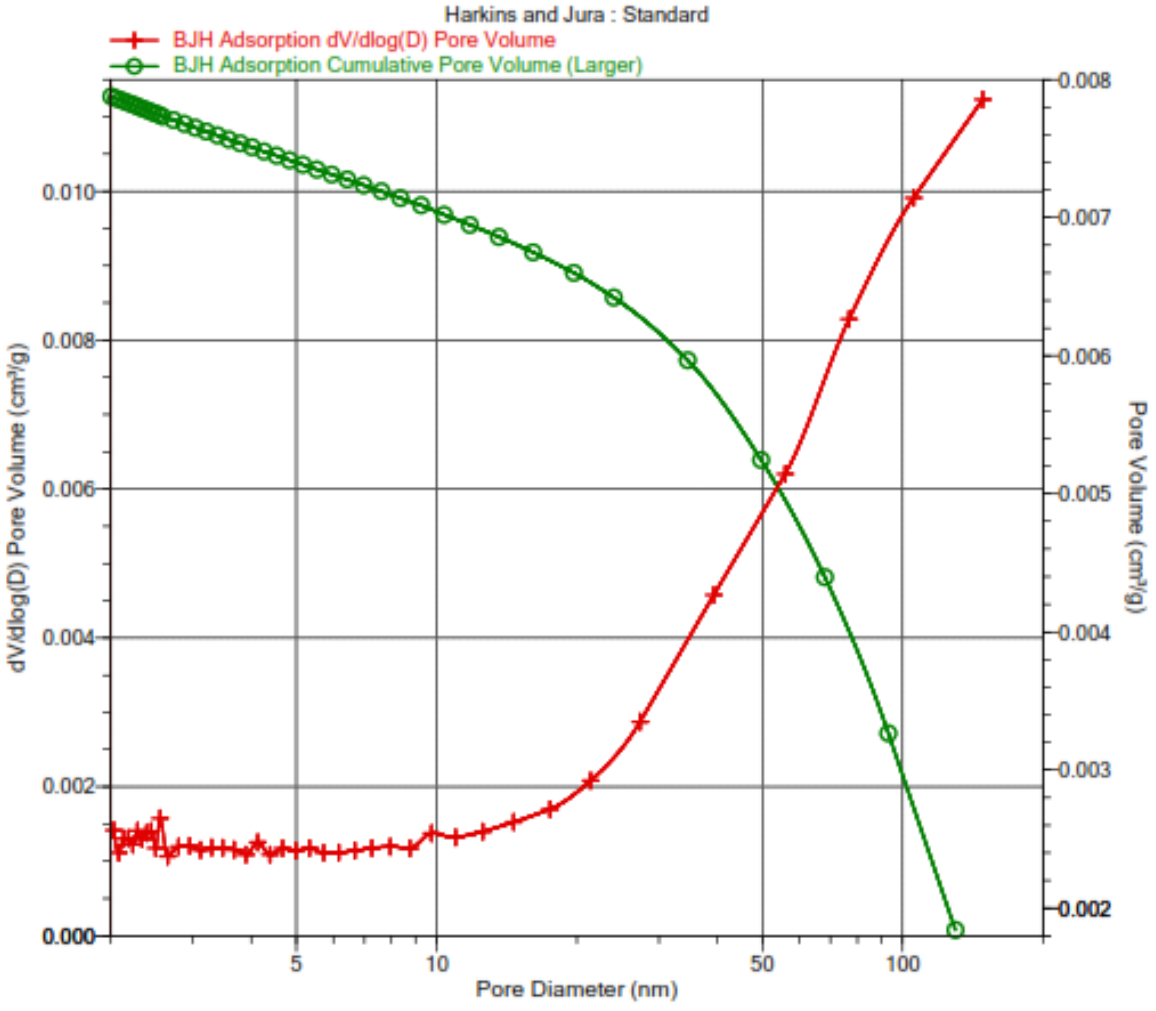
Full Report Set

Sample: whole CPH 38-63micron
Operator: Lee Stevens
Submitter:
Bar Code:
File: C:\Users\SRD\OneDrive - T...\whole CPH 38-63micron.SMP

Started:	29/02/2020 16:39:40	Analysis adsorptive:	N2
Completed:	01/03/2020 03:31:36	Analysis bath temp.:	-195.850 °C
Report time:	18/10/2022 09:50:41	Thermal correction:	No
Sample mass:	1.7385 g	Ambient free space:	15.4201 cm³ Measured
Analysis free space:	42.8893 cm³	Equilibration interval:	30 s
Low pressure dose:	None	Sample density:	1.000 g/cm³
Automatic degas:	Yes		

BJH Adsorption dV/dlog(D) Pore Volume



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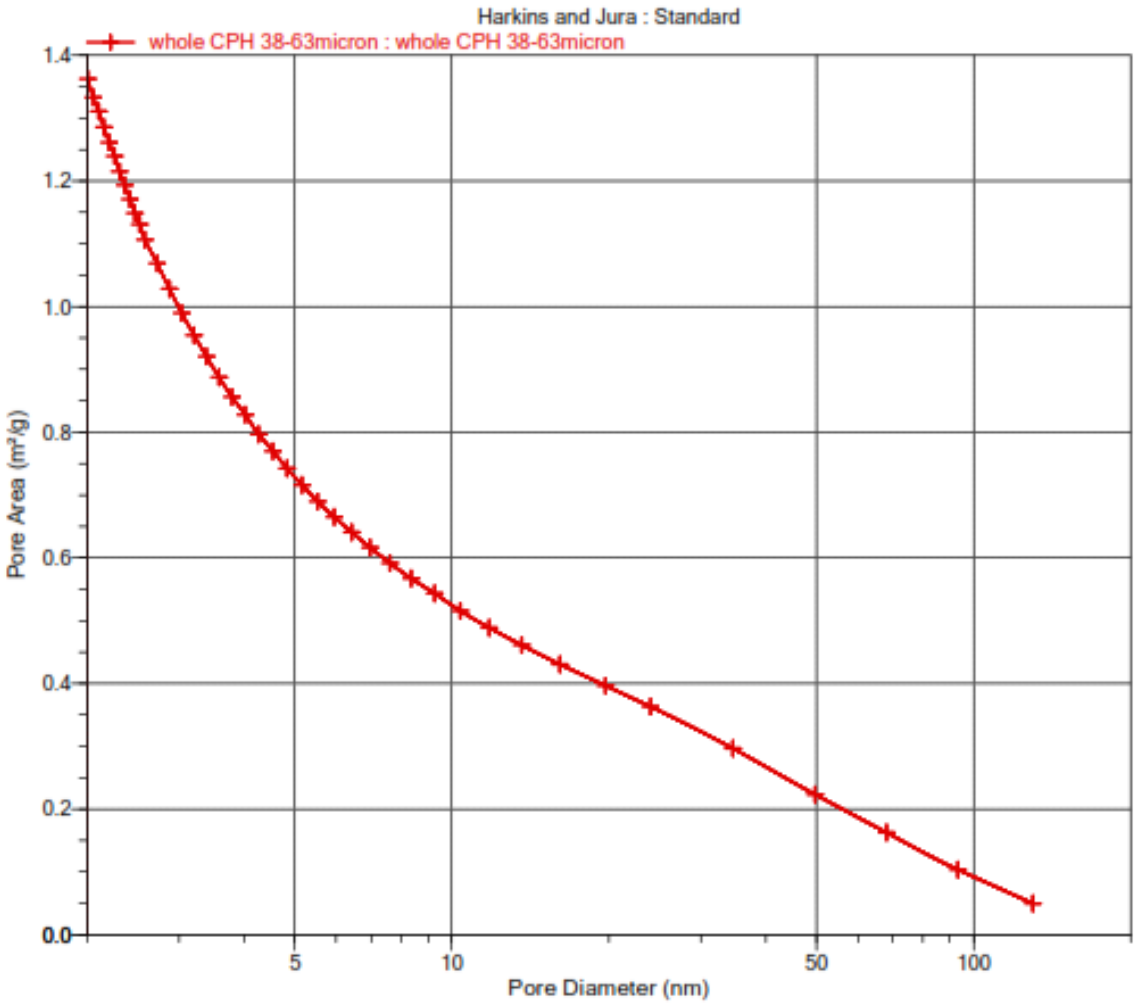
Full Report Set

Sample: whole CPH 38-63micron
Operator: Lee Stevens
Submitter:
Bar Code:
File: C:\Users\SRD\OneDrive - T...\whole CPH 38-63micron.SMP

Started:	29/02/2020 16:39:40	Analysis adsorptive:	N2
Completed:	01/03/2020 03:31:36	Analysis bath temp.:	-195.850 °C
Report time:	18/10/2022 09:50:41	Thermal correction:	No
Sample mass:	1.7385 g	Ambient free space:	15.4201 cm³ Measured
Analysis free space:	42.8893 cm³	Equilibration interval:	30 s
Low pressure dose:	None	Sample density:	1.000 g/cm³
Automatic degas:	Yes		

BJH Adsorption Cumulative Pore Area (Larger)



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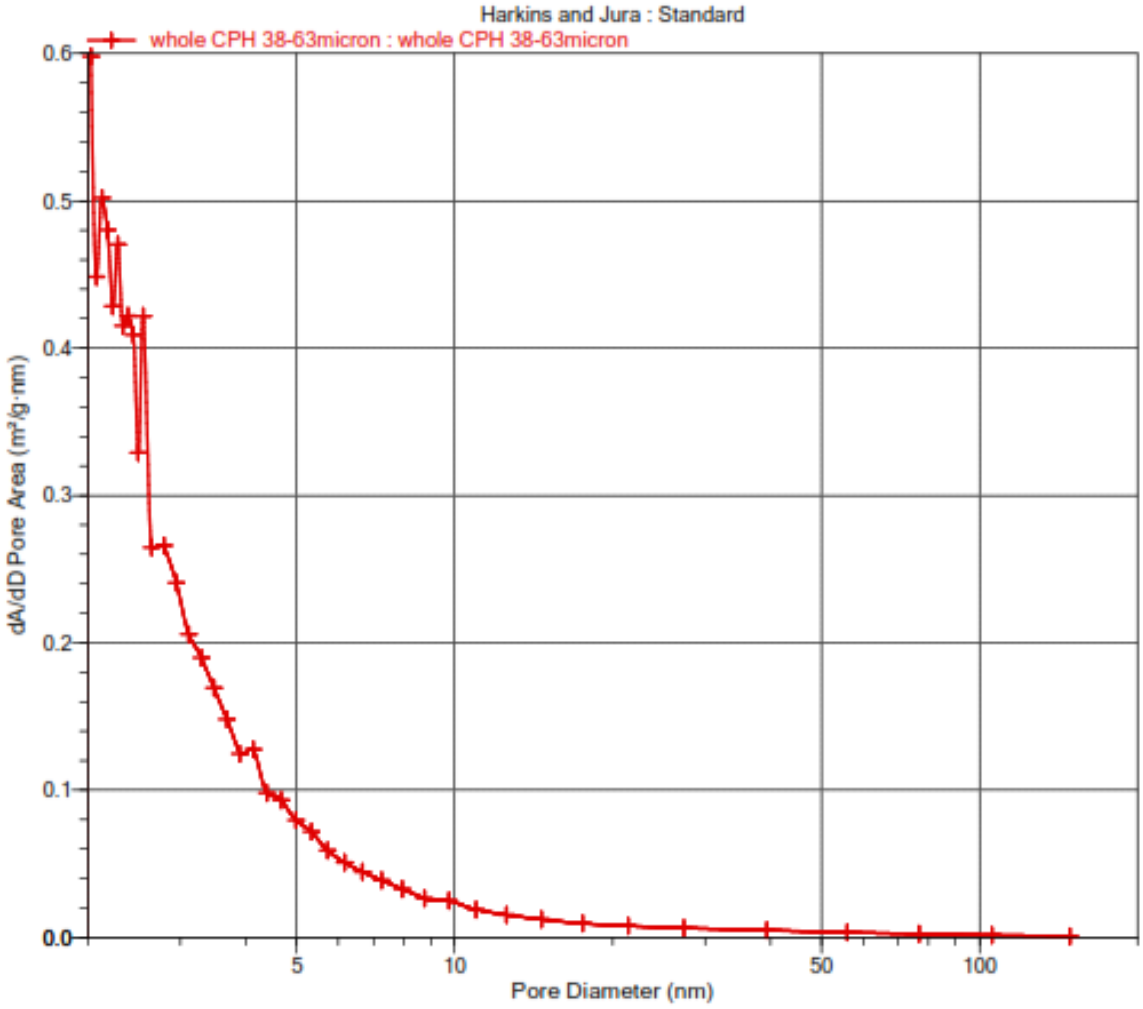
Full Report Set

Sample: whole CPH 38-63micron
Operator: Lee Stevens
Submitter:
Bar Code:
File: C:\Users\SRD\OneDrive - T...\whole CPH 38-63micron.SMP

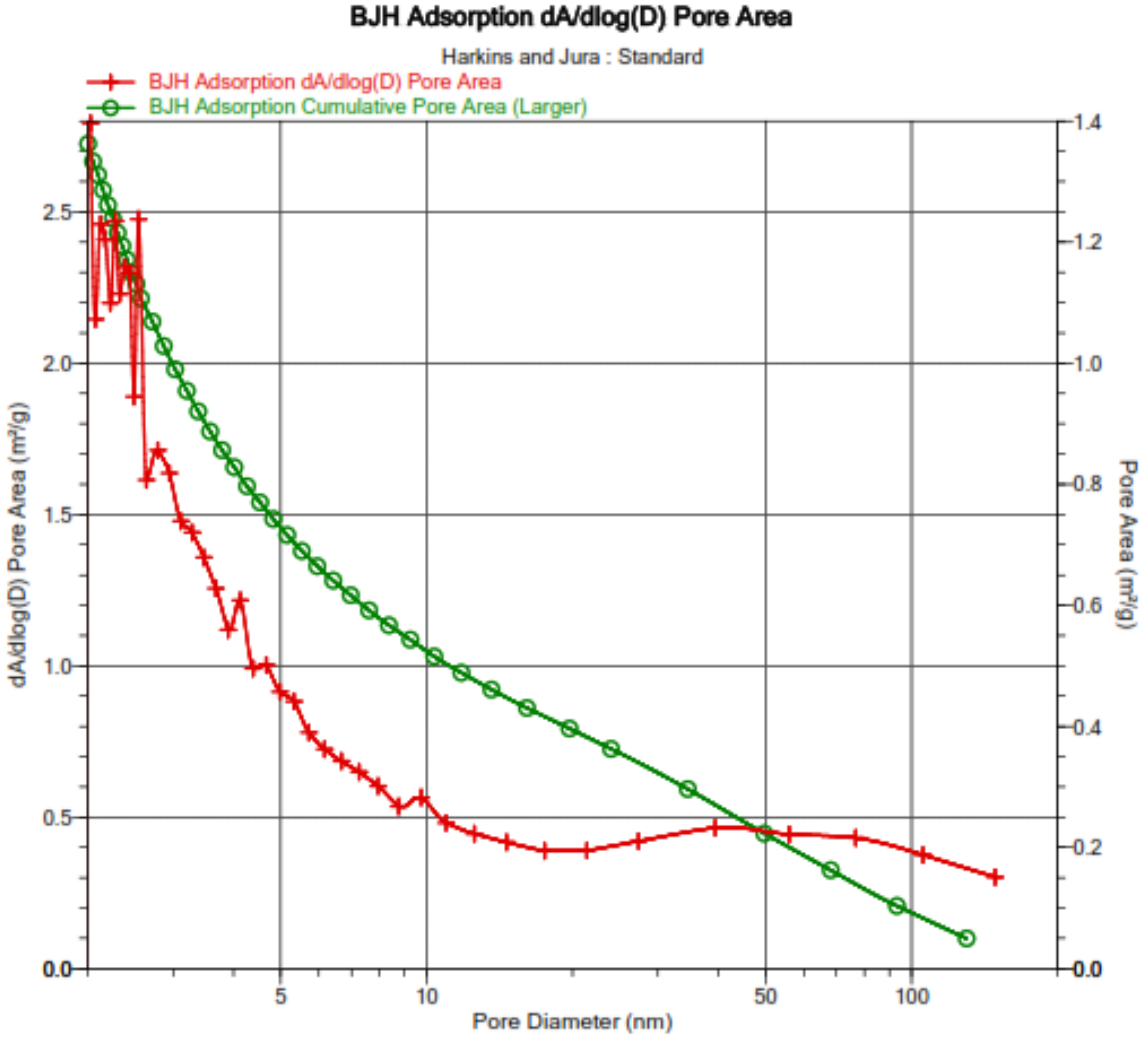
Started:	29/02/2020 16:39:40	Analysis adsorptive:	N2
Completed:	01/03/2020 03:31:36	Analysis bath temp.:	-195.850 °C
Report time:	18/10/2022 09:50:41	Thermal correction:	No
Sample mass:	1.7385 g	Ambient free space:	15.4201 cm³ Measured
Analysis free space:	42.8893 cm³	Equilibration interval:	30 s
Low pressure dose:	None	Sample density:	1.000 g/cm³
Automatic degas:	Yes		

BJH Adsorption dA/dD Pore Area



Sample: whole CPH 38-63micron
Operator: Lee Stevens
Submitter:
Bar Code:
File: C:\Users\SRD\OneDrive - T...\whole CPH 38-63micron.SMP

Started:	29/02/2020 16:39:40	Analysis adsorptive:	N2
Completed:	01/03/2020 03:31:36	Analysis bath temp.:	-195.850 °C
Report time:	18/10/2022 09:50:41	Thermal correction:	No
Sample mass:	1.7385 g	Ambient free space:	15.4201 cm³ Measured
Analysis free space:	42.8893 cm³	Equilibration interval:	30 s
Low pressure dose:	None	Sample density:	1.000 g/cm³
Automatic degas:	Yes		



Sample: whole CPH 38-63micron
Operator: Lee Stevens
Submitter:
Bar Code:
File: C:\Users\SRD\OneDrive - T...\whole CPH 38-63micron.SMP

Started: 29/02/2020 16:39:40
Completed: 01/03/2020 03:31:36
Report time: 18/10/2022 09:50:41
Sample mass: 1.7385 g
Analysis free space: 42.8893 cm³
Low pressure dose: None
Automatic degas: Yes

Analysis adsorptive: N2
Analysis bath temp.: -195.850 °C
Thermal correction: No
Ambient free space: 15.4201 cm³ Measured
Equilibration interval: 30 s
Sample density: 1.000 g/cm³

BJH Desorption Pore Distribution Report

Standard
Harkins and Jura
t = [13.99 / (0.034 - log(P/Po))] ^ 0.5

Diameter range: 2.0000 to 400.0000 nm
Adsorbate property factor: 0.95300 nm
Density conversion factor: 0.0015468
Fraction of pores open at both ends: 0.00

Pore Diameter Range (nm)	Average Diameter (nm)	Incremental Pore Volume (cm³/g)	Cumulative Pore Volume (cm³/g)	Incremental Pore Area (m²/g)	Cumulative Pore Area (m²/g)
64.9 - 44.2	50.6	0.001295	0.001295	0.102	0.102
44.2 - 30.1	34.5	0.000914	0.002209	0.106	0.208
30.1 - 23.1	25.7	0.000447	0.002655	0.070	0.278
23.1 - 19.4	20.9	0.000222	0.002877	0.042	0.321
19.4 - 17.6	18.4	0.000108	0.002986	0.024	0.344
17.6 - 14.1	15.4	0.000202	0.003187	0.052	0.396
14.1 - 11.8	12.7	0.000140	0.003327	0.044	0.440
11.8 - 10.1	10.8	0.000108	0.003435	0.040	0.480
10.1 - 8.9	9.4	0.000084	0.003519	0.036	0.516
8.9 - 7.9	8.3	0.000074	0.003593	0.036	0.551
7.9 - 7.1	7.4	0.000062	0.003655	0.033	0.585
7.1 - 6.4	6.7	0.000056	0.003711	0.034	0.618
6.4 - 5.8	6.1	0.000053	0.003764	0.035	0.653
5.8 - 5.3	5.5	0.000053	0.003817	0.038	0.691
5.3 - 4.7	5.0	0.000069	0.003886	0.056	0.747
4.7 - 4.4	4.5	0.000047	0.003933	0.041	0.788
4.4 - 4.0	4.2	0.000042	0.003975	0.040	0.829
4.0 - 3.8	3.9	0.000045	0.004020	0.046	0.875
3.8 - 3.5	3.6	0.000038	0.004058	0.042	0.917
3.5 - 3.3	3.4	0.000038	0.004096	0.045	0.962
3.3 - 3.1	3.2	0.000038	0.004134	0.048	1.009
3.1 - 2.9	3.0	0.000032	0.004166	0.043	1.053
2.9 - 2.8	2.8	0.000031	0.004198	0.044	1.097
2.8 - 2.6	2.7	0.000033	0.004231	0.049	1.146
2.6 - 2.5	2.5	0.000033	0.004264	0.053	1.198
2.5 - 2.3	2.4	0.000034	0.004298	0.057	1.255
2.3 - 2.2	2.3	0.000033	0.004332	0.059	1.314
2.2 - 2.1	2.1	0.000036	0.004368	0.068	1.381
2.1 - 2.0	2.0	0.000039	0.004407	0.077	1.459

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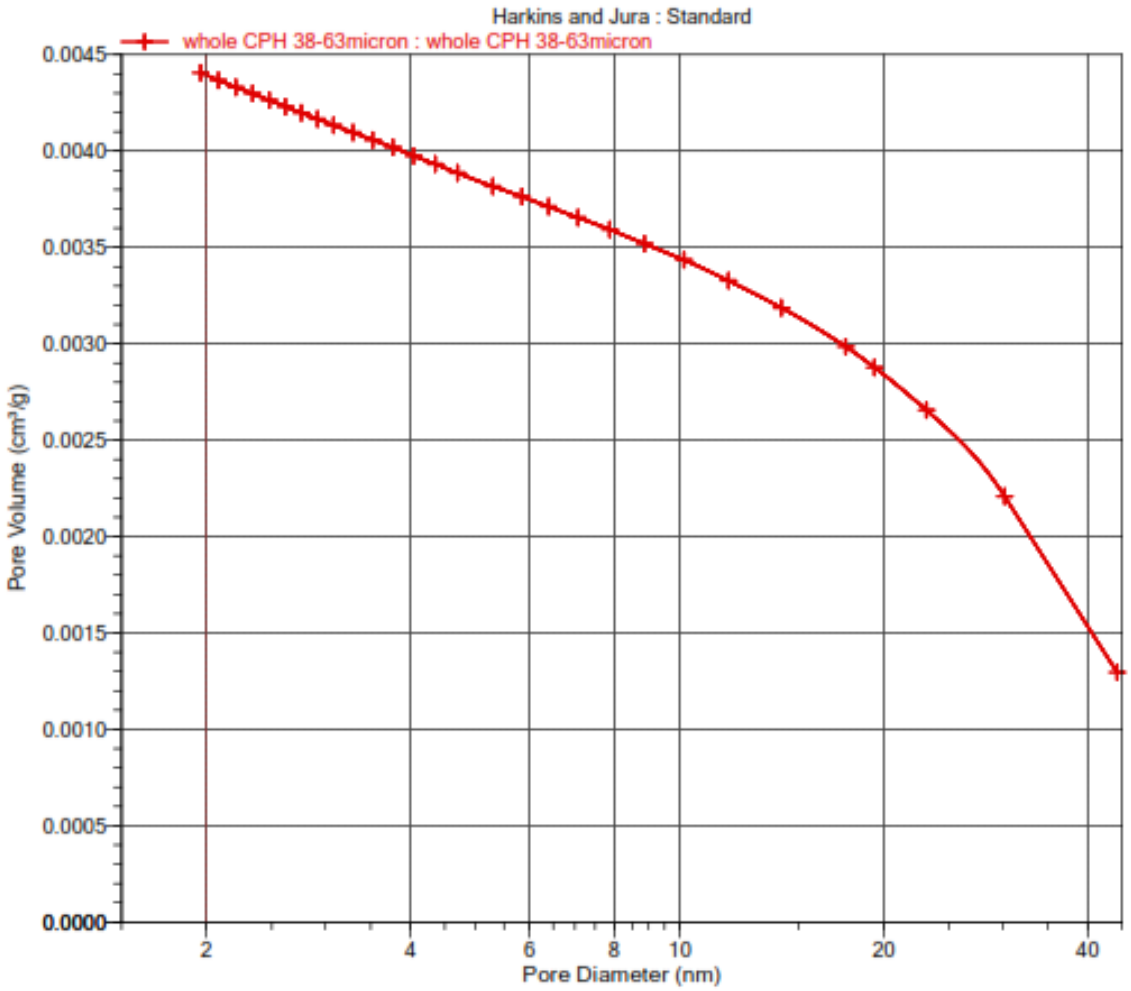
Full Report Set

Sample: whole CPH 38-63micron
Operator: Lee Stevens
Submitter:
Bar Code:
File: C:\Users\SRD\OneDrive - T...\whole CPH 38-63micron.SMP

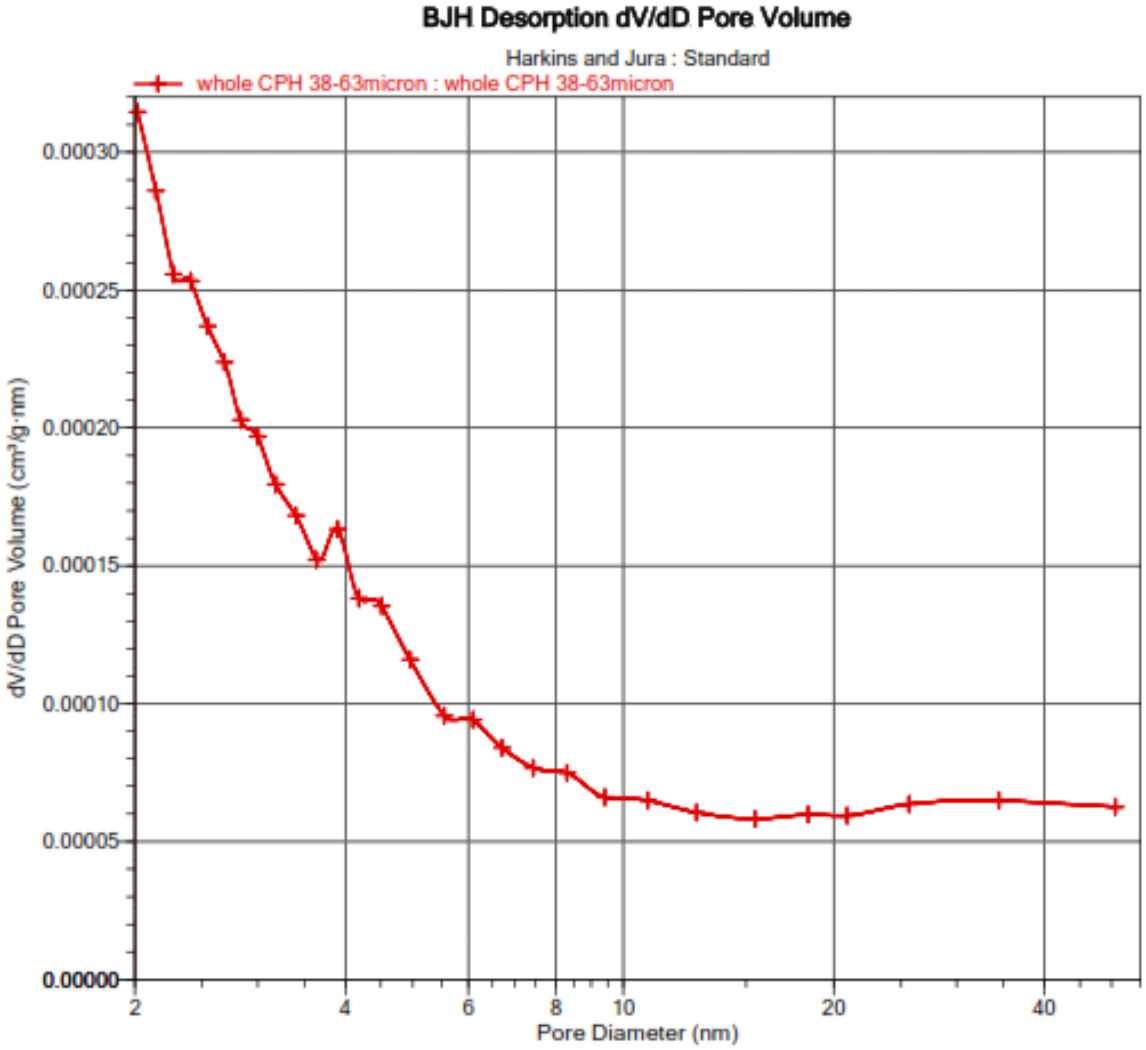
Started:	29/02/2020 16:39:40	Analysis adsorptive:	N2
Completed:	01/03/2020 03:31:36	Analysis bath temp.:	-195.850 °C
Report time:	18/10/2022 09:50:41	Thermal correction:	No
Sample mass:	1.7385 g	Ambient free space:	15.4201 cm³ Measured
Analysis free space:	42.8893 cm³	Equilibration interval:	30 s
Low pressure dose:	None	Sample density:	1.000 g/cm³
Automatic degas:	Yes		

BJH Desorption Cumulative Pore Volume (Larger)



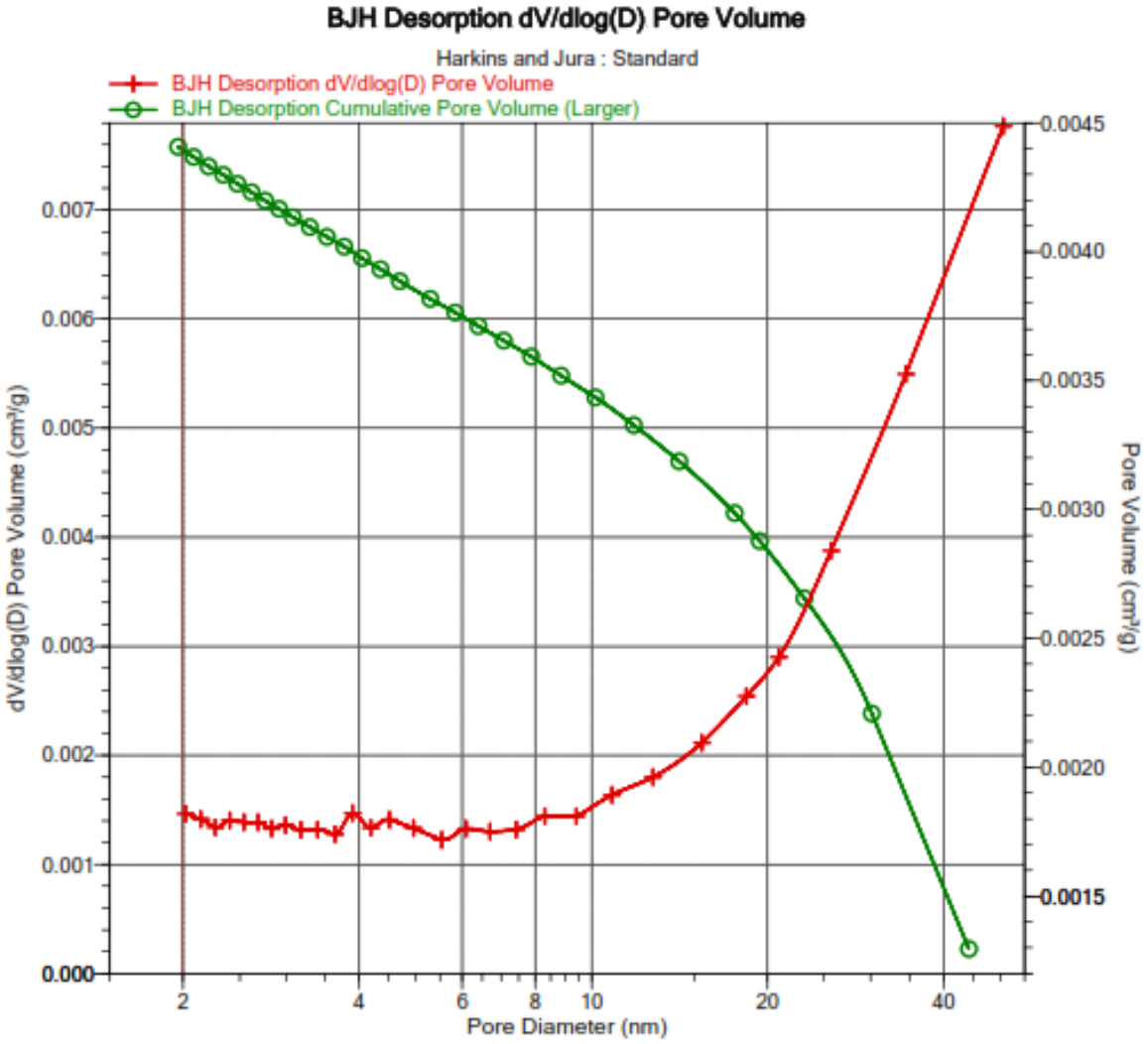
Sample: whole CPH 38-63micron
Operator: Lee Stevens
Submitter:
Bar Code:
File: C:\Users\SRD\OneDrive - T...\whole CPH 38-63micron.SMP

Started:	29/02/2020 16:39:40	Analysis adsorptive:	N2
Completed:	01/03/2020 03:31:36	Analysis bath temp.:	-195.850 °C
Report time:	18/10/2022 09:50:41	Thermal correction:	No
Sample mass:	1.7385 g	Ambient free space:	15.4201 cm³ Measured
Analysis free space:	42.8893 cm³	Equilibration interval:	30 s
Low pressure dose:	None	Sample density:	1.000 g/cm³
Automatic degas:	Yes		



Sample: whole CPH 38-63micron
Operator: Lee Stevens
Submitter:
Bar Code:
File: C:\Users\SRD\OneDrive - T...\whole CPH 38-63micron.SMP

Started:	29/02/2020 16:39:40	Analysis adsorptive:	N2
Completed:	01/03/2020 03:31:36	Analysis bath temp.:	-195.850 °C
Report time:	18/10/2022 09:50:41	Thermal correction:	No
Sample mass:	1.7385 g	Ambient free space:	15.4201 cm³ Measured
Analysis free space:	42.8893 cm³	Equilibration interval:	30 s
Low pressure dose:	None	Sample density:	1.000 g/cm³
Automatic degas:	Yes		



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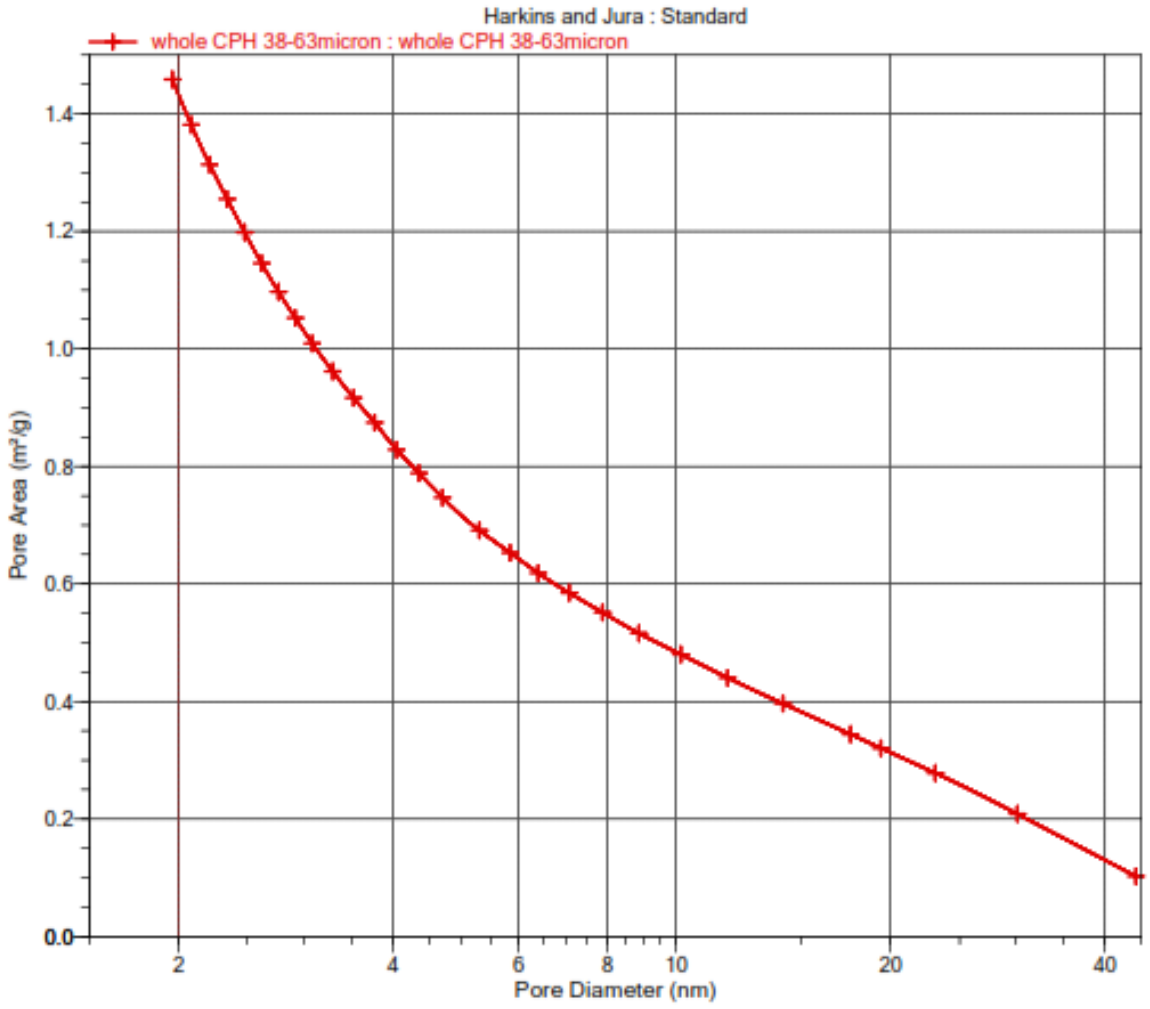
Full Report Set

Sample: whole CPH 38-63micron
Operator: Lee Stevens
Submitter:
Bar Code:
File: C:\Users\SRD\OneDrive - T...\whole CPH 38-63micron.SMP

Started:	29/02/2020 16:39:40	Analysis adsorptive:	N2
Completed:	01/03/2020 03:31:36	Analysis bath temp.:	-195.850 °C
Report time:	18/10/2022 09:50:41	Thermal correction:	No
Sample mass:	1.7385 g	Ambient free space:	15.4201 cm³ Measured
Analysis free space:	42.8893 cm³	Equilibration interval:	30 s
Low pressure dose:	None	Sample density:	1.000 g/cm³
Automatic degas:	Yes		

BJH Desorption Cumulative Pore Area (Larger)



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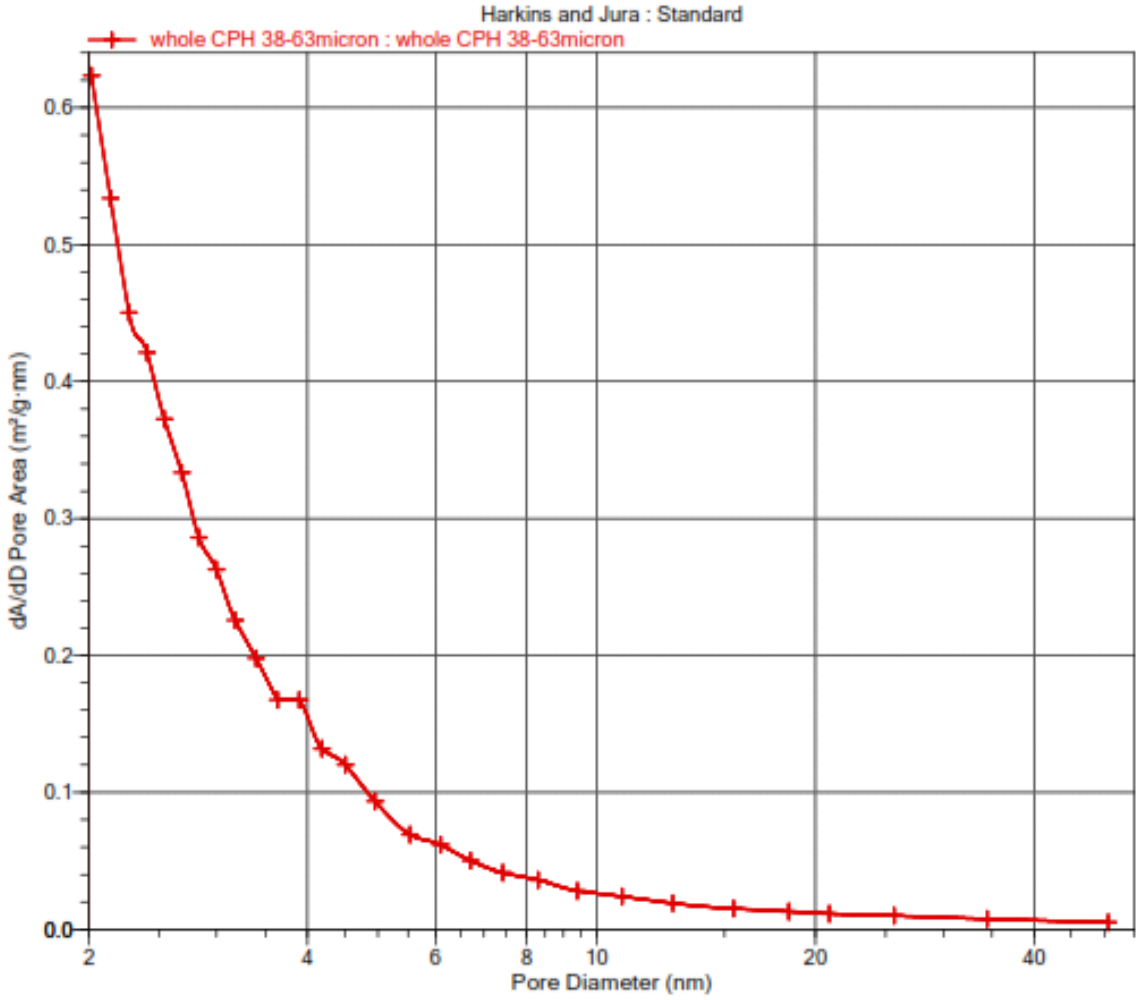
Full Report Set

Sample: whole CPH 38-63micron
Operator: Lee Stevens
Submitter:
Bar Code:
File: C:\Users\SRD\OneDrive - T...\whole CPH 38-63micron.SMP

Started:	29/02/2020 16:39:40	Analysis adsorptive:	N2
Completed:	01/03/2020 03:31:36	Analysis bath temp.:	-195.850 °C
Report time:	18/10/2022 09:50:41	Thermal correction:	No
Sample mass:	1.7385 g	Ambient free space:	15.4201 cm³ Measured
Analysis free space:	42.8893 cm³	Equilibration interval:	30 s
Low pressure dose:	None	Sample density:	1.000 g/cm³
Automatic degas:	Yes		

BJH Desorption dA/dD Pore Area



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Full Report Set

Sample: whole CPH 38-63micron
Operator: Lee Stevens
Submitter:
Bar Code:
File: C:\Users\SRD\OneDrive - T...\whole CPH 38-63micron.SMP

Started: 29/02/2020 16:39:40
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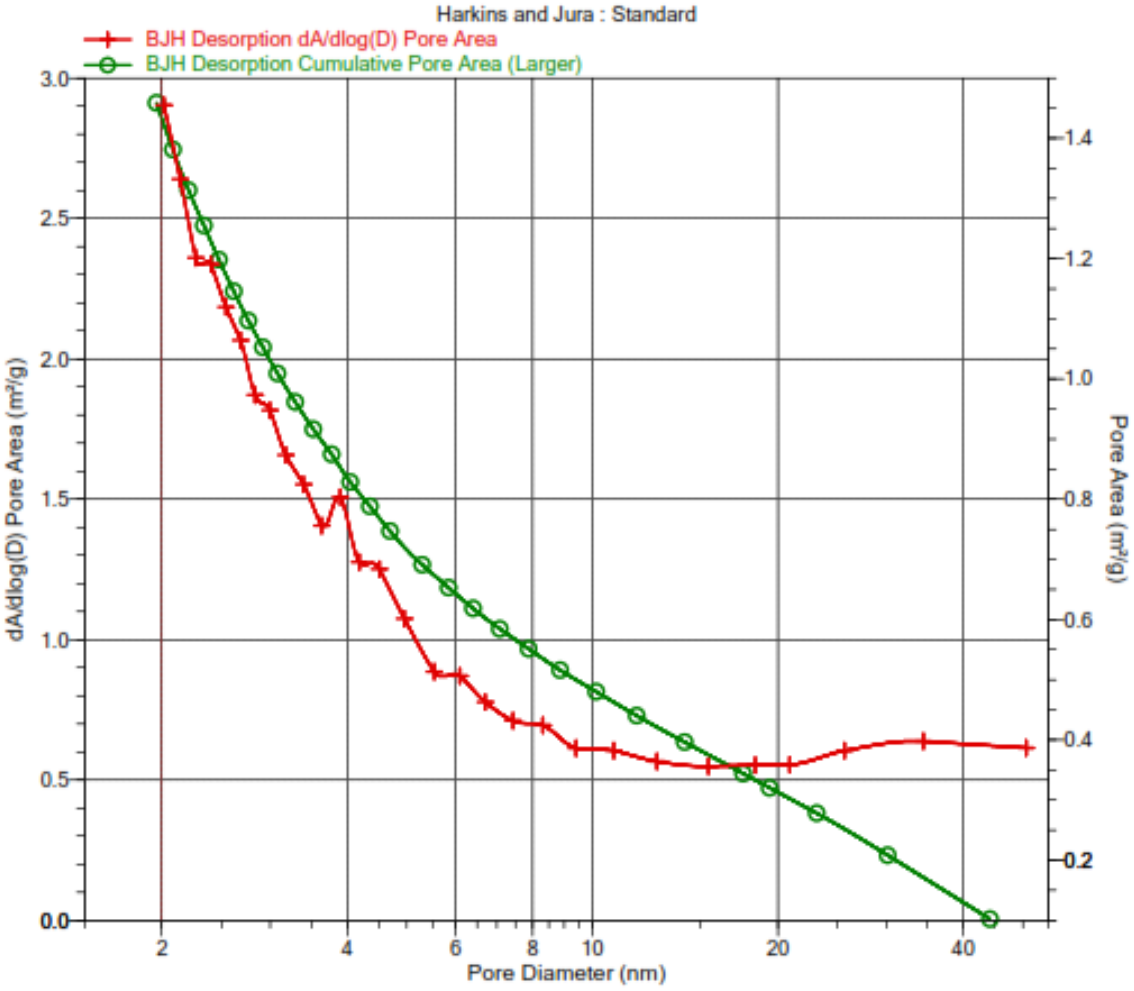
Report time: 18/10/2022 09:50:41
Sample mass: 1.7385 g
Analysis free space: 42.8893 cm³
Low pressure dose: None
Automatic degas: Yes

Analysis adsorptive: N2
Analysis bath temp.: -195.850 °C
Thermal correction: No
Ambient free space: 15.4201 cm³ Measured
Equilibration interval: 30 s
Sample density: 1.000 g/cm³

BJH Desorption dA/dlog(D) Pore Area



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Full Report Set

Sample: whole CPH 38-63micron
Operator: Lee Stevens
Submitter:
Bar Code:
File: C:\Users\SRD\OneDrive - T...\whole CPH 38-63micron.SMP

Started: 29/02/2020 16:39:40
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Report time: 18/10/2022 09:50:41
Sample mass: 1.7385 g
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Low pressure dose: None
Automatic degas: Yes

Analysis adsorptive: N2
Analysis bath temp.: -195.850 °C
Thermal correction: No
Ambient free space: 15.4201 cm³ Measured
Equilibration interval: 30 s
Sample density: 1.000 g/cm³

Dubinin-Radushkevich Tabular Report

Slope: -0.121397 ± 0.001661
Y-intercept: -0.593634 ± 0.009592
Correlation coefficient: 0.999626
Radushkevich fitted relative pressure range: 0.000500000 to 0.013551534 P/Po

Characteristic energy: 8.482832 kJ/mol
Limiting micropore capacity:
Limiting micropore volume: 0.0004 ± 0.0000 cm³/g STP
Equivalent surface area: 1.109462 m²/g

Affinity coefficient (beta): 0.33000

Density conversion factor: 0.0015468
Molecular cross-sectional area: 0.162 nm²

Absolute Pressure (mmHg)	Relative Pressure (P/Po)	Quantity Adsorbed (cm³/g STP)	Log Quantity Adsorbed	Log (Po/P)^2.0000
0.691454	0.000936582	0.0199	-1.7019	9.171534
2.251896	0.003050021	0.0429	-1.3673	6.328732
4.089542	0.005540337	0.0605	-1.2185	5.091629
5.346825	0.007244924	0.0701	-1.1541	4.579455
7.500092	0.010162878	0.0842	-1.0745	3.971982
9.082336	0.012308172	0.0942	-1.0261	3.647361
11.048721	0.014973308	0.1056	-0.9763	3.329465
12.810577	0.017359278	0.1148	-0.9401	3.099249
14.689754	0.019908729	0.1229	-0.9104	2.893253
16.523247	0.022388235	0.1296	-0.8873	2.722435
18.347397	0.024862707	0.1367	-0.8644	2.574265
20.197502	0.027376044	0.1439	-0.8419	2.44181
22.034111	0.029867687	0.1504	-0.8229	2.32501
23.880062	0.032366036	0.1567	-0.8050	2.219833
25.697983	0.034833304	0.1623	-0.7896	2.12578
27.533554	0.037317616	0.1685	-0.7735	2.03943
29.369125	0.039805123	0.1736	-0.7606	1.960171
36.593048	0.049598503	0.1936	-0.7130	1.701802
44.092102	0.059765553	0.2115	-0.6747	1.497072
51.269302	0.069487972	0.2269	-0.6442	1.341173
58.762131	0.079635997	0.2415	-0.6171	1.207561
66.058731	0.089543830	0.2549	-0.5936	1.098229
73.413467	0.099500000	0.2664	-0.5745	1.004359
80.870995	0.109594037	0.2771	-0.5573	0.9220091
88.247536	0.119580750	0.2873	-0.5417	0.8507087
95.556595	0.129484604	0.2965	-0.5279	0.7881566

Sample: whole CPH 38-63micron
Operator: Lee Stevens
Submitter:
Bar Code:
File: C:\Users\SRD\OneDrive - T...\whole CPH 38-63micron.SMP

Started: 29/02/2020 16:39:40
Completed: 01/03/2020 03:31:36
Report time: 18/10/2022 09:50:41
Sample mass: 1.7385 g
Analysis free space: 42.8893 cm³
Low pressure dose: None
Automatic degas: Yes

Analysis adsorptive: N2
Analysis bath temp.: -195.850 °C
Thermal correction: No
Ambient free space: 15.4201 cm³ Measured
Equilibration interval: 30 s
Sample density: 1.000 g/cm³

Absolute Pressure (mmHg)	Relative Pressure (P/Po)	Quantity Adsorbed (cm³/g STP)	Log Quantity Adsorbed	Log (Po/P)^2.0000
102.835541	0.139331118	0.3058	-0.5145	0.7326536
110.192352	0.149303215	0.3152	-0.5015	0.6821618
117.540863	0.159233063	0.3240	-0.4894	0.6367509
124.895615	0.169193957	0.3319	-0.4790	0.5953899
132.248276	0.179150186	0.3394	-0.4692	0.5576845
139.579132	0.189082278	0.3480	-0.4585	0.523234
146.900650	0.199004083	0.3558	-0.4489	0.4915945
154.254349	0.208972195	0.3628	-0.4403	0.4622796
161.575867	0.218875436	0.3701	-0.4317	0.43534
168.950333	0.228852863	0.3774	-0.4232	0.4101681
176.309235	0.238831677	0.3845	-0.4151	0.3867697
183.629700	0.248739720	0.3918	-0.4069	0.3651239
190.992752	0.258654165	0.3989	-0.3992	0.3448984
198.392136	0.268695278	0.4061	-0.3913	0.3257441
205.690811	0.278564687	0.4133	-0.3837	0.3081071
213.063202	0.288510873	0.4200	-0.3768	0.2914249
220.529037	0.298649828	0.4277	-0.3688	0.2754546
238.797516	0.323357040	0.4435	-0.3531	0.2404114
257.134552	0.348206402	0.4602	-0.3371	0.2099136
275.494385	0.373018014	0.4770	-0.3215	0.1834154
293.958069	0.398071503	0.4938	-0.3064	0.1600311
312.155975	0.422652279	0.5108	-0.2918	0.1398886
330.538666	0.447575398	0.5283	-0.2771	0.1218944
348.902679	0.472431099	0.5460	-0.2628	0.1060554
367.228271	0.497270029	0.5638	-0.2489	0.09205624
385.555939	0.522039338	0.5834	-0.2341	0.07969147
404.001953	0.547041255	0.6025	-0.2201	0.06863348
422.265259	0.571782843	0.6229	-0.2056	0.05893673
440.610596	0.596611406	0.6441	-0.1911	0.05031428
458.964203	0.621370576	0.6666	-0.1761	0.04270394
477.440338	0.646441831	0.6901	-0.1611	0.03589909
495.720245	0.671195180	0.7148	-0.1458	0.02998133
514.123718	0.696059268	0.7417	-0.1298	0.02476021
532.518860	0.720982282	0.7713	-0.1128	0.02018542
550.840271	0.745772036	0.8035	-0.0950	0.01622921
569.223999	0.770603973	0.8386	-0.0764	0.01280717
587.565186	0.795384676	0.8815	-0.0548	0.009884889
606.041321	0.820424625	0.9291	-0.0319	0.007389347
624.260010	0.845091621	0.9850	-0.0066	0.005343055
642.575195	0.869905344	1.0552	0.0233	0.003663639
660.798035	0.894544899	1.1469	0.0595	0.002342353
675.382935	0.914211853	1.2537	0.0982	0.001517348
695.400818	0.941326970	1.5145	0.1803	0.0006895612

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Full Report Set

Sample: whole CPH 38-63micron
Operator: Lee Stevens
Submitter:
Bar Code:
File: C:\Users\SRD\OneDrive - T...\whole CPH 38-63micron.SMP

Started: 29/02/2020 16:39:40
Completed: 01/03/2020 03:31:36
Report time: 18/10/2022 09:50:41
Sample mass: 1.7385 g
Analysis free space: 42.8893 cm³
Low pressure dose: None
Automatic degas: Yes

Analysis adsorptive: N2
Analysis bath temp.: -195.850 °C
Thermal correction: No
Ambient free space: 15.4201 cm³ Measured
Equilibration interval: 30 s
Sample density: 1.000 g/cm³

Absolute Pressure (mmHg)	Relative Pressure (P/Po)	Quantity Adsorbed (cm³/g STP)	Log Quantity Adsorbed	Log (Po/P)^2.0000
709.109436	0.959756723	1.9257	0.2846	0.0003182241
717.343567	0.970950521	2.4199	0.3838	0.0001639143
723.327820	0.978970519	3.0899	0.4899	8.520003e-05
727.878357	0.985004759	3.9501	0.5966	4.305553e-05
731.341858	0.989782176	5.0808	0.7059	1.989491e-05

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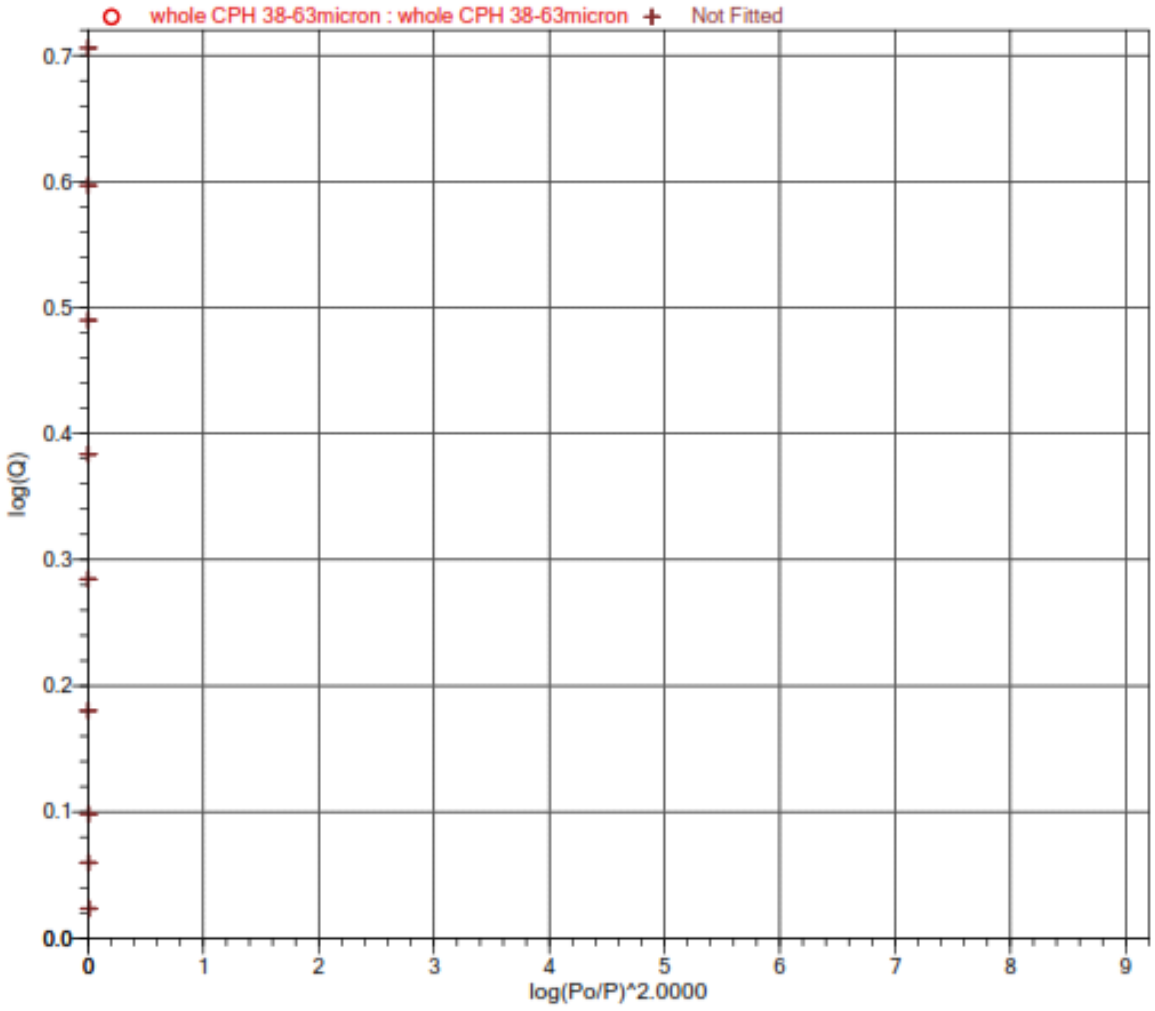
Started:	29/02/2020 16:39:40	Analysis adsorptive:	N2
Completed:	01/03/2020 03:31:36	Analysis bath temp.:	-195.850 °C
Report time:	18/10/2022 09:50:41	Thermal correction:	No
Sample mass:	1.7385 g	Ambient free space:	15.4201 cm³ Measured
Analysis free space:	42.8893 cm³	Equilibration interval:	30 s
Low pressure dose:	None	Sample density:	1.000 g/cm³
Automatic degas:	Yes		

Dubinin-Radushkevich Transformed Isotherm Plot



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Analysis adsorptive: N2
Analysis bath temp.: -195.850 °C
Thermal correction: No
Ambient free space: 15.4201 cm³ Measured
Equilibration interval: 30 s
Sample density: 1.000 g/cm³

Sample Information

Sample: whole CPH 38-63micron
Operator: Lee Stevens
Submitter:
Bar Code:
Mass type: Entered
Sample mass: 1.7385 g
Density: 1.000 g/cm³
Type of data: Automatically collected
Instrument type: MicroActive
Original instrument type: 2420
Comments:

Sample Tube

Sample tube: Sample tube 6
Ambient free space: 17.6678 cm³
Analysis free space: 51.6255 cm³
Non-ideality factor: 0.0000570
Use isothermal jacket: Yes
Use filler rod: Yes
Vacuum seal type: Seal Frit

Degas Conditions

Degas conditions: Degas Conditions

Evacuation Phase

Temperature ramp rate: 5.0 °C/min
Target temperature: 50 °C
Evacuation rate: 5.0 mmHg/s
Unrest. evacuation from: 5.0 mmHg
Vacuum level: 1e-01 mmHg
Evacuation time: 30 min

Heating Phase

Sample prep: Stage	Temperature (°C)	Ramp Rate (°C/min)	Time (min)
1	90	5.0	1,440

Evacuation and Heating Phases

Hold pressure: 5 mmHg

Sample: whole CPH 38-63micron
Operator: Lee Stevens
Submitter:
Bar Code:
File: C:\Users\SRD\OneDrive - T...\whole CPH 38-63micron.SMP

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Low pressure dose: None
Automatic degas: Yes

Analysis adsorptive: N2
Analysis bath temp.: -195.850 °C
Thermal correction: No
Ambient free space: 15.4201 cm³ Measured
Equilibration interval: 30 s
Sample density: 1.000 g/cm³

Backfill

Backfill sample tube: Yes

Analysis Conditions

Analysis conditions: Micropore Run
Isotherm collection: Target Pressure
Absolute pressure dosing: No

Pressure Table

Starting Pressure (P/Po)	Pressure Increment (P/Po)	Ending Pressure (P/Po)
0.000000000		0.001000000
0.001000000		0.003000000
0.003000000		0.005000000
0.005000000		0.007500000
0.007500000		0.010000000
0.010000000		0.012500000
0.012500000		0.015000000
0.015000000		0.017500000
0.017500000		0.020000000
0.020000000		0.022500000
0.022500000		0.025000000
0.025000000		0.027500000
0.027500000		0.030000000
0.030000000		0.032500000
0.032500000		0.035000000
0.035000000		0.037500000
0.037500000		0.040000000
0.040000000		0.050000000
0.050000000		0.060000000
0.060000000		0.070000000
0.070000000		0.080000000
0.080000000		0.090000000
0.090000000		0.100000000
0.100000000		0.110000000
0.110000000		0.120000000
0.120000000		0.130000000
0.130000000		0.140000000
0.140000000		0.150000000
0.150000000		0.160000000
0.160000000		0.170000000
0.170000000		0.180000000
0.180000000		0.190000000
0.190000000		0.200000000

Sample: whole CPH 38-63micron
Operator: Lee Stevens
Submitter:
Bar Code:
File: C:\Users\SRD\OneDrive - T...\whole CPH 38-63micron.SMP

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Analysis adsorptive: N2
Analysis bath temp.: -195.850 °C
Thermal correction: No
Ambient free space: 15.4201 cm³ Measured
Equilibration interval: 30 s
Sample density: 1.000 g/cm³

Pressure Table

Starting Pressure (P/Po)	Pressure Increment (P/Po)	Ending Pressure (P/Po)
0.200000000		0.210000000
0.210000000		0.220000000
0.220000000		0.230000000
0.230000000		0.240000000
0.240000000		0.250000000
0.250000000		0.260000000
0.260000000		0.270000000
0.270000000		0.280000000
0.280000000		0.290000000
0.290000000		0.300000000
0.300000000		0.325000000
0.325000000		0.350000000
0.350000000		0.375000000
0.375000000		0.400000000
0.400000000		0.425000000
0.425000000		0.450000000
0.450000000		0.475000000
0.475000000		0.500000000
0.500000000		0.525000000
0.525000000		0.550000000
0.550000000		0.575000000
0.575000000		0.600000000
0.600000000		0.625000000
0.625000000		0.650000000
0.650000000		0.675000000
0.675000000		0.700000000
0.700000000		0.725000000
0.725000000		0.750000000
0.750000000		0.775000000
0.775000000		0.800000000
0.800000000		0.825000000
0.825000000		0.850000000
0.850000000		0.875000000
0.875000000		0.900000000
0.900000000		0.920000000
0.920000000		0.950000000
0.950000000		0.970000000
0.970000000		0.980000000
0.980000000		0.990000000
0.990000000		0.995000000
0.995000000		0.998000000
0.998000000		0.970000000

Sample: whole CPH 38-63micron
Operator: Lee Stevens
Submitter:
Bar Code:
File: C:\Users\SRD\OneDrive - T...\whole CPH 38-63micron.SMP

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Automatic degas: Yes

Analysis adsorptive: N2
Analysis bath temp.: -195.850 °C
Thermal correction: No
Ambient free space: 15.4201 cm³ Measured
Equilibration interval: 30 s
Sample density: 1.000 g/cm³

Pressure Table

Starting Pressure (P/Po)	Pressure Increment (P/Po)	Ending Pressure (P/Po)
0.970000000		0.955000000
0.955000000		0.940000000
0.940000000		0.925000000
0.925000000		0.910000000
0.910000000		0.880000000
0.880000000		0.850000000
0.850000000		0.820000000
0.820000000		0.790000000
0.790000000		0.760000000
0.760000000		0.730000000
0.730000000		0.700000000
0.700000000		0.670000000
0.670000000		0.640000000
0.640000000		0.625000000
0.625000000		0.610000000
0.610000000		0.580000000
0.580000000		0.550000000
0.550000000		0.520000000
0.520000000		0.505000000
0.505000000		0.490000000
0.490000000		0.460000000
0.460000000		0.430000000
0.430000000		0.400000000
0.400000000		0.375000000
0.375000000		0.350000000
0.350000000		0.325000000
0.325000000		0.300000000
0.300000000		0.275000000
0.275000000		0.250000000
0.250000000		0.225000000
0.225000000		0.200000000
0.200000000		0.175000000
0.175000000		0.150000000
0.150000000		0.125000000
0.125000000		0.100000000
0.100000000		0.075000000
0.075000000		0.050000000
0.050000000		0.030000000
0.030000000		0.010000000

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Full Report Set

Sample: whole CPH 38-63micron
Operator: Lee Stevens
Submitter:
Bar Code:
File: C:\Users\SRD\OneDrive - T...\whole CPH 38-63micron.SMP

Started: 29/02/2020 16:39:40
Completed: 01/03/2020 03:31:36
Report time: 18/10/2022 09:50:41
Sample mass: 1.7385 g
Analysis free space: 42.8893 cm³
Low pressure dose: None
Automatic degas: Yes

Analysis adsorptive: N2
Analysis bath temp.: -195.850 °C
Thermal correction: No
Ambient free space: 15.4201 cm³ Measured
Equilibration interval: 30 s
Sample density: 1.000 g/cm³

Preparation

Fast evacuation: No
Evacuation rate: 5.0 mmHg/s
Unrestricted evacuation from: 0.5 mmHg
Vacuum setpoint: 10 µmHg
Evacuation time: 0.50 h

Leak test: No
Use TranSeal: No

Free Space

Measured before analysis
Lower Dewar for evacuation: Yes
Evacuation time: 0.15 h
Outgas test: No

Po and Temperature

Po type: Measured in Psat tube, adjusted by measurement over sample
Temperature type: Entered
Temperature: -195.850 °C

Dosing

Use first pressure fixed dose: No
Use maximum volume increment: No
Target tolerance: 7.0% or 5.000 mmHg
Low pressure dosing: No

Equilibration

	Relative Pressure (P/Po)	Equilibration Interval (s)
1	0.010000000	30
2	0.100000000	25
3	0.300000000	20
4	1.000000000	15

Minimum equilibration delay at P/Po >= 0.995: 600 s

Sample Backfill

Backfill at start of analysis: Yes
Backfill at end of analysis: Yes
Backfill gas: N2

ɔrptive Properties

Sample: whole CPH 38-63micron
Operator: Lee Stevens
Submitter:
Bar Code:
File: C:\Users\SRD\OneDrive - T...\whole CPH 38-63micron.SMP

Started: 29/02/2020 16:39:40
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Sample mass: 1.7385 g
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Low pressure dose: None
Automatic degas: Yes

Analysis adsorptive: N2
Analysis bath temp.: -195.850 °C
Thermal correction: No
Ambient free space: 15.4201 cm³ Measured
Equilibration interval: 30 s
Sample density: 1.000 g/cm³

Adsorptive: Nitrogen @ 77.35 K (N2)
Non-condensing adsorptive: No
Maximum manifold pressure: 925.00 mmHg
Therm. tran. hard-sphere diameter: 0.38600 nm
Molecular cross-sectional area: 0.162 nm²
Adsorbate molecular weight: 28.01
Thermal conductivity: 1.00
Non-ideality factor: 0.0000620
Density conversion factor: 0.0015468
Dosing method: Normal

Psat vs. Temperature Table

	Saturation Pressure (mmHg)	Temperature (°C)
1	600.193	-197.75
2	634.512	-197.30
3	674.383	-196.80
4	720.420	-196.25
5	742.119	-196.00
6	759.833	-195.80
7	777.867	-195.60
8	805.525	-195.30
9	853.268	-194.80
10	903.122	-194.30

Calculation BET surface area and pore characteristics of untreated CPH 38-63 micron (Measurement 1) using MicroActive:

1. BET Surface area = 1.457 m^2/g
2. Vmicro = micropore volume of Dubinin-Radeskevich (DR) Tabular report = 0.40 mm^3/g
3. Vmeso = Pore Volume of $BJH_{adsorption}$ (2 – 50 nm) = 2.63 mm^3/g
4. Vtotal = Pore Volume of $BJH_{adsorption}$ (2 – 400 nm) + Vmicro = 7.87 mm^3/g + 0.40 mm^3/g = 8.27 mm^3/g
5. Average pore diameter = $\frac{4xV_{total}}{BET_{SA}} x1000 = \frac{4x8.27}{1.4578} = \frac{33.1}{1.4578} = 22.71\,nm$

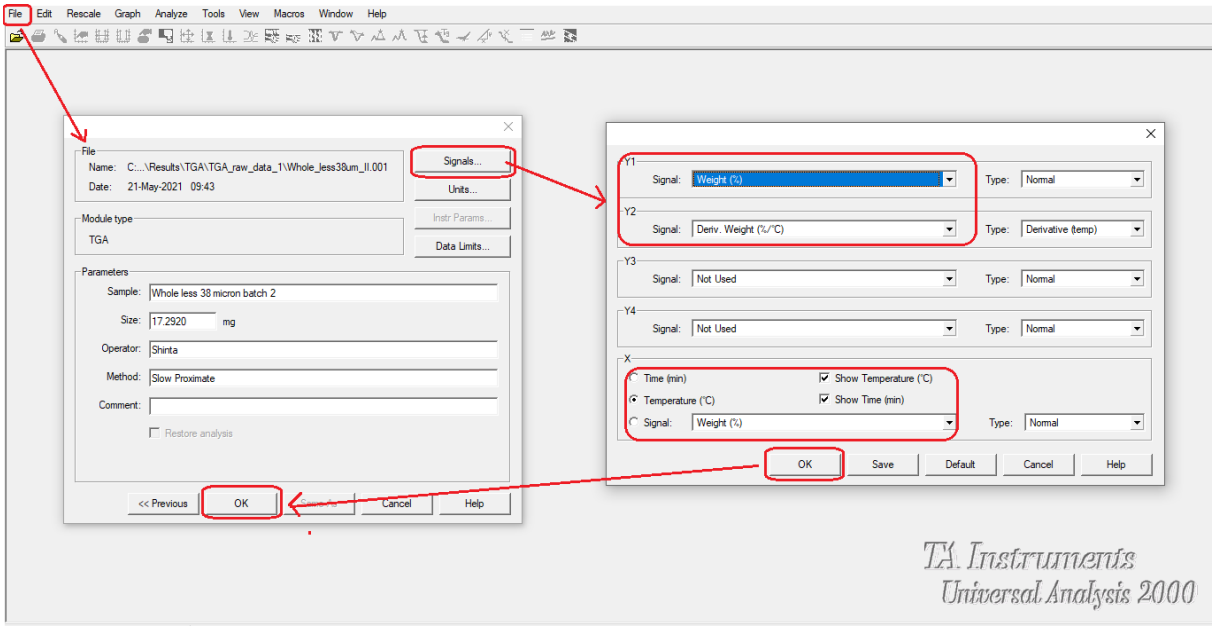
Calculation of BET surface area and pore characteristics of untreated CPH 38-63 micron (duplicate analysis):

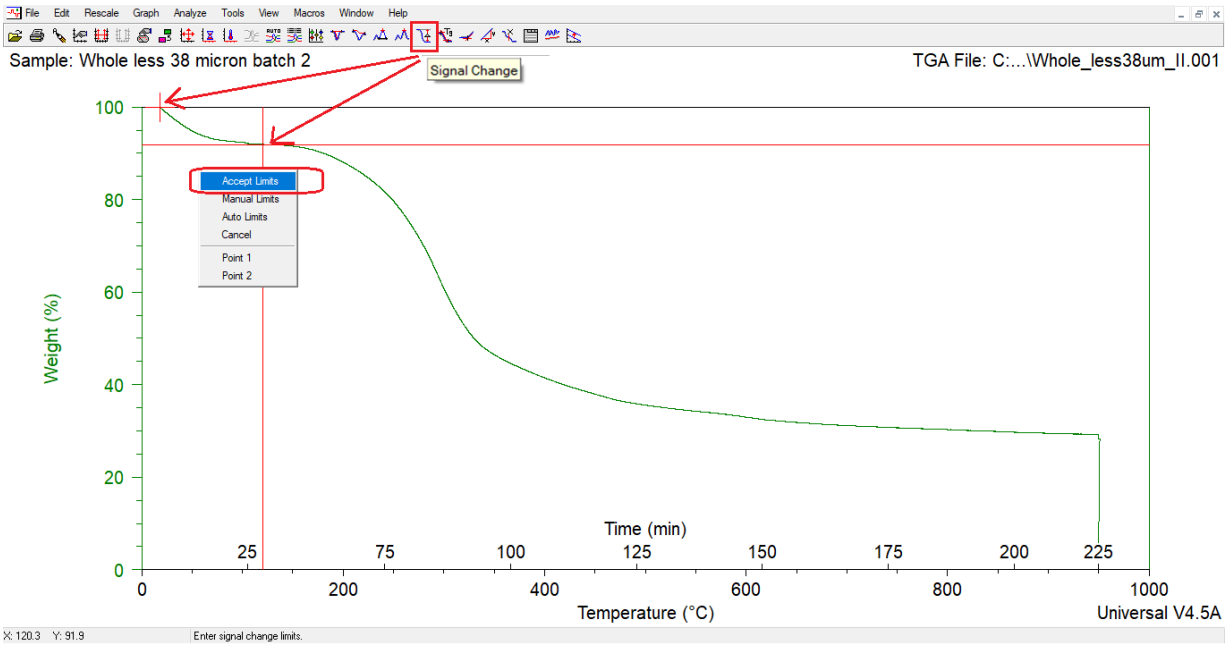
Table S.8 BET surface area and pore characteristics of untreated CPH 38-63 micron

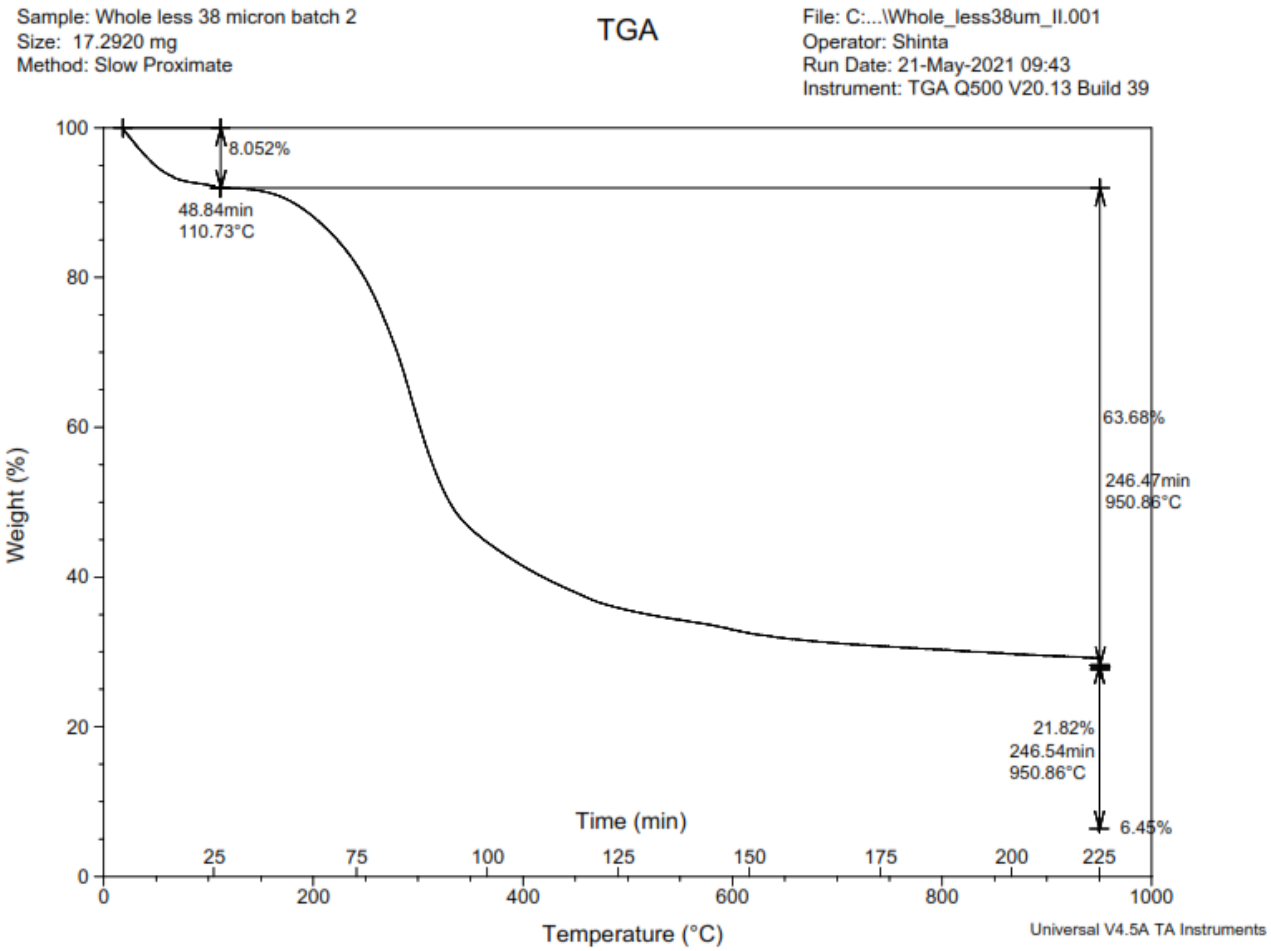
Untreated CPH ≤38 micron	**BET Surface Area (m^2/g)**	**Micropore volume (mm^3/g)**	**Mesopore volume (mm^3/g)**	**Total pore volume (mm^3/g)**	**Average pore diameter (4V/A) (nm)**
Measurement 1	***1.46***	***0.40***	***2.63***	***8.27***	***22.71***
Measurement 2	1.54	0.45	2.62	7.96	20.66
Average	1.50 ± 0.06	0.42 ± 0.05	2.63 ± 0.01	8.11 ± 0.22	21.68 ± 1.45

Appendix E: Calculation of proximate contents (TGA)

Calculation of proximate contents using TA Instruments Universal Analysis 2000 Software – an example data for untreated CPH ≤38 micron (Measurement 1):







Calculation of proximate contents of untreated CPH ≤38 micron (duplicate analysis):

Table S.9 Proximate analysis of untreated CPH ≤38 micron

Untreated CPH ≤38 micron	**Proximate (%)**			
	Moisture content (MC)	**Volatile matter (VM)**	**Fixed carbon (FC)**	**Ash (A)**
Measurement 1	8.05	63.68	21.82	6.45
Measurement 2	9.23	62.82	22.24	5.71
Average	8.64 ± 0.83	63.25 ± 0.61	22.03 ± 0.30	6.08 ± 0.52

Appendix F: Statistic Tests of Significance

Appendix F.1 Test of Significance of the effect of drying and size reduction on Total Phenolic Content (TPC), Total Monomeric Anthocyanin (TMA), and Antioxidant Activity (AOA)

F.1.1 Total Phenolic Content (TPC)

ANOVA - Total Phenolic Content

Cases	Sum of Squares	df	Mean Square	F	p	η^2
Size reduction	34858.687	1	34858.687	130.302	< 0.001***	0.236
Drying treatment	85597.896	1	85597.896	319.965	< 0.001***	0.580
Size reduction ✻ Drying treatment	18519.439	1	18519.439	69.226	< 0.001***	0.126
Residuals	8560.716	32	267.522			

Note. Type III Sum of Squares

Post Hoc Tests

Post Hoc Comparisons - Size reduction

		Mean Difference	SE	t	p_{tukey}
with size reduction	without size reduction	62.235	5.452	11.415	< 0.001 ***

*** p < 0.001

Note. Results are averaged over the levels of: Drying treatment

Post Hoc Comparisons - Drying treatment

		Mean Difference	SE	t	p_{tukey}
dry	fresh	-97.524	5.452	-17.888	< 0.001 ***

*** p < 0.001

Note. Results are averaged over the levels of: Size reduction

F.1.2 Total Monomeric Anthocyanin (TMA)

ANOVA - Total Monomeric Anthocyanin

Cases	Sum of Squares	df	Mean Square	F	p	η^2
Size reduction	0.125	1	0.125	133.956	< 0.001***	0.395
Drying treatment	0.160	1	0.160	171.787	< 0.001***	0.507
Size reduction ✻ Drying treatment	9.428×10^{-4}	1	9.428×10^{-4}	1.011	0.322	0.003
Residuals	0.030	32	9.327×10^{-4}			

Note. Type III Sum of Squares

Post Hoc Tests

Post Hoc Comparisons - Size reduction

		Mean Difference	SE	t	p_{tukey}
with size reduction	without size reduction	0.118	0.010	11.574	< 0.001***

* p < 0.05, ** p < 0.01, *** p < 0.001

Note. Results are averaged over the levels of: Drying treatment

Post Hoc Comparisons - Drying treatment

		Mean Difference	SE	t	p_{tukey}
dry	fresh	-0.133	0.010	-13.107	< 0.001***

* p < 0.05, ** p < 0.01, *** p < 0.001

Note. Results are averaged over the levels of: Size reduction

F.1.3 Antioxidant Activity (AOA)

ANOVA - Antioxidant Activity

Cases	Sum of Squares	df	Mean Square	F	p	η^2
Size reduction	7.452	1	7.452	35.352	< 0.001***	0.002
Drying treatment	3207.182	1	3207.182	15214.217	< 0.001***	0.993
Size reduction ✻ Drying treatment	9.833	1	9.833	46.643	< 0.001***	0.003
Residuals	6.746	32	0.211			

Note. Type III Sum of Squares

Post Hoc Tests

Post Hoc Comparisons - Size reduction

		Mean Difference	SE	t	p_{tukey}
with size reduction	without size reduction	-0.910	0.153	-5.946	< 0.001***

* p < 0.05, ** p < 0.01, *** p < 0.001

Note. Results are averaged over the levels of: Drying treatment

Post Hoc Comparisons - Drying treatment

		Mean Difference	SE	t	p_{tukey}
dry	fresh	-18.877	0.153	-123.346	< 0.001***

* p < 0.05, ** p < 0.01, *** p < 0.001

Note. Results are averaged over the levels of: Size reduction

F.1.4 Correlation between TPC, TMA, and AOA on drying effect

Pearson's Correlations

Variable		Total Phenolic Content	Total Monomeric Anthocyanin	Antioxidant Activity
1. Total Phenolic Content	Pearson's r	—		
	p-value	—		
2. Total Monomeric Anthocyanin	Pearson's r	0.891***	—	
	p-value	< 0.001	—	
3. Antioxidant Activity	Pearson's r	0.720***	0.673***	—
	p-value	< 0.001	< 0.001	—

* p < 0.05, ** p < 0.01, *** p < 0.001

Appendix F.2 Test of Significance the effect of solvent type on Total Phenolic Content (TPC), Total Monomeric Anthocyanin (TMA), and Antioxidant Activity (AOA)

F.2.1 Total Phenolic Content (TPC)

ANOVA – Total Phenolic Content

Cases	Sum of Squares	df	Mean Square	F	p	η^2
Solvent Type	44926.639	7	6418.091	3014.622	< 0.001***	0.997
Residuals	136.255	64	2.129			

Note. Type III Sum of Squares

Post Hoc Tests

Post Hoc Comparisons – Solvent Type

		Mean Difference	SE	t	p_{tukey}	
(100% 1-Butanol)	(100% 1-Pentanol)	3.188	0.688	4.635	< 0.001	***
	(100% 1-Propanol)	-12.347	0.688	-17.951	< 0.001	***
	100%Ethanol	-34.807	0.688	-50.604	< 0.001	***
	100%Methanol	-55.440	0.688	-80.602	< 0.001	***
	50%Ethanol	-65.534	0.688	-95.277	< 0.001	***
	50%Methanol	-57.236	0.688	-83.213	< 0.001	***
	Deionised Water	-37.098	0.688	-53.935	< 0.001	***
(100% 1-Pentanol)	(100% 1-Propanol)	-15.535	0.688	-22.586	< 0.001	***
	100%Ethanol	-37.996	0.688	-55.240	< 0.001	***
	100%Methanol	-58.629	0.688	-85.237	< 0.001	***
	50%Ethanol	-68.723	0.688	-99.913	< 0.001	***
	50%Methanol	-60.424	0.688	-87.848	< 0.001	***
	Deionised Water	-40.287	0.688	-58.571	< 0.001	***
(100% 1-Propanol)	100%Ethanol	-22.460	0.688	-32.654	< 0.001	***
	100%Methanol	-43.093	0.688	-62.651	< 0.001	***
	50%Ethanol	-53.187	0.688	-77.326	< 0.001	***
	50%Methanol	-44.889	0.688	-65.262	< 0.001	***
	Deionised Water	-24.751	0.688	-35.985	< 0.001	***
100%Ethanol	100%Methanol	-20.633	0.688	-29.997	< 0.001	***
	50%Ethanol	-30.727	0.688	-44.673	< 0.001	***
	50%Methanol	-22.429	0.688	-32.608	< 0.001	***
	Deionised Water	-2.291	0.688	-3.331	0.029	*
100%Methanol	50%Ethanol	-10.094	0.688	-14.675	< 0.001	***
	50%Methanol	-1.796	0.688	-2.611	0.171	
	Deionised Water	18.342	0.688	26.667	< 0.001	***
50%Ethanol	50%Methanol	8.298	0.688	12.064	< 0.001	***
	Deionised Water	28.436	0.688	41.342	< 0.001	***
50%Methanol	Deionised Water	20.138	0.688	29.277	< 0.001	***

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Note. P-value adjusted for comparing a family of 8

F.2.2 Total Monomeric Anthocyanin (TMA)

ANOVA – Total Monomeric Anthocyanin

Cases	Sum of Squares	df	Mean Square	F	p	η^2
Solvent Type	0.125	5	0.025	161.500	< 0.001***	0.944
Residuals	0.007	48	1.550×10^{-4}			

Note. Type III Sum of Squares

Post Hoc Tests

Post Hoc Comparisons – Solvent Type

		Mean Difference	SE	t	p_{tukey}	
(100% 1-Propanol)	100%Ethanol	-0.032	0.006	-5.390	< 0.001	***
	100%Methanol	-0.060	0.006	-10.224	< 0.001	***
	50%Ethanol	-0.138	0.006	-23.559	< 0.001	***
	50%Methanol	-0.102	0.006	-17.454	< 0.001	***
	Deionised Water	-0.020	0.006	-3.438	0.015	*
100%Ethanol	100%Methanol	-0.028	0.006	-4.834	< 0.001	***
	50%Ethanol	-0.107	0.006	-18.168	< 0.001	***
	50%Methanol	-0.071	0.006	-12.063	< 0.001	***
	Deionised Water	0.011	0.006	1.952	0.384	
100%Methanol	50%Ethanol	-0.078	0.006	-13.335	< 0.001	***
	50%Methanol	-0.042	0.006	-7.230	< 0.001	***
	Deionised Water	0.040	0.006	6.786	< 0.001	***
50%Ethanol	50%Methanol	0.036	0.006	6.105	< 0.001	***
	Deionised Water	0.118	0.006	20.121	< 0.001	***
50%Methanol	Deionised Water	0.082	0.006	14.015	< 0.001	***

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Note. P-value adjusted for comparing a family of 6

F.2.3 Antioxidant Activity (AOA)

ANOVA - Antioxidant Activity

Cases	Sum of Squares	df	Mean Square	F	p	η^2
Solvent Type	72.911	7	10.416	893.554	< 0.001***	0.990
Residuals	0.746	64	0.012			

Note. Type III Sum of Squares

Post Hoc Tests

Post Hoc Comparisons - Solvent Type

		Mean Difference	SE	t	p_{tukey}	
(100% 1-Butanol)	(100% 1-Pentanol)	1.527	0.051	29.998	< 0.001	***
	(100% 1-Propanol)	-1.296	0.051	-25.469	< 0.001	***
	100%Ethanol	-1.226	0.051	-24.095	< 0.001	***
	100%Methanol	-1.015	0.051	-19.941	< 0.001	***
	50%Ethanol	0.633	0.051	12.430	< 0.001	***
	50%Methanol	0.583	0.051	11.457	< 0.001	***
	Deionised Water	0.904	0.051	17.761	< 0.001	***
(100% 1-Pentanol)	(100% 1-Propanol)	-2.823	0.051	-55.467	< 0.001	***
	100%Ethanol	-2.753	0.051	-54.093	< 0.001	***
	100%Methanol	-2.542	0.051	-49.938	< 0.001	***
	50%Ethanol	-0.894	0.051	-17.567	< 0.001	***
	50%Methanol	-0.944	0.051	-18.540	< 0.001	***
	Deionised Water	-0.623	0.051	-12.237	< 0.001	***
(100% 1-Propanol)	100%Ethanol	0.070	0.051	1.374	0.865	
	100%Methanol	0.281	0.051	5.529	< 0.001	***
	50%Ethanol	1.929	0.051	37.900	< 0.001	***
	50%Methanol	1.879	0.051	36.927	< 0.001	***
	Deionised Water	2.200	0.051	43.231	< 0.001	***
100%Ethanol	100%Methanol	0.211	0.051	4.155	0.002	**
	50%Ethanol	1.859	0.051	36.526	< 0.001	***
	50%Methanol	1.809	0.051	35.553	< 0.001	***
	Deionised Water	2.130	0.051	41.856	< 0.001	***
100%Methanol	50%Ethanol	1.648	0.051	32.371	< 0.001	***
	50%Methanol	1.598	0.051	31.398	< 0.001	***
	Deionised Water	1.919	0.051	37.702	< 0.001	***
50%Ethanol	50%Methanol	-0.050	0.051	-0.973	0.977	
	Deionised Water	0.271	0.051	5.331	< 0.001	***
50%Methanol	Deionised Water	0.321	0.051	6.304	< 0.001	***

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Note. P-value adjusted for comparing a family of 8

F.2.4 Correlation between TPC, TMA, and AOA on solvent type effect

Pearson's Correlations

Variable		Total Phenolic Content	Total Monomeric Anthocyanin	Antioxidant Activity
1. Total Phenolic Content	Pearson's r	—		
	p-value	—		
2. Total Monomeric Anthocyanin	Pearson's r	0.910 *	—	
	p-value	0.012	—	
3. Antioxidant Activity	Pearson's r	0.075	-0.542	—
	p-value	0.861	0.267	—

*p < 0.05, **p < 0.01, ***p < 0.001

Appendix F.3 Test of Significance the effect of extraction method (microwave and conventional heating) on Total Phenolic Content (TPC), Total Monomeric Anthocyanin (TMA), and Antioxidant Activity (AOA)

F.3.1 Total Phenolic Content (TPC)

ANOVA - Total Phenolic Content

Cases	Sum of Squares	df	Mean Square	F	p	η^2
Extraction Method	2190.857	3	730.286	99.209	< 0.001***	0.903
Residuals	235.554	32	7.361			

Note. Type III Sum of Squares

Post Hoc Tests

Post Hoc Comparisons - Extraction Method

		Mean Difference	SE	t	p_{tukey}
CSE	MAE	-10.428	1.279	-8.153	< 0.001***
	Maceration	11.560	1.279	9.039	< 0.001***
	Reflux	-1.025	1.279	-0.802	0.853
MAE	Maceration	21.988	1.279	17.192	< 0.001***
	Reflux	9.403	1.279	7.352	< 0.001***
Maceration	Reflux	-12.586	1.279	-9.840	< 0.001***

*** p < 0.001

Note. P-value adjusted for comparing a family of 4

F.3.2 Total Monomeric Anthocyanin (TMA)

ANOVA - Total Monomeric Anthocyanin

Cases	Sum of Squares	df	Mean Square	F	p	η^2
Extraction Method	0.086	3	0.029	59.226	< 0.001***	0.847
Residuals	0.015	32	4.835×10^{-4}			

Note. Type III Sum of Squares

Post Hoc Tests

Post Hoc Comparisons - Extraction Method

		Mean Difference	SE	t	p_{tukey}
CSE	MAE	-0.079	0.010	-7.623	< 0.001***
	Maceration	0.057	0.010	5.457	< 0.001***
	Reflux	0.011	0.010	1.107	0.688
MAE	Maceration	0.136	0.010	13.080	< 0.001***
	Reflux	0.090	0.010	8.730	< 0.001***
Maceration	Reflux	-0.045	0.010	-4.350	< 0.001***

** p < 0.01, *** p < 0.001

Note. P-value adjusted for comparing a family of 4

F.3.3 Antioxidant Activity (AOA)

ANOVA - Antioxidant Activity

Cases	Sum of Squares	df	Mean Square	F	p	η^2
Extraction Method	0.125	3	0.042	0.650	0.589	0.057
Residuals	2.043	32	0.064			

Note. Type III Sum of Squares

F.3.4 Correlation between TPC, TMA, and AOA on extraction method investigation

Pearson's Correlations

Variable		Total Phenolic Content	Total Monomeric Anthocyanin	Antioxidant Activity
1. Total Phenolic Content	Pearson's r	—		
	p-value	—		
2. Total Monomeric Anthocyanin	Pearson's r	0.875***	—	
	p-value	< 0.001	—	
3. Antioxidant Activity	Pearson's r	-0.070	-0.149	—
	p-value	0.686	0.386	—

* p < 0.05, ** p < 0.01, *** p < 0.001

Appendix F.4 Test of Significance the effect of CPH particle size on Total Phenolic Content (TPC), Total Monomeric Anthocyanin (TMA), and Antioxidant Activity (AOA)

F.4.1 Total Phenolic Content (TPC)

ANOVA - Total Phenolic Content

Cases	Sum of Squares	df	Mean Square	F	p	η^2
CPH Particle Size	40080.478	3	13360.159	14114.372	< 0.001***	0.999
Residuals	30.290	32	0.947			

Note. Type III Sum of Squares

Post Hoc Tests

Post Hoc Comparisons - CPH Particle Size

		Mean Difference	SE	t	p_{tukey}	
≤ 38 micron	(125-150 micron)	61.492	0.459	134.077	< 0.001	***
	(63-90 micron)	38.238	0.459	83.372	< 0.001	***
	without grinding (0.5x0.5 cm)	91.270	0.459	199.003	< 0.001	***
(125-150 micron)	(63-90 micron)	-23.255	0.459	-50.705	< 0.001	***
	without grinding (0.5x0.5 cm)	29.777	0.459	64.926	< 0.001	***
(63-90 micron)	without grinding (0.5x0.5 cm)	53.032	0.459	115.630	< 0.001	***

*** p < 0.001

Note. P-value adjusted for comparing a family of 4

F.4.2 Total Monomeric Anthocyanin (TMA)

ANOVA - Total Monomeric Anthocyanin

Cases	Sum of Squares	df	Mean Square	F	p	η^2
CPH Particle Size	0.161	3	0.054	152.943	< 0.001***	0.935
Residuals	0.011	32	3.503×10^{-4}			

Note. Type III Sum of Squares

Post Hoc Tests

Post Hoc Comparisons - CPH Particle Size

		Mean Difference	SE	t	p_{tukey}
≤ 38 micron	(125-150 micron)	0.090	0.009	10.183	< 0.001 ***
	(63-90 micron)	0.077	0.009	8.674	< 0.001 ***
	without grinding (0.5x0.5 cm)	0.188	0.009	21.297	< 0.001 ***
(125-150 micron)	(63-90 micron)	-0.013	0.009	-1.509	0.444
	without grinding (0.5x0.5 cm)	0.098	0.009	11.114	< 0.001 ***
(63-90 micron)	without grinding (0.5x0.5 cm)	0.111	0.009	12.623	< 0.001 ***

*** p < 0.001

Note. P-value adjusted for comparing a family of 4

F.4.3 Antioxidant Activity (AOA)

ANOVA - Antioxidant Activity

Cases	Sum of Squares	df	Mean Square	F	p	η^2
CPH Particle Size	1.143	3	0.381	14.211	< 0.001***	0.571
Residuals	0.858	32	0.027			

Note. Type III Sum of Squares

Post Hoc Tests

Post Hoc Comparisons - CPH Particle Size

		Mean Difference	SE	t	p_{tukey}
≤ 38 micron	(125-150 micron)	-0.357	0.077	-4.619	< 0.001***
	(63-90 micron)	-0.125	0.077	-1.614	0.385
	without grinding (0.5x0.5 cm)	-0.447	0.077	-5.788	< 0.001***
(125-150 micron)	(63-90 micron)	0.232	0.077	3.006	0.025*
	without grinding (0.5x0.5 cm)	-0.090	0.077	-1.169	0.651
(63-90 micron)	without grinding (0.5x0.5 cm)	-0.322	0.077	-4.174	0.001**

* p < 0.05, ** p < 0.01, *** p < 0.001

Note. P-value adjusted for comparing a family of 4

F.4.4 Correlation between TPC, TMA, and AOA on CPH particle size effect

Pearson's Correlations

Variable		Total Phenolic Content	Total Monomeric Anthocyanin	Antioxidant Activity
1. Total Phenolic Content	Pearson's r	—		
	p-value	—		
2. Total Monomeric Anthocyanin	Pearson's r	0.975*	—	
	p-value	0.025	—	
3. Antioxidant Activity	Pearson's r	-0.979*	-0.929	—
	p-value	0.021	0.071	—

* p < 0.05, ** p < 0.01, *** p < 0.001

Appendix F.5 Test of Significance the effect of extraction time (1, 5, 10, 15, and 30 min), temperature (50, 60, and 70 °C) and heating method (MAE and CSE) on Total Phenolic Content (TPC), Total Monomeric Anthocyanin (TMA), and Antioxidant Activity (AOA)

F.5.1 Total Phenolic Content (TPC)

ANOVA - Total Phenolic Content

Cases	Sum of Squares	df	Mean Square	F	p	η^2
Time	2355.448	4	588.862	176.683	< 0.001***	0.330
Temperature	1702.581	2	851.291	255.422	< 0.001***	0.239
Heating method	0.940	1	0.940	0.282	0.596	1.318×10^{-4}
Time ✻ Temperature	843.739	8	105.467	31.645	< 0.001***	0.118
Time ✻ Heating method	384.985	4	96.246	28.878	< 0.001***	0.054
Temperature ✻ Heating method	694.722	2	347.361	104.222	< 0.001***	0.097
Time ✻ Temperature ✻ Heating method	346.080	8	43.260	12.980	< 0.001***	0.049
Residuals	799.891	240	3.333			

Note. Type III Sum of Squares

Post Hoc Tests

Post Hoc Comparisons - Time

		Mean Difference	SE	t	p_{tukey}
1	5	-6.178	0.351	-17.585	< 0.001***
	10	-3.106	0.351	-8.839	< 0.001***
	15	-1.604	0.351	-4.565	< 0.001***
	30	2.625	0.351	7.470	< 0.001***
5	10	3.073	0.351	8.745	< 0.001***
	15	4.574	0.351	13.020	< 0.001***
	30	8.803	0.351	25.055	< 0.001***
10	15	1.502	0.351	4.274	< 0.001***
	30	5.730	0.351	16.309	< 0.001***
15	30	4.228	0.351	12.035	< 0.001***

** p < 0.01, *** p <0.001

Note. P-value adjusted for comparing a family of 5

Note. Results are averaged over the levels of: Temperature, Heating method

Post Hoc Comparisons - Temperature

		Mean Difference	SE	t	p_{tukey}
50	60	-6.106	0.272	-22.435	< 0.001***
	70	-3.698	0.272	-13.589	< 0.001***
60	70	2.408	0.272	8.847	< 0.001***

*** p < 0.001

Note. P-value adjusted for comparing a family of 3

Note. Results are averaged over the levels of: Time, Heating method

Post Hoc Comparisons - Heating method

		Mean Difference	SE	t	p_{tukey}
CSE	MAE	0.118	0.222	0.531	0.596

Note. Results are averaged over the levels of: Time, Temperature

Post Hoc Comparisons - Time ✻ Temperature

		Mean Difference	SE	t	p_{tukey}
1 50	5 50	-3.250	0.609	-5.341	< 0.001 ***
	10 50	0.276	0.609	0.454	1.000
	15 50	1.762	0.609	2.895	0.206
	30 50	8.809	0.609	14.475	< 0.001 ***
	1 60	-3.239	0.609	-5.322	< 0.001 ***
	5 60	-7.803	0.609	-12.823	< 0.001 ***
	10 60	-4.979	0.609	-8.182	< 0.001 ***
	15 60	-3.740	0.609	-6.145	< 0.001 ***
	30 60	-3.171	0.609	-5.211	< 0.001 ***
	1 70	2.951	0.609	4.849	< 0.001 ***
	5 70	-7.769	0.609	-12.767	< 0.001 ***
	10 70	-4.902	0.609	-8.056	< 0.001 ***
	15 70	-3.121	0.609	-5.129	< 0.001 ***
	30 70	1.948	0.609	3.201	0.096
5 50	10 50	3.526	0.609	5.795	< 0.001 ***
	15 50	5.012	0.609	8.236	< 0.001 ***
	30 50	12.059	0.609	19.816	< 0.001 ***
	1 60	0.011	0.609	0.019	1.000
	5 60	-4.553	0.609	-7.483	< 0.001 ***
	10 60	-1.729	0.609	-2.841	0.233
	15 60	-0.490	0.609	-0.805	1.000
	30 60	0.079	0.609	0.129	1.000
	1 70	6.201	0.609	10.189	< 0.001 ***
	5 70	-4.519	0.609	-7.426	< 0.001 ***
	10 70	-1.652	0.609	-2.715	0.302
	15 70	0.129	0.609	0.211	1.000
	30 70	5.198	0.609	8.542	< 0.001 ***
10 50	15 50	1.485	0.609	2.441	0.487
	30 50	8.532	0.609	14.021	< 0.001 ***
	1 60	-3.515	0.609	-5.776	< 0.001 ***
	5 60	-8.080	0.609	-13.277	< 0.001 ***
	10 60	-5.255	0.609	-8.636	< 0.001 ***
	15 60	-4.016	0.609	-6.599	< 0.001 ***
	30 60	-3.447	0.609	-5.665	< 0.001 ***
	1 70	2.674	0.609	4.395	0.002 **
	5 70	-8.045	0.609	-13.221	< 0.001 ***
	10 70	-5.178	0.609	-8.510	< 0.001 ***
	15 70	-3.398	0.609	-5.583	< 0.001 ***
	30 70	1.672	0.609	2.747	0.284
15 50	30 50	7.047	0.609	11.580	< 0.001 ***
	1 60	-5.000	0.609	-8.217	< 0.001 ***
	5 60	-9.565	0.609	-15.718	< 0.001 ***
	10 60	-6.741	0.609	-11.077	< 0.001 ***
	15 60	-5.502	0.609	-9.041	< 0.001 ***
	30 60	-4.933	0.609	-8.106	< 0.001 ***

Post Hoc Comparisons - Time ✻ Temperature

		Mean Difference	SE	t	p_{tukey}	
	1 70	1.189	0.609	1.954	0.823	
	5 70	-9.531	0.609	-15.662	< 0.001	***
	10 70	-6.664	0.609	-10.951	< 0.001	***
	15 70	-4.883	0.609	-8.024	< 0.001	***
	30 70	0.186	0.609	0.306	1.000	
30 50	1 60	-12.047	0.609	-19.797	< 0.001	***
	5 60	-16.612	0.609	-27.298	< 0.001	***
	10 60	-13.788	0.609	-22.657	< 0.001	***
	15 60	-12.549	0.609	-20.621	< 0.001	***
	30 60	-11.980	0.609	-19.686	< 0.001	***
	1 70	-5.858	0.609	-9.626	< 0.001	***
	5 70	-16.578	0.609	-27.242	< 0.001	***
	10 70	-13.711	0.609	-22.531	< 0.001	***
	15 70	-11.930	0.609	-19.605	< 0.001	***
	30 70	-6.861	0.609	-11.274	< 0.001	***
1 60	5 60	-4.565	0.609	-7.501	< 0.001	***
	10 60	-1.740	0.609	-2.860	0.223	
	15 60	-0.501	0.609	-0.824	1.000	
	30 60	0.067	0.609	0.111	1.000	
	1 70	6.189	0.609	10.171	< 0.001	***
	5 70	-4.531	0.609	-7.445	< 0.001	***
	10 70	-1.664	0.609	-2.734	0.291	
	15 70	0.117	0.609	0.192	1.000	
	30 70	5.187	0.609	8.523	< 0.001	***
5 60	10 60	2.824	0.609	4.641	< 0.001	***
	15 60	4.064	0.609	6.678	< 0.001	***
	30 60	4.632	0.609	7.612	< 0.001	***
	1 70	10.754	0.609	17.672	< 0.001	***
	5 70	0.034	0.609	0.056	1.000	
	10 70	2.901	0.609	4.768	< 0.001	***
	15 70	4.682	0.609	7.694	< 0.001	***
	30 70	9.751	0.609	16.024	< 0.001	***
10 60	15 60	1.239	0.609	2.036	0.775	
	30 60	1.808	0.609	2.971	0.173	
	1 70	7.930	0.609	13.031	< 0.001	***
	5 70	-2.790	0.609	-4.585	< 0.001	***
	10 70	0.077	0.609	0.126	1.000	
	15 70	1.858	0.609	3.052	0.142	
	30 70	6.927	0.609	11.383	< 0.001	***
15 60	30 60	0.569	0.609	0.934	1.000	
	1 70	6.690	0.609	10.994	< 0.001	***
	5 70	-4.029	0.609	-6.621	< 0.001	***
	10 70	-1.162	0.609	-1.910	0.846	
	15 70	0.618	0.609	1.016	1.000	
	30 70	5.688	0.609	9.347	< 0.001	***
30 60	1 70	6.122	0.609	10.060	< 0.001	***
	5 70	-4.598	0.609	-7.556	< 0.001	***
	10 70	-1.731	0.609	-2.844	0.231	
	15 70	0.050	0.609	0.082	1.000	
	30 70	5.119	0.609	8.412	< 0.001	***
1 70	5 70	-10.720	0.609	-17.616	< 0.001	***

Post Hoc Comparisons - Time ✻ Temperature

		Mean Difference	SE	t	p_{tukey}	
	10 70	-7.853	0.609	-12.904	< 0.001	***
	15 70	-6.072	0.609	-9.978	< 0.001	***
	30 70	-1.003	0.609	-1.648	0.946	
5 70	10 70	2.867	0.609	4.711	< 0.001	***
	15 70	4.648	0.609	7.637	< 0.001	***
	30 70	9.717	0.609	15.968	< 0.001	***
10 70	15 70	1.781	0.609	2.926	0.192	
	30 70	6.850	0.609	11.257	< 0.001	***
15 70	30 70	5.069	0.609	8.331	< 0.001	***

* p < 0.05, ** p <0.01, *** p < 0.001

Note. P-value adjusted for comparing a family of 15

Note. Results are averaged over the levels of: Heating method

Post Hoc Comparisons - Time ✻ Heating method

		Mean Difference	SE	t	p_{tukey}	
1 CSE	5 CSE	-4.208	0.497	-8.468	< 0.001	***
	10 CSE	-1.356	0.497	-2.729	0.168	
	15 CSE	1.434	0.497	2.887	0.115	
	30 CSE	2.592	0.497	5.216	< 0.001	***
	1 MAE	2.808	0.497	5.652	< 0.001	***
	5 MAE	-5.340	0.497	-10.748	< 0.001	***
	10 MAE	-2.047	0.497	-4.120	0.002	**
	15 MAE	-1.834	0.497	-3.691	0.010	*
	30 MAE	5.466	0.497	11.000	< 0.001	***
5 CSE	10 CSE	2.852	0.497	5.740	< 0.001	***
	15 CSE	5.642	0.497	11.355	< 0.001	***
	30 CSE	6.799	0.497	13.684	< 0.001	***
	1 MAE	7.016	0.497	14.120	< 0.001	***
	5 MAE	-1.133	0.497	-2.280	0.406	
	10 MAE	2.161	0.497	4.348	< 0.001	***
	15 MAE	2.374	0.497	4.778	< 0.001	***
	30 MAE	9.673	0.497	19.468	< 0.001	***
10 CSE	15 CSE	2.790	0.497	5.615	< 0.001	***
	30 CSE	3.948	0.497	7.945	< 0.001	***
	1 MAE	4.164	0.497	8.381	< 0.001	***
	5 MAE	-3.985	0.497	-8.019	< 0.001	***
	10 MAE	-0.691	0.497	-1.391	0.929	
	15 MAE	-0.478	0.497	-0.962	0.994	
	30 MAE	6.821	0.497	13.729	< 0.001	***
15 CSE	30 CSE	1.157	0.497	2.329	0.374	
	1 MAE	1.374	0.497	2.765	0.155	
	5 MAE	-6.775	0.497	-13.635	< 0.001	***
	10 MAE	-3.482	0.497	-7.007	< 0.001	***
	15 MAE	-3.268	0.497	-6.577	< 0.001	***
	30 MAE	4.031	0.497	8.113	< 0.001	***
30 CSE	1 MAE	0.217	0.497	0.436	1.000	
	5 MAE	-7.932	0.497	-15.964	< 0.001	***
	10 MAE	-4.639	0.497	-9.336	< 0.001	***
	15 MAE	-4.425	0.497	-8.907	< 0.001	***
	30 MAE	2.874	0.497	5.784	< 0.001	***

Post Hoc Comparisons - Time ✻ Heating method

		Mean Difference	SE	t	p_{tukey}	
1 MAE	5 MAE	-8.149	0.497	-16.400	< 0.001	***
	10 MAE	-4.855	0.497	-9.772	< 0.001	***
	15 MAE	-4.642	0.497	-9.342	< 0.001	***
	30 MAE	2.657	0.497	5.348	< 0.001	***
5 MAE	10 MAE	3.293	0.497	6.628	< 0.001	***
	15 MAE	3.507	0.497	7.058	< 0.001	***
	30 MAE	10.806	0.497	21.748	< 0.001	***
10 MAE	15 MAE	0.213	0.497	0.430	1.000	
	30 MAE	7.513	0.497	15.120	< 0.001	***
15 MAE	30 MAE	7.299	0.497	14.691	< 0.001	***

Note. Results are averaged over the levels of: Temperature

Note. P-value adjusted for comparing a family of 10

* p < 0.05, ** p < 0.01, *** p < 0.001

Post Hoc Comparisons - Temperature ✻ Heating method

		Mean Difference	SE	t	p_{tukey}	
50 CSE	60 CSE	-4.564	0.385	-11.858	< 0.001	***
	70 CSE	-6.057	0.385	-15.738	< 0.001	***
	50 MAE	-0.427	0.385	-1.109	0.877	
	60 MAE	-8.074	0.385	-20.979	< 0.001	***
	70 MAE	-1.766	0.385	-4.588	< 0.001	***
60 CSE	70 CSE	-1.493	0.385	-3.880	0.002	**
	50 MAE	4.137	0.385	10.749	< 0.001	***
	60 MAE	-3.510	0.385	-9.121	< 0.001	***
	70 MAE	2.798	0.385	7.270	< 0.001	***
70 CSE	50 MAE	5.630	0.385	14.629	< 0.001	***
	60 MAE	-2.017	0.385	-5.241	< 0.001	***
	70 MAE	4.291	0.385	11.149	< 0.001	***
50 MAE	60 MAE	-7.648	0.385	-19.870	< 0.001	***
	70 MAE	-1.339	0.385	-3.480	0.008	**
60 MAE	70 MAE	6.308	0.385	16.391	< 0.001	***

* p < 0.05, ** p < 0.01, *** p < 0.001

Note. P-value adjusted for comparing a family of 6

Note. Results are averaged over the levels of: Time

Post Hoc Comparisons - Time ✻ Temperature ✻ Heating method

		Mean Difference	SE	t	p_{tukey}	
1 50 CSE	5 50 CSE	-4.027	0.861	-4.680	0.002	**
	10 50 CSE	0.059	0.861	0.068	1.000	
	15 50 CSE	2.971	0.861	3.453	0.138	
	30 50 CSE	5.672	0.861	6.591	< 0.001	***
	1 60 CSE	-2.805	0.861	-3.259	0.226	
	5 60 CSE	-6.247	0.861	-7.259	< 0.001	***
	10 60 CSE	-4.492	0.861	-5.219	< 0.001	***
	15 60 CSE	-2.072	0.861	-2.408	0.834	
	30 60 CSE	-2.528	0.861	-2.938	0.439	
	1 70 CSE	-4.089	0.861	-4.751	0.001	**
	5 70 CSE	-9.242	0.861	-10.739	< 0.001	***
	10 70 CSE	-6.528	0.861	-7.585	< 0.001	***

Post Hoc Comparisons - Time ✻ Temperature ✻ Heating method

		Mean Difference	SE	t	p_{tukey}
	15 70 CSE	-3.490	0.861	-4.055	0.021 *
	30 70 CSE	-2.262	0.861	-2.629	0.684
	1 50 MAE	-1.595	0.861	-1.854	0.991
	5 50 MAE	-4.068	0.861	-4.727	0.001 **
	10 50 MAE	-1.102	0.861	-1.280	1.000
	15 50 MAE	-1.043	0.861	-1.212	1.000
	30 50 MAE	10.350	0.861	12.026	< 0.001 ***
	1 60 MAE	-5.268	0.861	-6.121	< 0.001 ***
	5 60 MAE	-10.955	0.861	-12.729	< 0.001 ***
	10 60 MAE	-7.061	0.861	-8.205	< 0.001 ***
	15 60 MAE	-7.003	0.861	-8.137	< 0.001 ***
	30 60 MAE	-5.409	0.861	-6.286	< 0.001 ***
	1 70 MAE	8.395	0.861	9.754	< 0.001 ***
	5 70 MAE	-7.892	0.861	-9.170	< 0.001 ***
	10 70 MAE	-4.872	0.861	-5.661	< 0.001 ***
	15 70 MAE	-4.349	0.861	-5.053	< 0.001 ***
	30 70 MAE	4.563	0.861	5.302	< 0.001 ***
5 50 CSE	10 50 CSE	4.086	0.861	4.748	0.001 **
	15 50 CSE	6.999	0.861	8.132	< 0.001 ***
	30 50 CSE	9.700	0.861	11.271	< 0.001 ***
	1 60 CSE	1.223	0.861	1.421	1.000
	5 60 CSE	-2.220	0.861	-2.580	0.721
	10 60 CSE	-0.465	0.861	-0.540	1.000
	15 60 CSE	1.955	0.861	2.272	0.902
	30 60 CSE	1.499	0.861	1.742	0.997
	1 70 CSE	-0.061	0.861	-0.071	1.000
	5 70 CSE	-5.214	0.861	-6.059	< 0.001 ***
	10 70 CSE	-2.500	0.861	-2.905	0.464
	15 70 CSE	0.538	0.861	0.625	1.000
	30 70 CSE	1.765	0.861	2.051	0.968
	1 50 MAE	2.432	0.861	2.826	0.528
	5 50 MAE	-0.041	0.861	-0.047	1.000
	10 50 MAE	2.926	0.861	3.400	0.159
	15 50 MAE	2.984	0.861	3.467	0.133
	30 50 MAE	14.377	0.861	16.706	< 0.001 ***
	1 60 MAE	-1.240	0.861	-1.441	1.000
	5 60 MAE	-6.927	0.861	-8.050	< 0.001 ***
	10 60 MAE	-3.034	0.861	-3.526	0.113
	15 60 MAE	-2.976	0.861	-3.457	0.137
	30 60 MAE	-1.382	0.861	-1.606	0.999
	1 70 MAE	12.422	0.861	14.434	< 0.001 ***
	5 70 MAE	-3.865	0.861	-4.490	0.004 **
	10 70 MAE	-0.845	0.861	-0.981	1.000
	15 70 MAE	-0.321	0.861	-0.373	1.000
	30 70 MAE	8.590	0.861	9.982	< 0.001 ***
10 50 CSE	15 50 CSE	2.913	0.861	3.384	0.166
	30 50 CSE	5.613	0.861	6.523	< 0.001 ***
	1 60 CSE	-2.864	0.861	-3.327	0.192
	5 60 CSE	-6.306	0.861	-7.328	< 0.001 ***
	10 60 CSE	-4.551	0.861	-5.288	< 0.001 ***
	15 60 CSE	-2.131	0.861	-2.476	0.792

Post Hoc Comparisons - Time ✻ Temperature ✻ Heating method

		Mean Difference	SE	t	p_{tukey}
	30 60 CSE	-2.587	0.861	-3.006	0.388
	1 70 CSE	-4.147	0.861	-4.819	< 0.001 ***
	5 70 CSE	-9.301	0.861	-10.807	< 0.001 ***
	10 70 CSE	-6.587	0.861	-7.653	< 0.001 ***
	15 70 CSE	-3.548	0.861	-4.123	0.016 *
	30 70 CSE	-2.321	0.861	-2.697	0.631
	1 50 MAE	-1.654	0.861	-1.922	0.986
	5 50 MAE	-4.127	0.861	-4.795	0.001 **
	10 50 MAE	-1.160	0.861	-1.348	1.000
	15 50 MAE	-1.102	0.861	-1.281	1.000
	30 50 MAE	10.291	0.861	11.958	< 0.001 ***
	1 60 MAE	-5.327	0.861	-6.189	< 0.001 ***
	5 60 MAE	-11.014	0.861	-12.798	< 0.001 ***
	10 60 MAE	-7.120	0.861	-8.274	< 0.001 ***
	15 60 MAE	-7.062	0.861	-8.205	< 0.001 ***
	30 60 MAE	-5.468	0.861	-6.354	< 0.001 ***
	1 70 MAE	8.336	0.861	9.686	< 0.001 ***
	5 70 MAE	-7.951	0.861	-9.238	< 0.001 ***
	10 70 MAE	-4.931	0.861	-5.729	< 0.001 ***
	15 70 MAE	-4.407	0.861	-5.121	< 0.001 ***
	30 70 MAE	4.504	0.861	5.234	< 0.001 ***
15 50 CSE	30 50 CSE	2.701	0.861	3.138	0.297
	1 60 CSE	-5.776	0.861	-6.712	< 0.001 ***
	5 60 CSE	-9.219	0.861	-10.712	< 0.001 ***
	10 60 CSE	-7.463	0.861	-8.672	< 0.001 ***
	15 60 CSE	-5.043	0.861	-5.860	< 0.001 ***
	30 60 CSE	-5.500	0.861	-6.390	< 0.001 ***
	1 70 CSE	-7.060	0.861	-8.204	< 0.001 ***
	5 70 CSE	-12.213	0.861	-14.191	< 0.001 ***
	10 70 CSE	-9.499	0.861	-11.038	< 0.001 ***
	15 70 CSE	-6.461	0.861	-7.508	< 0.001 ***
	30 70 CSE	-5.234	0.861	-6.081	< 0.001 ***
	1 50 MAE	-4.567	0.861	-5.307	< 0.001 ***
	5 50 MAE	-7.039	0.861	-8.180	< 0.001 ***
	10 50 MAE	-4.073	0.861	-4.733	0.001 **
	15 50 MAE	-4.015	0.861	-4.665	0.002 **
	30 50 MAE	7.378	0.861	8.574	< 0.001 ***
	1 60 MAE	-8.239	0.861	-9.574	< 0.001 ***
	5 60 MAE	-13.926	0.861	-16.182	< 0.001 ***
	10 60 MAE	-10.033	0.861	-11.658	< 0.001 ***
	15 60 MAE	-9.974	0.861	-11.590	< 0.001 ***
	30 60 MAE	-8.381	0.861	-9.738	< 0.001 ***
	1 70 MAE	5.423	0.861	6.302	< 0.001 ***
	5 70 MAE	-10.863	0.861	-12.623	< 0.001 ***
	10 70 MAE	-7.843	0.861	-9.114	< 0.001 ***
	15 70 MAE	-7.320	0.861	-8.506	< 0.001 ***
	30 70 MAE	1.592	0.861	1.849	0.992
30 50 CSE	1 60 CSE	-8.477	0.861	-9.850	< 0.001 ***
	5 60 CSE	-11.920	0.861	-13.850	< 0.001 ***
	10 60 CSE	-10.164	0.861	-11.810	< 0.001 ***
	15 60 CSE	-7.744	0.861	-8.999	< 0.001 ***

Post Hoc Comparisons - Time ✻ Temperature ✻ Heating method

		Mean Difference	SE	t	p_{tukey}
	30 60 CSE	-8.200	0.861	-9.529	< 0.001 ***
	1 70 CSE	-9.761	0.861	-11.342	< 0.001 ***
	5 70 CSE	-14.914	0.861	-17.330	< 0.001 ***
	10 70 CSE	-12.200	0.861	-14.176	< 0.001 ***
	15 70 CSE	-9.162	0.861	-10.646	< 0.001 ***
	30 70 CSE	-7.934	0.861	-9.220	< 0.001 ***
	1 50 MAE	-7.268	0.861	-8.445	< 0.001 ***
	5 50 MAE	-9.740	0.861	-11.318	< 0.001 ***
	10 50 MAE	-6.774	0.861	-7.871	< 0.001 ***
	15 50 MAE	-6.715	0.861	-7.803	< 0.001 ***
	30 50 MAE	4.678	0.861	5.435	< 0.001 ***
	1 60 MAE	-10.940	0.861	-12.712	< 0.001 ***
	5 60 MAE	-16.627	0.861	-19.320	< 0.001 ***
	10 60 MAE	-12.734	0.861	-14.796	< 0.001 ***
	15 60 MAE	-12.675	0.861	-14.728	< 0.001 ***
	30 60 MAE	-11.082	0.861	-12.877	< 0.001 ***
	1 70 MAE	2.722	0.861	3.163	0.281
	5 70 MAE	-13.564	0.861	-15.761	< 0.001 ***
	10 70 MAE	-10.544	0.861	-12.252	< 0.001 ***
	15 70 MAE	-10.021	0.861	-11.644	< 0.001 ***
	30 70 MAE	-1.109	0.861	-1.289	1.000
1 60 CSE	5 60 CSE	-3.443	0.861	-4.000	0.025 *
	10 60 CSE	-1.687	0.861	-1.960	0.982
	15 60 CSE	0.733	0.861	0.851	1.000
	30 60 CSE	0.276	0.861	0.321	1.000
	1 70 CSE	-1.284	0.861	-1.492	1.000
	5 70 CSE	-6.437	0.861	-7.480	< 0.001 ***
	10 70 CSE	-3.723	0.861	-4.326	0.008 **
	15 70 CSE	-0.685	0.861	-0.796	1.000
	30 70 CSE	0.542	0.861	0.630	1.000
	1 50 MAE	1.209	0.861	1.405	1.000
	5 50 MAE	-1.263	0.861	-1.468	1.000
	10 50 MAE	1.703	0.861	1.979	0.979
	15 50 MAE	1.761	0.861	2.047	0.968
	30 50 MAE	13.155	0.861	15.285	< 0.001 ***
	1 60 MAE	-2.463	0.861	-2.862	0.499
	5 60 MAE	-8.150	0.861	-9.470	< 0.001 ***
	10 60 MAE	-4.257	0.861	-4.946	< 0.001 ***
	15 60 MAE	-4.198	0.861	-4.878	< 0.001 ***
	30 60 MAE	-2.605	0.861	-3.027	0.373
	1 70 MAE	11.199	0.861	13.013	< 0.001 ***
	5 70 MAE	-5.087	0.861	-5.911	< 0.001 ***
	10 70 MAE	-2.067	0.861	-2.402	0.837
	15 70 MAE	-1.544	0.861	-1.794	0.995
	30 70 MAE	7.368	0.861	8.561	< 0.001 ***
5 60 CSE	10 60 CSE	1.756	0.861	2.040	0.970
	15 60 CSE	4.175	0.861	4.852	< 0.001 ***
	30 60 CSE	3.719	0.861	4.322	0.008 **
	1 70 CSE	2.159	0.861	2.508	0.771
	5 70 CSE	-2.994	0.861	-3.479	0.129
	10 70 CSE	-0.280	0.861	-0.326	1.000

Post Hoc Comparisons - Time ✻ Temperature ✻ Heating method

		Mean Difference	SE	t	p_{tukey}
	15 70 CSE	2.758	0.861	3.204	0.257
	30 70 CSE	3.985	0.861	4.631	0.002 **
	1 50 MAE	4.652	0.861	5.405	< 0.001 ***
	5 50 MAE	2.179	0.861	2.532	0.754
	10 50 MAE	5.146	0.861	5.979	< 0.001 ***
	15 50 MAE	5.204	0.861	6.047	< 0.001 ***
	30 50 MAE	16.597	0.861	19.286	< 0.001 ***
	1 60 MAE	0.980	0.861	1.138	1.000
	5 60 MAE	-4.707	0.861	-5.470	< 0.001 ***
	10 60 MAE	-0.814	0.861	-0.946	1.000
	15 60 MAE	-0.755	0.861	-0.878	1.000
	30 60 MAE	0.838	0.861	0.974	1.000
	1 70 MAE	14.642	0.861	17.014	< 0.001 ***
	5 70 MAE	-1.644	0.861	-1.911	0.987
	10 70 MAE	1.376	0.861	1.598	0.999
	15 70 MAE	1.899	0.861	2.206	0.927
	30 70 MAE	10.810	0.861	12.561	< 0.001 ***
10 60 CSE	15 60 CSE	2.420	0.861	2.812	0.539
	30 60 CSE	1.964	0.861	2.282	0.898
	1 70 CSE	0.403	0.861	0.469	1.000
	5 70 CSE	-4.750	0.861	-5.519	< 0.001 ***
	10 70 CSE	-2.036	0.861	-2.366	0.857
	15 70 CSE	1.002	0.861	1.164	1.000
	30 70 CSE	2.230	0.861	2.591	0.712
	1 50 MAE	2.896	0.861	3.366	0.174
	5 50 MAE	0.424	0.861	0.493	1.000
	10 50 MAE	3.390	0.861	3.939	0.031 *
	15 50 MAE	3.449	0.861	4.007	0.024 *
	30 50 MAE	14.842	0.861	17.246	< 0.001 ***
	1 60 MAE	-0.776	0.861	-0.902	1.000
	5 60 MAE	-6.463	0.861	-7.510	< 0.001 ***
	10 60 MAE	-2.570	0.861	-2.986	0.403
	15 60 MAE	-2.511	0.861	-2.918	0.455
	30 60 MAE	-0.918	0.861	-1.066	1.000
	1 70 MAE	12.886	0.861	14.974	< 0.001 ***
	5 70 MAE	-3.400	0.861	-3.951	0.030 *
	10 70 MAE	-0.380	0.861	-0.442	1.000
	15 70 MAE	0.143	0.861	0.167	1.000
	30 70 MAE	9.055	0.861	10.521	< 0.001 ***
15 60 CSE	30 60 CSE	-0.456	0.861	-0.530	1.000
	1 70 CSE	-2.017	0.861	-2.343	0.869
	5 70 CSE	-7.170	0.861	-8.331	< 0.001 ***
	10 70 CSE	-4.456	0.861	-5.177	< 0.001 ***
	15 70 CSE	-1.418	0.861	-1.647	0.999
	30 70 CSE	-0.190	0.861	-0.221	1.000
	1 50 MAE	0.477	0.861	0.554	1.000
	5 50 MAE	-1.996	0.861	-2.319	0.881
	10 50 MAE	0.970	0.861	1.128	1.000
	15 50 MAE	1.029	0.861	1.195	1.000
	30 50 MAE	12.422	0.861	14.434	< 0.001 ***
	1 60 MAE	-3.196	0.861	-3.713	0.065

Post Hoc Comparisons - Time ✻ Temperature ✻ Heating method

		Mean Difference	SE	t	p_{tukey}
	5 60 MAE	-8.883	0.861	-10.322	< 0.001 ***
	10 60 MAE	-4.989	0.861	-5.798	< 0.001 ***
	15 60 MAE	-4.931	0.861	-5.729	< 0.001 ***
	30 60 MAE	-3.337	0.861	-3.878	0.038 *
	1 70 MAE	10.467	0.861	12.162	< 0.001 ***
	5 70 MAE	-5.820	0.861	-6.762	< 0.001 ***
	10 70 MAE	-2.800	0.861	-3.253	0.229
	15 70 MAE	-2.277	0.861	-2.645	0.671
	30 70 MAE	6.635	0.861	7.710	< 0.001 ***
30 60 CSE	1 70 CSE	-1.560	0.861	-1.813	0.994
	5 70 CSE	-6.713	0.861	-7.801	< 0.001 ***
	10 70 CSE	-4.000	0.861	-4.647	0.002 **
	15 70 CSE	-0.961	0.861	-1.117	1.000
	30 70 CSE	0.266	0.861	0.309	1.000
	1 50 MAE	0.933	0.861	1.084	1.000
	5 50 MAE	-1.540	0.861	-1.789	0.995
	10 50 MAE	1.427	0.861	1.658	0.998
	15 50 MAE	1.485	0.861	1.726	0.997
	30 50 MAE	12.878	0.861	14.964	< 0.001 ***
	1 60 MAE	-2.739	0.861	-3.183	0.269
	5 60 MAE	-8.427	0.861	-9.791	< 0.001 ***
	10 60 MAE	-4.533	0.861	-5.267	< 0.001 ***
	15 60 MAE	-4.475	0.861	-5.199	< 0.001 ***
	30 60 MAE	-2.881	0.861	-3.348	0.182
	1 70 MAE	10.923	0.861	12.692	< 0.001 ***
	5 70 MAE	-5.364	0.861	-6.232	< 0.001 ***
	10 70 MAE	-2.344	0.861	-2.723	0.610
	15 70 MAE	-1.820	0.861	-2.115	0.954
	30 70 MAE	7.091	0.861	8.240	< 0.001 ***
1 70 CSE	5 70 CSE	-5.153	0.861	-5.988	< 0.001 ***
	10 70 CSE	-2.439	0.861	-2.834	0.521
	15 70 CSE	0.599	0.861	0.696	1.000
	30 70 CSE	1.826	0.861	2.122	0.952
	1 50 MAE	2.493	0.861	2.897	0.471
	5 50 MAE	0.021	0.861	0.024	1.000
	10 50 MAE	2.987	0.861	3.471	0.132
	15 50 MAE	3.045	0.861	3.539	0.109
	30 50 MAE	14.439	0.861	16.777	< 0.001 ***
	1 60 MAE	-1.179	0.861	-1.370	1.000
	5 60 MAE	-6.866	0.861	-7.978	< 0.001 ***
	10 60 MAE	-2.973	0.861	-3.454	0.138
	15 60 MAE	-2.914	0.861	-3.386	0.165
	30 60 MAE	-1.321	0.861	-1.535	1.000
	1 70 MAE	12.483	0.861	14.505	< 0.001 ***
	5 70 MAE	-3.803	0.861	-4.419	0.005 **
	10 70 MAE	-0.783	0.861	-0.910	1.000
	15 70 MAE	-0.260	0.861	-0.302	1.000
	30 70 MAE	8.652	0.861	10.053	< 0.001 ***
5 70 CSE	10 70 CSE	2.714	0.861	3.154	0.288
	15 70 CSE	5.752	0.861	6.684	< 0.001 ***
	30 70 CSE	6.979	0.861	8.110	< 0.001 ***

Post Hoc Comparisons - Time ✻ Temperature ✻ Heating method

		Mean Difference	SE	t	p_{tukey}
	1 50 MAE	7.646	0.861	8.885	< 0.001 ***
	5 50 MAE	5.174	0.861	6.012	< 0.001 ***
	10 50 MAE	8.140	0.861	9.459	< 0.001 ***
	15 50 MAE	8.198	0.861	9.526	< 0.001 ***
	30 50 MAE	19.592	0.861	22.765	< 0.001 ***
	1 60 MAE	3.974	0.861	4.618	0.002 **
	5 60 MAE	-1.713	0.861	-1.991	0.978
	10 60 MAE	2.180	0.861	2.533	0.754
	15 60 MAE	2.239	0.861	2.602	0.704
	30 60 MAE	3.832	0.861	4.453	0.005 **
	1 70 MAE	17.636	0.861	20.493	< 0.001 ***
	5 70 MAE	1.350	0.861	1.569	0.999
	10 70 MAE	4.370	0.861	5.078	< 0.001 ***
	15 70 MAE	4.893	0.861	5.686	< 0.001 ***
	30 70 MAE	13.805	0.861	16.041	< 0.001 ***
10 70 CSE	15 70 CSE	3.038	0.861	3.530	0.112
	30 70 CSE	4.266	0.861	4.956	< 0.001 ***
	1 50 MAE	4.932	0.861	5.731	< 0.001 ***
	5 50 MAE	2.460	0.861	2.858	0.502
	10 50 MAE	5.426	0.861	6.305	< 0.001 ***
	15 50 MAE	5.485	0.861	6.373	< 0.001 ***
	30 50 MAE	16.878	0.861	19.611	< 0.001 ***
	1 60 MAE	1.260	0.861	1.464	1.000
	5 60 MAE	-4.427	0.861	-5.144	< 0.001 ***
	10 60 MAE	-0.534	0.861	-0.620	1.000
	15 60 MAE	-0.475	0.861	-0.552	1.000
	30 60 MAE	1.118	0.861	1.300	1.000
	1 70 MAE	14.922	0.861	17.339	< 0.001 ***
	5 70 MAE	-1.364	0.861	-1.585	0.999
	10 70 MAE	1.656	0.861	1.924	0.986
	15 70 MAE	2.179	0.861	2.532	0.755
	30 70 MAE	11.091	0.861	12.887	< 0.001 ***
15 70 CSE	30 70 CSE	1.227	0.861	1.426	1.000
	1 50 MAE	1.894	0.861	2.201	0.929
	5 50 MAE	-0.578	0.861	-0.672	1.000
	10 50 MAE	2.388	0.861	2.775	0.568
	15 50 MAE	2.446	0.861	2.843	0.514
	30 50 MAE	13.840	0.861	16.081	< 0.001 ***
	1 60 MAE	-1.778	0.861	-2.066	0.965
	5 60 MAE	-7.465	0.861	-8.674	< 0.001 ***
	10 60 MAE	-3.572	0.861	-4.150	0.015 *
	15 60 MAE	-3.513	0.861	-4.082	0.019 *
	30 60 MAE	-1.920	0.861	-2.231	0.918
	1 70 MAE	11.884	0.861	13.809	< 0.001 ***
	5 70 MAE	-4.402	0.861	-5.115	< 0.001 ***
	10 70 MAE	-1.382	0.861	-1.606	0.999
	15 70 MAE	-0.859	0.861	-0.998	1.000
	30 70 MAE	8.053	0.861	9.357	< 0.001 ***
30 70 CSE	1 50 MAE	0.667	0.861	0.775	1.000
	5 50 MAE	-1.806	0.861	-2.098	0.958
	10 50 MAE	1.161	0.861	1.349	1.000

Post Hoc Comparisons - Time ✻ Temperature ✻ Heating method

		Mean Difference	SE	t	p_{tukey}
	15 50 MAE	1.219	0.861	1.416	1.000
	30 50 MAE	12.612	0.861	14.655	< 0.001 ***
	1 60 MAE	-3.006	0.861	-3.492	0.124
	5 60 MAE	-8.693	0.861	-10.101	< 0.001 ***
	10 60 MAE	-4.799	0.861	-5.577	< 0.001 ***
	15 60 MAE	-4.741	0.861	-5.508	< 0.001 ***
	30 60 MAE	-3.147	0.861	-3.657	0.077
	1 70 MAE	10.657	0.861	12.383	< 0.001 ***
	5 70 MAE	-5.630	0.861	-6.541	< 0.001 ***
	10 70 MAE	-2.610	0.861	-3.032	0.369
	15 70 MAE	-2.086	0.861	-2.424	0.824
	30 70 MAE	6.825	0.861	7.931	< 0.001 ***
1 50 MAE	5 50 MAE	-2.473	0.861	-2.873	0.490
	10 50 MAE	0.494	0.861	0.574	1.000
	15 50 MAE	0.552	0.861	0.642	1.000
	30 50 MAE	11.945	0.861	13.880	< 0.001 ***
	1 60 MAE	-3.672	0.861	-4.267	0.009 **
	5 60 MAE	-9.359	0.861	-10.875	< 0.001 ***
	10 60 MAE	-5.466	0.861	-6.351	< 0.001 ***
	15 60 MAE	-5.407	0.861	-6.283	< 0.001 ***
	30 60 MAE	-3.814	0.861	-4.432	0.005 **
	1 70 MAE	9.990	0.861	11.608	< 0.001 ***
	5 70 MAE	-6.296	0.861	-7.316	< 0.001 ***
	10 70 MAE	-3.276	0.861	-3.807	0.048 *
	15 70 MAE	-2.753	0.861	-3.199	0.260
	30 70 MAE	6.158	0.861	7.156	< 0.001 ***
5 50 MAE	10 50 MAE	2.966	0.861	3.447	0.141
	15 50 MAE	3.025	0.861	3.515	0.117
	30 50 MAE	14.418	0.861	16.753	< 0.001 ***
	1 60 MAE	-1.200	0.861	-1.394	1.000
	5 60 MAE	-6.887	0.861	-8.002	< 0.001 ***
	10 60 MAE	-2.993	0.861	-3.478	0.129
	15 60 MAE	-2.935	0.861	-3.410	0.155
	30 60 MAE	-1.341	0.861	-1.559	0.999
	1 70 MAE	12.463	0.861	14.481	< 0.001 ***
	5 70 MAE	-3.824	0.861	-4.443	0.005 **
	10 70 MAE	-0.804	0.861	-0.934	1.000
	15 70 MAE	-0.281	0.861	-0.326	1.000
	30 70 MAE	8.631	0.861	10.029	< 0.001 ***
10 50 MAE	15 50 MAE	0.058	0.861	0.068	1.000
	30 50 MAE	11.452	0.861	13.306	< 0.001 ***
	1 60 MAE	-4.166	0.861	-4.841	< 0.001 ***
	5 60 MAE	-9.853	0.861	-11.449	< 0.001 ***
	10 60 MAE	-5.960	0.861	-6.925	< 0.001 ***
	15 60 MAE	-5.901	0.861	-6.857	< 0.001 ***
	30 60 MAE	-4.308	0.861	-5.006	< 0.001 ***
	1 70 MAE	9.496	0.861	11.034	< 0.001 ***
	5 70 MAE	-6.790	0.861	-7.890	< 0.001 ***
	10 70 MAE	-3.770	0.861	-4.381	0.006 **
	15 70 MAE	-3.247	0.861	-3.773	0.054
	30 70 MAE	5.665	0.861	6.582	< 0.001 ***

Post Hoc Comparisons - Time ✻ Temperature ✻ Heating method

		Mean Difference	SE	t	p_{tukey}
15 50 MAE	30 50 MAE	11.393	0.861	13.239	< 0.001 ***
	1 60 MAE	-4.225	0.861	-4.909	< 0.001 ***
	5 60 MAE	-9.912	0.861	-11.517	< 0.001 ***
	10 60 MAE	-6.018	0.861	-6.993	< 0.001 ***
	15 60 MAE	-5.960	0.861	-6.925	< 0.001 ***
	30 60 MAE	-4.366	0.861	-5.073	< 0.001 ***
	1 70 MAE	9.438	0.861	10.966	< 0.001 ***
	5 70 MAE	-6.849	0.861	-7.958	< 0.001 ***
	10 70 MAE	-3.829	0.861	-4.449	0.005 **
	15 70 MAE	-3.305	0.861	-3.841	0.043 *
	30 70 MAE	5.606	0.861	6.514	< 0.001 ***
30 50 MAE	1 60 MAE	-15.618	0.861	-18.147	< 0.001 ***
	5 60 MAE	-21.305	0.861	-24.756	< 0.001 ***
	10 60 MAE	-17.411	0.861	-20.231	< 0.001 ***
	15 60 MAE	-17.353	0.861	-20.163	< 0.001 ***
	30 60 MAE	-15.759	0.861	-18.312	< 0.001 ***
	1 70 MAE	-1.955	0.861	-2.272	0.902
	5 70 MAE	-18.242	0.861	-21.196	< 0.001 ***
	10 70 MAE	-15.222	0.861	-17.687	< 0.001 ***
	15 70 MAE	-14.698	0.861	-17.079	< 0.001 ***
	30 70 MAE	-5.787	0.861	-6.724	< 0.001 ***
1 60 MAE	5 60 MAE	-5.687	0.861	-6.608	< 0.001 ***
	10 60 MAE	-1.794	0.861	-2.084	0.961
	15 60 MAE	-1.735	0.861	-2.016	0.974
	30 60 MAE	-0.142	0.861	-0.165	1.000
	1 70 MAE	13.662	0.861	15.875	< 0.001 ***
	5 70 MAE	-2.624	0.861	-3.049	0.357
	10 70 MAE	0.396	0.861	0.460	1.000
	15 70 MAE	0.919	0.861	1.068	1.000
	30 70 MAE	9.831	0.861	11.423	< 0.001 ***
5 60 MAE	10 60 MAE	3.893	0.861	4.524	0.003 **
	15 60 MAE	3.952	0.861	4.592	0.003 **
	30 60 MAE	5.545	0.861	6.444	< 0.001 ***
	1 70 MAE	19.349	0.861	22.483	< 0.001 ***
	5 70 MAE	3.063	0.861	3.559	0.103
	10 70 MAE	6.083	0.861	7.068	< 0.001 ***
	15 70 MAE	6.606	0.861	7.676	< 0.001 ***
	30 70 MAE	15.518	0.861	18.031	< 0.001 ***
10 60 MAE	15 60 MAE	0.059	0.861	0.068	1.000
	30 60 MAE	1.652	0.861	1.920	0.986
	1 70 MAE	15.456	0.861	17.959	< 0.001 ***
	5 70 MAE	-0.830	0.861	-0.965	1.000
	10 70 MAE	2.190	0.861	2.544	0.746
	15 70 MAE	2.713	0.861	3.152	0.288
	30 70 MAE	11.624	0.861	13.507	< 0.001 ***
15 60 MAE	30 60 MAE	1.593	0.861	1.852	0.992
	1 70 MAE	15.397	0.861	17.891	< 0.001 ***
	5 70 MAE	-0.889	0.861	-1.033	1.000
	10 70 MAE	2.131	0.861	2.476	0.792
	15 70 MAE	2.654	0.861	3.084	0.333
	30 70 MAE	11.566	0.861	13.439	< 0.001 ***

Post Hoc Comparisons - Time ✻ Temperature ✻ Heating method

		Mean Difference	SE	t	p_{tukey}
30 60 MAE	1 70 MAE	13.804	0.861	16.040	< 0.001 ***
	5 70 MAE	-2.482	0.861	-2.885	0.481
	10 70 MAE	0.538	0.861	0.625	1.000
	15 70 MAE	1.061	0.861	1.233	1.000
	30 70 MAE	9.972	0.861	11.588	< 0.001 ***
1 70 MAE	5 70 MAE	-16.286	0.861	-18.924	< 0.001 ***
	10 70 MAE	-13.266	0.861	-15.415	< 0.001 ***
	15 70 MAE	-12.743	0.861	-14.807	< 0.001 ***
	30 70 MAE	-3.832	0.861	-4.452	0.005 **
5 70 MAE	10 70 MAE	3.020	0.861	3.509	0.119
	15 70 MAE	3.543	0.861	4.117	0.017 *
	30 70 MAE	12.455	0.861	14.472	< 0.001 ***
10 70 MAE	15 70 MAE	0.523	0.861	0.608	1.000
	30 70 MAE	9.435	0.861	10.963	< 0.001 ***
15 70 MAE	30 70 MAE	8.912	0.861	10.355	< 0.001 ***

Note. P-value adjusted for comparing a family of 30

* p < 0.05, ** p < 0.01, *** p < 0.001

F.5.2 Total Monomeric Anthocyanin (TMA)

ANOVA - Total Monomeric Anthocyanin

Cases	Sum of Squares	df	Mean Square	F	p	η²
Time	1.331	4	0.333	555.984	< 0.001***	0.629
Temperature	0.387	2	0.194	323.657	< 0.001***	0.183
Heating method	0.024	1	0.024	39.698	< 0.001***	0.011
Time ✻ Temperature	0.143	8	0.018	29.916	< 0.001***	0.068
Time ✻ Heating method	0.031	4	0.008	12.951	< 0.001***	0.015
Temperature ✻ Heating method	0.015	2	0.008	12.937	< 0.001***	0.007
Time ✻ Temperature ✻ Heating method	0.039	8	0.005	8.227	< 0.001***	0.019
Residuals	0.144	240	5.986×10^{-4}			

Note. Type III Sum of Squares

Post Hoc Comparisons - Time

		Mean Difference	SE	t	p_{tukey}
1	5	-0.152	0.005	-32.203	< 0.001***
	10	-0.038	0.005	-8.055	< 0.001***
	15	0.003	0.005	0.716	0.953
	30	0.059	0.005	12.487	< 0.001***
5	10	0.114	0.005	24.148	< 0.001***
	15	0.155	0.005	32.919	< 0.001***
	30	0.210	0.005	44.690	< 0.001***
10	15	0.041	0.005	8.771	< 0.001***
	30	0.097	0.005	20.542	< 0.001***
15	30	0.055	0.005	11.771	< 0.001***

*** p < 0.001

Note. P-value adjusted for comparing a family of 5

Note. Results are averaged over the levels of: Temperature, Heating method

Post Hoc Comparisons - Temperature

		Mean Difference	SE	t	p_{tukey}
50	60	0.053	0.004	14.495	< 0.001***
	70	0.092	0.004	25.356	< 0.001***
60	70	0.040	0.004	10.861	< 0.001***

*** p < 0.001

Note. P-value adjusted for comparing a family of 3

Note. Results are averaged over the levels of: Time, Heating method

Post Hoc Comparisons – Heating method

		Mean Difference	SE	t	p_{tukey}
CSE	MAE	-0.019	0.003	-6.301	< 0.001 ***

*** p < 0.001

Note. Results are averaged over the levels of: Time, Temperature

Post Hoc Comparisons - Time ✻ Temperature

		Mean Difference	SE	t	p_{tukey}
1 50	5 50	-0.129	0.008	-15.784	< 0.001 ***
	10 50	-0.058	0.008	-7.091	< 0.001 ***
	15 50	-0.030	0.008	-3.672	0.023 *
	30 50	0.030	0.008	3.726	0.019 *
	1 60	-0.007	0.008	-0.838	1.000
	5 60	-0.113	0.008	-13.876	< 0.001 ***
	10 60	0.013	0.008	1.655	0.944
	15 60	0.058	0.008	7.132	< 0.001 ***
	30 60	0.127	0.008	15.518	< 0.001 ***
	1 70	0.117	0.008	14.339	< 0.001 ***
	5 70	-0.103	0.008	-12.616	< 0.001 ***
	10 70	0.041	0.008	4.986	< 0.001 ***
	15 70	0.092	0.008	11.281	< 0.001 ***
	30 70	0.130	0.008	15.886	< 0.001 ***
5 50	10 50	0.071	0.008	8.692	< 0.001 ***
	15 50	0.099	0.008	12.112	< 0.001 ***
	30 50	0.159	0.008	19.510	< 0.001 ***
	1 60	0.122	0.008	14.946	< 0.001 ***
	5 60	0.016	0.008	1.907	0.847
	10 60	0.142	0.008	17.439	< 0.001 ***
	15 60	0.187	0.008	22.916	< 0.001 ***
	30 60	0.255	0.008	31.302	< 0.001 ***
	1 70	0.246	0.008	30.123	< 0.001 ***
	5 70	0.026	0.008	3.168	0.105
	10 70	0.169	0.008	20.770	< 0.001 ***
	15 70	0.221	0.008	27.064	< 0.001 ***
	30 70	0.258	0.008	31.669	< 0.001 ***
10 50	15 50	0.028	0.008	3.420	0.051
	30 50	0.088	0.008	10.818	< 0.001 ***
	1 60	0.051	0.008	6.254	< 0.001 ***
	5 60	-0.055	0.008	-6.785	< 0.001 ***
	10 60	0.071	0.008	8.747	< 0.001 ***
	15 60	0.116	0.008	14.224	< 0.001 ***
	30 60	0.184	0.008	22.609	< 0.001 ***
	1 70	0.175	0.008	21.431	< 0.001 ***
	5 70	-0.045	0.008	-5.525	< 0.001 ***
	10 70	0.099	0.008	12.078	< 0.001 ***
	15 70	0.150	0.008	18.372	< 0.001 ***
	30 70	0.187	0.008	22.977	< 0.001 ***
15 50	30 50	0.060	0.008	7.398	< 0.001 ***
	1 60	0.023	0.008	2.834	0.236
	5 60	-0.083	0.008	-10.205	< 0.001 ***
	10 60	0.043	0.008	5.327	< 0.001 ***
	15 60	0.088	0.008	10.804	< 0.001 ***

Post Hoc Comparisons - Time ✻ Temperature

		Mean Difference	SE	t	p_{tukey}	
	30 60	0.157	0.008	19.190	< 0.001	***
	1 70	0.147	0.008	18.011	< 0.001	***
	5 70	-0.073	0.008	-8.944	< 0.001	***
	10 70	0.071	0.008	8.658	< 0.001	***
	15 70	0.122	0.008	14.953	< 0.001	***
	30 70	0.160	0.008	19.558	< 0.001	***
30 50	1 60	-0.037	0.008	-4.564	< 0.001	***
	5 60	-0.144	0.008	-17.602	< 0.001	***
	10 60	-0.017	0.008	-2.071	0.753	
	15 60	0.028	0.008	3.406	0.053	
	30 60	0.096	0.008	11.792	< 0.001	***
	1 70	0.087	0.008	10.613	< 0.001	***
	5 70	-0.133	0.008	-16.342	< 0.001	***
	10 70	0.010	0.008	1.260	0.995	
	15 70	0.062	0.008	7.555	< 0.001	***
	30 70	0.099	0.008	12.160	< 0.001	***
1 60	5 60	-0.106	0.008	-13.038	< 0.001	***
	10 60	0.020	0.008	2.493	0.449	
	15 60	0.065	0.008	7.970	< 0.001	***
	30 60	0.133	0.008	16.356	< 0.001	***
	1 70	0.124	0.008	15.177	< 0.001	***
	5 70	-0.096	0.008	-11.778	< 0.001	***
	10 70	0.047	0.008	5.824	< 0.001	***
	15 70	0.099	0.008	12.119	< 0.001	***
	30 70	0.136	0.008	16.724	< 0.001	***
5 60	10 60	0.127	0.008	15.532	< 0.001	***
	15 60	0.171	0.008	21.009	< 0.001	***
	30 60	0.240	0.008	29.394	< 0.001	***
	1 70	0.230	0.008	28.216	< 0.001	***
	5 70	0.010	0.008	1.260	0.995	
	10 70	0.154	0.008	18.863	< 0.001	***
	15 70	0.205	0.008	25.157	< 0.001	***
	30 70	0.243	0.008	29.762	< 0.001	***
10 60	15 60	0.045	0.008	5.477	< 0.001	***
	30 60	0.113	0.008	13.863	< 0.001	***
	1 70	0.103	0.008	12.684	< 0.001	***
	5 70	-0.116	0.008	-14.271	< 0.001	***
	10 70	0.027	0.008	3.331	0.067	
	15 70	0.078	0.008	9.625	< 0.001	***
	30 70	0.116	0.008	14.230	< 0.001	***
15 60	30 60	0.068	0.008	8.386	< 0.001	***
	1 70	0.059	0.008	7.207	< 0.001	***
	5 70	-0.161	0.008	-19.748	< 0.001	***
	10 70	-0.017	0.008	-2.146	0.703	
	15 70	0.034	0.008	4.149	0.004	**
	30 70	0.071	0.008	8.754	< 0.001	***
30 60	1 70	-0.010	0.008	-1.178	0.998	
	5 70	-0.229	0.008	-28.134	< 0.001	***
	10 70	-0.086	0.008	-10.531	< 0.001	***
	15 70	-0.035	0.008	-4.237	0.003	**
	30 70	0.003	0.008	0.368	1.000	

Post Hoc Comparisons - Time ✻ Temperature

		Mean Difference	SE	t	p_{tukey}	
1 70	5 70	-0.220	0.008	-26.955	< 0.001	***
	10 70	-0.076	0.008	-9.353	< 0.001	***
	15 70	-0.025	0.008	-3.059	0.139	
	30 70	0.013	0.008	1.546	0.968	
5 70	10 70	0.144	0.008	17.602	< 0.001	***
	15 70	0.195	0.008	23.897	< 0.001	***
	30 70	0.232	0.008	28.502	< 0.001	***
10 70	15 70	0.051	0.008	6.294	< 0.001	***
	30 70	0.089	0.008	10.899	< 0.001	***
15 70	30 70	0.038	0.008	4.605	< 0.001	***

Note. Results are averaged over the levels of: Heating method
Note. P-value adjusted for comparing a family of 15
* p < 0.05, ** p < 0.01, *** p < 0.001

Post Hoc Comparisons - Time ✻ Heating method

		Mean Difference	SE	t	p_{tukey}	
1 CSE	5 CSE	-0.135	0.007	-20.285	< 0.001	***
	10 CSE	-0.018	0.007	-2.631	0.209	
	15 CSE	0.033	0.007	5.023	< 0.001	***
	30 CSE	0.064	0.007	9.645	< 0.001	***
	1 MAE	0.010	0.007	1.535	0.877	
	5 MAE	-0.158	0.007	-23.722	< 0.001	***
	10 MAE	-0.048	0.007	-7.225	< 0.001	***
	15 MAE	-0.016	0.007	-2.475	0.287	
	30 MAE	0.064	0.007	9.550	< 0.001	***
5 CSE	10 CSE	0.118	0.007	17.654	< 0.001	***
	15 CSE	0.169	0.007	25.307	< 0.001	***
	30 CSE	0.199	0.007	29.929	< 0.001	***
	1 MAE	0.145	0.007	21.820	< 0.001	***
	5 MAE	-0.023	0.007	-3.437	0.024	*
	10 MAE	0.087	0.007	13.060	< 0.001	***
	15 MAE	0.119	0.007	17.810	< 0.001	***
	30 MAE	0.199	0.007	29.835	< 0.001	***
10 CSE	15 CSE	0.051	0.007	7.653	< 0.001	***
	30 CSE	0.082	0.007	12.275	< 0.001	***
	1 MAE	0.028	0.007	4.166	0.002	**
	5 MAE	-0.140	0.007	-21.091	< 0.001	***
	10 MAE	-0.031	0.007	-4.594	< 0.001	***
	15 MAE	0.001	0.007	0.156	1.000	
	30 MAE	0.081	0.007	12.181	< 0.001	***
15 CSE	30 CSE	0.031	0.007	4.622	< 0.001	***
	1 MAE	-0.023	0.007	-3.487	0.020	*
	5 MAE	-0.191	0.007	-28.745	< 0.001	***
	10 MAE	-0.082	0.007	-12.248	< 0.001	***
	15 MAE	-0.050	0.007	-7.498	< 0.001	***
	30 MAE	0.030	0.007	4.528	< 0.001	***
30 CSE	1 MAE	-0.054	0.007	-8.109	< 0.001	***
	5 MAE	-0.222	0.007	-33.367	< 0.001	***
	10 MAE	-0.112	0.007	-16.870	< 0.001	***

Post Hoc Comparisons - Time ✻ Heating method

		Mean Difference	SE	t	p_{tukey}	
	15 MAE	-0.081	0.007	-12.120	< 0.001	***
	30 MAE	-6.296×10^{-4}	0.007	-0.095	1.000	
1 MAE	5 MAE	-0.168	0.007	-25.257	< 0.001	***
	10 MAE	-0.058	0.007	-8.760	< 0.001	***
	15 MAE	-0.027	0.007	-4.010	0.003	**
	30 MAE	0.053	0.007	8.015	< 0.001	***
5 MAE	10 MAE	0.110	0.007	16.497	< 0.001	***
	15 MAE	0.141	0.007	21.247	< 0.001	***
	30 MAE	0.222	0.007	33.272	< 0.001	***
10 MAE	15 MAE	0.032	0.007	4.750	< 0.001	***
	30 MAE	0.112	0.007	16.775	< 0.001	***
15 MAE	30 MAE	0.080	0.007	12.025	< 0.001	***

Note. Results are averaged over the levels of: Temperature

Note. P-value adjusted for comparing a family of 10

* p < 0.05, ** p < 0.01, *** p < 0.001

Post Hoc Comparisons - Temperature ✻ Heating method

		Mean Difference	SE	t	p_{tukey}	
50 CSE	60 CSE	0.051	0.005	9.896	< 0.001	***
	70 CSE	0.076	0.005	14.653	< 0.001	***
	50 MAE	-0.031	0.005	-6.058	< 0.001	***
	60 MAE	0.023	0.005	4.545	< 0.001	***
	70 MAE	0.078	0.005	15.148	< 0.001	***
60 CSE	70 CSE	0.025	0.005	4.756	< 0.001	***
	50 MAE	-0.082	0.005	-15.954	< 0.001	***
	60 MAE	-0.028	0.005	-5.351	< 0.001	***
	70 MAE	0.027	0.005	5.252	< 0.001	***
70 CSE	50 MAE	-0.107	0.005	-20.710	< 0.001	***
	60 MAE	-0.052	0.005	-10.107	< 0.001	***
	70 MAE	0.003	0.005	0.495	0.996	
50 MAE	60 MAE	0.055	0.005	10.603	< 0.001	***
	70 MAE	0.109	0.005	21.206	< 0.001	***
60 MAE	70 MAE	0.055	0.005	10.603	< 0.001	***

** p < 0.01, *** p < 0.001

Note. P-value adjusted for comparing a family of 6

Note. Results are averaged over the levels of: Time

Post Hoc Comparisons - Time ✻ Temperature ✻ Heating method

		Mean Difference	SE	t	p_{tukey}	
1 50 CSE	5 50 CSE	-0.131	0.012	-11.320	< 0.001	***
	10 50 CSE	-0.057	0.012	-4.961	< 0.001	***
	15 50 CSE	-0.039	0.012	-3.353	0.180	
	30 50 CSE	0.016	0.012	1.358	1.000	
	1 60 CSE	-0.020	0.012	-1.696	0.998	
	5 60 CSE	-0.124	0.012	-10.751	< 0.001	***
	10 60 CSE	0.003	0.012	0.289	1.000	
	15 60 CSE	0.081	0.012	7.023	< 0.001	***
	30 60 CSE	0.104	0.012	8.988	< 0.001	***
	1 70 CSE	0.053	0.012	4.566	0.003	**
	5 70 CSE	-0.118	0.012	-10.193	< 0.001	***

Post Hoc Comparisons - Time ✻ Temperature ✻ Heating method

		Mean Difference	SE	t	p_{tukey}	
	10 70 CSE	0.034	0.012	2.986	0.402	
	15 70 CSE	0.091	0.012	7.900	< 0.001	***
	30 70 CSE	0.106	0.012	9.229	< 0.001	***
	1 50 MAE	-0.041	0.012	-3.564	0.101	
	5 50 MAE	-0.168	0.012	-14.566	< 0.001	***
	10 50 MAE	-0.100	0.012	-8.632	< 0.001	***
	15 50 MAE	-0.062	0.012	-5.405	< 0.001	***
	30 50 MAE	0.004	0.012	0.347	1.000	
	1 60 MAE	-0.035	0.012	-3.054	0.354	
	5 60 MAE	-0.143	0.012	-12.437	< 0.001	***
	10 60 MAE	-0.017	0.012	-1.512	1.000	
	15 60 MAE	-0.006	0.012	-0.501	1.000	
	30 60 MAE	0.108	0.012	9.393	< 0.001	***
	1 70 MAE	0.140	0.012	12.148	< 0.001	***
	5 70 MAE	-0.129	0.012	-11.214	< 0.001	***
	10 70 MAE	0.006	0.012	0.501	1.000	
	15 70 MAE	0.052	0.012	4.489	0.004	**
	30 70 MAE	0.112	0.012	9.672	< 0.001	***
5 50 CSE	10 50 CSE	0.073	0.012	6.358	< 0.001	***
	15 50 CSE	0.092	0.012	7.967	< 0.001	***
	30 50 CSE	0.146	0.012	12.678	< 0.001	***
	1 60 CSE	0.111	0.012	9.624	< 0.001	***
	5 60 CSE	0.007	0.012	0.568	1.000	
	10 60 CSE	0.134	0.012	11.609	< 0.001	***
	15 60 CSE	0.212	0.012	18.343	< 0.001	***
	30 60 CSE	0.234	0.012	20.308	< 0.001	***
	1 70 CSE	0.183	0.012	15.886	< 0.001	***
	5 70 CSE	0.013	0.012	1.127	1.000	
	10 70 CSE	0.165	0.012	14.306	< 0.001	***
	15 70 CSE	0.222	0.012	19.219	< 0.001	***
	30 70 CSE	0.237	0.012	20.549	< 0.001	***
	1 50 MAE	0.089	0.012	7.755	< 0.001	***
	5 50 MAE	-0.037	0.012	-3.247	0.233	
	10 50 MAE	0.031	0.012	2.688	0.638	
	15 50 MAE	0.068	0.012	5.915	< 0.001	***
	30 50 MAE	0.135	0.012	11.666	< 0.001	***
	1 60 MAE	0.095	0.012	8.266	< 0.001	***
	5 60 MAE	-0.013	0.012	-1.118	1.000	
	10 60 MAE	0.113	0.012	9.807	< 0.001	***
	15 60 MAE	0.125	0.012	10.819	< 0.001	***
	30 60 MAE	0.239	0.012	20.713	< 0.001	***
	1 70 MAE	0.271	0.012	23.468	< 0.001	***
	5 70 MAE	0.001	0.012	0.106	1.000	
	10 70 MAE	0.136	0.012	11.821	< 0.001	***
	15 70 MAE	0.182	0.012	15.809	< 0.001	***
	30 70 MAE	0.242	0.012	20.992	< 0.001	***
10 50 CSE	15 50 CSE	0.019	0.012	1.609	0.999	
	30 50 CSE	0.073	0.012	6.320	< 0.001	***
	1 60 CSE	0.038	0.012	3.266	0.223	
	5 60 CSE	-0.067	0.012	-5.790	< 0.001	***
	10 60 CSE	0.061	0.012	5.250	< 0.001	***

Post Hoc Comparisons - Time ✻ Temperature ✻ Heating method

		Mean Difference	SE	t	p_{tukey}	
	15 60 CSE	0.138	0.012	11.984	< 0.001	***
	30 60 CSE	0.161	0.012	13.950	< 0.001	***
	1 70 CSE	0.110	0.012	9.528	< 0.001	***
	5 70 CSE	-0.060	0.012	-5.231	< 0.001	***
	10 70 CSE	0.092	0.012	7.948	< 0.001	***
	15 70 CSE	0.148	0.012	12.861	< 0.001	***
	30 70 CSE	0.164	0.012	14.191	< 0.001	***
	1 50 MAE	0.016	0.012	1.397	1.000	
	5 50 MAE	-0.111	0.012	-9.605	< 0.001	***
	10 50 MAE	-0.042	0.012	-3.670	0.074	
	15 50 MAE	-0.005	0.012	-0.443	1.000	
	30 50 MAE	0.061	0.012	5.308	< 0.001	***
	1 60 MAE	0.022	0.012	1.907	0.987	
	5 60 MAE	-0.086	0.012	-7.476	< 0.001	***
	10 60 MAE	0.040	0.012	3.449	0.140	
	15 60 MAE	0.051	0.012	4.460	0.004	**
	30 60 MAE	0.166	0.012	14.354	< 0.001	***
	1 70 MAE	0.197	0.012	17.110	< 0.001	***
	5 70 MAE	-0.072	0.012	-6.252	< 0.001	***
	10 70 MAE	0.063	0.012	5.462	< 0.001	***
	15 70 MAE	0.109	0.012	9.451	< 0.001	***
	30 70 MAE	0.169	0.012	14.634	< 0.001	***
15 50 CSE	30 50 CSE	0.054	0.012	4.711	0.002	**
	1 60 CSE	0.019	0.012	1.657	0.998	
	5 60 CSE	-0.085	0.012	-7.399	< 0.001	***
	10 60 CSE	0.042	0.012	3.642	0.081	
	15 60 CSE	0.120	0.012	10.376	< 0.001	***
	30 60 CSE	0.142	0.012	12.341	< 0.001	***
	1 70 CSE	0.091	0.012	7.919	< 0.001	***
	5 70 CSE	-0.079	0.012	-6.840	< 0.001	***
	10 70 CSE	0.073	0.012	6.339	< 0.001	***
	15 70 CSE	0.130	0.012	11.252	< 0.001	***
	30 70 CSE	0.145	0.012	12.582	< 0.001	***
	1 50 MAE	-0.002	0.012	-0.212	1.000	
	5 50 MAE	-0.129	0.012	-11.214	< 0.001	***
	10 50 MAE	-0.061	0.012	-5.279	< 0.001	***
	15 50 MAE	-0.024	0.012	-2.052	0.968	
	30 50 MAE	0.043	0.012	3.699	0.068	
	1 60 MAE	0.003	0.012	0.299	1.000	
	5 60 MAE	-0.105	0.012	-9.085	< 0.001	***
	10 60 MAE	0.021	0.012	1.840	0.992	
	15 60 MAE	0.033	0.012	2.852	0.507	
	30 60 MAE	0.147	0.012	12.745	< 0.001	***
	1 70 MAE	0.179	0.012	15.501	< 0.001	***
	5 70 MAE	-0.091	0.012	-7.861	< 0.001	***
	10 70 MAE	0.044	0.012	3.854	0.041	*
	15 70 MAE	0.090	0.012	7.842	< 0.001	***
	30 70 MAE	0.150	0.012	13.025	< 0.001	***
30 50 CSE	1 60 CSE	-0.035	0.012	-3.054	0.354	
	5 60 CSE	-0.140	0.012	-12.110	< 0.001	***
	10 60 CSE	-0.012	0.012	-1.069	1.000	

Post Hoc Comparisons - Time ✻ Temperature ✻ Heating method

		Mean Difference	SE	t	p_{tukey}	
	15 60 CSE	0.065	0.012	5.665	< 0.001	***
	30 60 CSE	0.088	0.012	7.630	< 0.001	***
	1 70 CSE	0.037	0.012	3.208	0.255	
	5 70 CSE	-0.133	0.012	-11.551	< 0.001	***
	10 70 CSE	0.019	0.012	1.628	0.999	
	15 70 CSE	0.075	0.012	6.541	< 0.001	***
	30 70 CSE	0.091	0.012	7.871	< 0.001	***
	1 50 MAE	-0.057	0.012	-4.923	< 0.001	***
	5 50 MAE	-0.184	0.012	-15.925	< 0.001	***
	10 50 MAE	-0.115	0.012	-9.990	< 0.001	***
	15 50 MAE	-0.078	0.012	-6.763	< 0.001	***
	30 50 MAE	-0.012	0.012	-1.012	1.000	
	1 60 MAE	-0.051	0.012	-4.412	0.005	**
	5 60 MAE	-0.159	0.012	-13.796	< 0.001	***
	10 60 MAE	-0.033	0.012	-2.871	0.492	
	15 60 MAE	-0.021	0.012	-1.859	0.991	
	30 60 MAE	0.093	0.012	8.035	< 0.001	***
	1 70 MAE	0.124	0.012	10.790	< 0.001	***
	5 70 MAE	-0.145	0.012	-12.572	< 0.001	***
	10 70 MAE	-0.010	0.012	-0.857	1.000	
	15 70 MAE	0.036	0.012	3.131	0.302	
	30 70 MAE	0.096	0.012	8.314	< 0.001	***
1 60 CSE	5 60 CSE	-0.104	0.012	-9.056	< 0.001	***
	10 60 CSE	0.023	0.012	1.985	0.979	
	15 60 CSE	0.101	0.012	8.719	< 0.001	***
	30 60 CSE	0.123	0.012	10.684	< 0.001	***
	1 70 CSE	0.072	0.012	6.262	< 0.001	***
	5 70 CSE	-0.098	0.012	-8.497	< 0.001	***
	10 70 CSE	0.054	0.012	4.682	0.002	**
	15 70 CSE	0.111	0.012	9.595	< 0.001	***
	30 70 CSE	0.126	0.012	10.925	< 0.001	***
	1 50 MAE	-0.022	0.012	-1.869	0.990	
	5 50 MAE	-0.148	0.012	-12.871	< 0.001	***
	10 50 MAE	-0.080	0.012	-6.936	< 0.001	***
	15 50 MAE	-0.043	0.012	-3.709	0.066	
	30 50 MAE	0.024	0.012	2.042	0.969	
	1 60 MAE	-0.016	0.012	-1.358	1.000	
	5 60 MAE	-0.124	0.012	-10.742	< 0.001	***
	10 60 MAE	0.002	0.012	0.183	1.000	
	15 60 MAE	0.014	0.012	1.195	1.000	
	30 60 MAE	0.128	0.012	11.088	< 0.001	***
	1 70 MAE	0.160	0.012	13.844	< 0.001	***
	5 70 MAE	-0.110	0.012	-9.518	< 0.001	***
	10 70 MAE	0.025	0.012	2.196	0.930	
	15 70 MAE	0.071	0.012	6.185	< 0.001	***
	30 70 MAE	0.131	0.012	11.368	< 0.001	***
5 60 CSE	10 60 CSE	0.127	0.012	11.040	< 0.001	***
	15 60 CSE	0.205	0.012	17.774	< 0.001	***
	30 60 CSE	0.228	0.012	19.740	< 0.001	***
	1 70 CSE	0.177	0.012	15.318	< 0.001	***
	5 70 CSE	0.006	0.012	0.559	1.000	

Post Hoc Comparisons - Time ✻ Temperature ✻ Heating method

		Mean Difference	SE	t	p_{tukey}	
	10 70 CSE	0.158	0.012	13.738	< 0.001	***
	15 70 CSE	0.215	0.012	18.651	< 0.001	***
	30 70 CSE	0.230	0.012	19.980	< 0.001	***
	1 50 MAE	0.083	0.012	7.187	< 0.001	***
	5 50 MAE	-0.044	0.012	-3.815	0.047	*
	10 50 MAE	0.024	0.012	2.119	0.953	
	15 50 MAE	0.062	0.012	5.347	< 0.001	***
	30 50 MAE	0.128	0.012	11.098	< 0.001	***
	1 60 MAE	0.089	0.012	7.697	< 0.001	***
	5 60 MAE	-0.019	0.012	-1.686	0.998	
	10 60 MAE	0.107	0.012	9.239	< 0.001	***
	15 60 MAE	0.118	0.012	10.250	< 0.001	***
	30 60 MAE	0.232	0.012	20.144	< 0.001	***
	1 70 MAE	0.264	0.012	22.899	< 0.001	***
	5 70 MAE	-0.005	0.012	-0.462	1.000	
	10 70 MAE	0.130	0.012	11.252	< 0.001	***
	15 70 MAE	0.176	0.012	15.241	< 0.001	***
	30 70 MAE	0.236	0.012	20.424	< 0.001	***
10 60 CSE	15 60 CSE	0.078	0.012	6.734	< 0.001	***
	30 60 CSE	0.100	0.012	8.699	< 0.001	***
	1 70 CSE	0.049	0.012	4.277	0.009	**
	5 70 CSE	-0.121	0.012	-10.482	< 0.001	***
	10 70 CSE	0.031	0.012	2.697	0.630	
	15 70 CSE	0.088	0.012	7.611	< 0.001	***
	30 70 CSE	0.103	0.012	8.940	< 0.001	***
	1 50 MAE	-0.044	0.012	-3.854	0.041	*
	5 50 MAE	-0.171	0.012	-14.855	< 0.001	***
	10 50 MAE	-0.103	0.012	-8.921	< 0.001	***
	15 50 MAE	-0.066	0.012	-5.694	< 0.001	***
	30 50 MAE	6.667×10^{-4}	0.012	0.058	1.000	
	1 60 MAE	-0.039	0.012	-3.343	0.184	
	5 60 MAE	-0.147	0.012	-12.726	< 0.001	***
	10 60 MAE	-0.021	0.012	-1.802	0.994	
	15 60 MAE	-0.009	0.012	-0.790	1.000	
	30 60 MAE	0.105	0.012	9.104	< 0.001	***
	1 70 MAE	0.137	0.012	11.859	< 0.001	***
	5 70 MAE	-0.133	0.012	-11.503	< 0.001	***
	10 70 MAE	0.002	0.012	0.212	1.000	
	15 70 MAE	0.048	0.012	4.200	0.012	*
	30 70 MAE	0.108	0.012	9.383	< 0.001	***
15 60 CSE	30 60 CSE	0.023	0.012	1.965	0.981	
	1 70 CSE	-0.028	0.012	-2.457	0.805	
	5 70 CSE	-0.199	0.012	-17.216	< 0.001	***
	10 70 CSE	-0.047	0.012	-4.037	0.022	*
	15 70 CSE	0.010	0.012	0.877	1.000	
	30 70 CSE	0.025	0.012	2.206	0.927	
	1 50 MAE	-0.122	0.012	-10.587	< 0.001	***
	5 50 MAE	-0.249	0.012	-21.589	< 0.001	***
	10 50 MAE	-0.181	0.012	-15.655	< 0.001	***
	15 50 MAE	-0.143	0.012	-12.428	< 0.001	***
	30 50 MAE	-0.077	0.012	-6.676	< 0.001	***

Post Hoc Comparisons - Time ✻ Temperature ✻ Heating method

		Mean Difference	SE	t	p_{tukey}	
	1 60 MAE	-0.116	0.012	-10.077	< 0.001	***
	5 60 MAE	-0.224	0.012	-19.460	< 0.001	***
	10 60 MAE	-0.098	0.012	-8.536	< 0.001	***
	15 60 MAE	-0.087	0.012	-7.524	< 0.001	***
	30 60 MAE	0.027	0.012	2.370	0.855	
	1 70 MAE	0.059	0.012	5.125	< 0.001	***
	5 70 MAE	-0.210	0.012	-18.237	< 0.001	***
	10 70 MAE	-0.075	0.012	-6.522	< 0.001	***
	15 70 MAE	-0.029	0.012	-2.534	0.754	
	30 70 MAE	0.031	0.012	2.649	0.668	
30 60 CSE	1 70 CSE	-0.051	0.012	-4.422	0.005	**
	5 70 CSE	-0.221	0.012	-19.181	< 0.001	***
	10 70 CSE	-0.069	0.012	-6.002	< 0.001	***
	15 70 CSE	-0.013	0.012	-1.089	1.000	
	30 70 CSE	0.003	0.012	0.241	1.000	
	1 50 MAE	-0.145	0.012	-12.553	< 0.001	***
	5 50 MAE	-0.272	0.012	-23.555	< 0.001	***
	10 50 MAE	-0.203	0.012	-17.620	< 0.001	***
	15 50 MAE	-0.166	0.012	-14.393	< 0.001	***
	30 50 MAE	-0.100	0.012	-8.641	< 0.001	***
	1 60 MAE	-0.139	0.012	-12.042	< 0.001	***
	5 60 MAE	-0.247	0.012	-21.425	< 0.001	***
	10 60 MAE	-0.121	0.012	-10.501	< 0.001	***
	15 60 MAE	-0.109	0.012	-9.489	< 0.001	***
	30 60 MAE	0.005	0.012	0.405	1.000	
	1 70 MAE	0.036	0.012	3.160	0.284	
	5 70 MAE	-0.233	0.012	-20.202	< 0.001	***
	10 70 MAE	-0.098	0.012	-8.487	< 0.001	***
	15 70 MAE	-0.052	0.012	-4.499	0.004	**
	30 70 MAE	0.008	0.012	0.684	1.000	
1 70 CSE	5 70 CSE	-0.170	0.012	-14.759	< 0.001	***
	10 70 CSE	-0.018	0.012	-1.580	0.999	
	15 70 CSE	0.038	0.012	3.333	0.189	
	30 70 CSE	0.054	0.012	4.663	0.002	**
	1 50 MAE	-0.094	0.012	-8.131	< 0.001	***
	5 50 MAE	-0.221	0.012	-19.133	< 0.001	***
	10 50 MAE	-0.152	0.012	-13.198	< 0.001	***
	15 50 MAE	-0.115	0.012	-9.971	< 0.001	***
	30 50 MAE	-0.049	0.012	-4.220	0.011	*
	1 60 MAE	-0.088	0.012	-7.620	< 0.001	***
	5 60 MAE	-0.196	0.012	-17.004	< 0.001	***
	10 60 MAE	-0.070	0.012	-6.079	< 0.001	***
	15 60 MAE	-0.058	0.012	-5.067	< 0.001	***
	30 60 MAE	0.056	0.012	4.827	< 0.001	***
	1 70 MAE	0.087	0.012	7.582	< 0.001	***
	5 70 MAE	-0.182	0.012	-15.780	< 0.001	***
	10 70 MAE	-0.047	0.012	-4.065	0.020	*
	15 70 MAE	-8.889×10^{-4}	0.012	-0.077	1.000	
	30 70 MAE	0.059	0.012	5.106	< 0.001	***
5 70 CSE	10 70 CSE	0.152	0.012	13.179	< 0.001	***
	15 70 CSE	0.209	0.012	18.092	< 0.001	***

Post Hoc Comparisons - Time ✻ Temperature ✻ Heating method

		Mean Difference	SE	t	p_{tukey}	
	30 70 CSE	0.224	0.012	19.422	< 0.001	***
	1 50 MAE	0.076	0.012	6.628	< 0.001	***
	5 50 MAE	-0.050	0.012	-4.374	0.006	**
	10 50 MAE	0.018	0.012	1.561	0.999	
	15 50 MAE	0.055	0.012	4.788	0.001	**
	30 50 MAE	0.122	0.012	10.539	< 0.001	***
	1 60 MAE	0.082	0.012	7.139	< 0.001	***
	5 60 MAE	-0.026	0.012	-2.245	0.913	
	10 60 MAE	0.100	0.012	8.680	< 0.001	***
	15 60 MAE	0.112	0.012	9.692	< 0.001	***
	30 60 MAE	0.226	0.012	19.585	< 0.001	***
	1 70 MAE	0.258	0.012	22.341	< 0.001	***
	5 70 MAE	-0.012	0.012	-1.021	1.000	
	10 70 MAE	0.123	0.012	10.693	< 0.001	***
	15 70 MAE	0.169	0.012	14.682	< 0.001	***
	30 70 MAE	0.229	0.012	19.865	< 0.001	***
10 70 CSE	15 70 CSE	0.057	0.012	4.913	< 0.001	***
	30 70 CSE	0.072	0.012	6.243	< 0.001	***
	1 50 MAE	-0.076	0.012	-6.551	< 0.001	***
	5 50 MAE	-0.202	0.012	-17.553	< 0.001	***
	10 50 MAE	-0.134	0.012	-11.618	< 0.001	***
	15 50 MAE	-0.097	0.012	-8.391	< 0.001	***
	30 50 MAE	-0.030	0.012	-2.640	0.676	
	1 60 MAE	-0.070	0.012	-6.040	< 0.001	***
	5 60 MAE	-0.178	0.012	-15.424	< 0.001	***
	10 60 MAE	-0.052	0.012	-4.499	0.004	**
	15 60 MAE	-0.040	0.012	-3.487	0.126	
	30 60 MAE	0.074	0.012	6.406	< 0.001	***
	1 70 MAE	0.106	0.012	9.162	< 0.001	***
	5 70 MAE	-0.164	0.012	-14.200	< 0.001	***
	10 70 MAE	-0.029	0.012	-2.486	0.786	
	15 70 MAE	0.017	0.012	1.503	1.000	
	30 70 MAE	0.077	0.012	6.686	< 0.001	***
15 70 CSE	30 70 CSE	0.015	0.012	1.329	1.000	
	1 50 MAE	-0.132	0.012	-11.464	< 0.001	***
	5 50 MAE	-0.259	0.012	-22.466	< 0.001	***
	10 50 MAE	-0.191	0.012	-16.532	< 0.001	***
	15 50 MAE	-0.153	0.012	-13.304	< 0.001	***
	30 50 MAE	-0.087	0.012	-7.553	< 0.001	***
	1 60 MAE	-0.126	0.012	-10.954	< 0.001	***
	5 60 MAE	-0.235	0.012	-20.337	< 0.001	***
	10 60 MAE	-0.109	0.012	-9.412	< 0.001	***
	15 60 MAE	-0.097	0.012	-8.401	< 0.001	***
	30 60 MAE	0.017	0.012	1.493	1.000	
	1 70 MAE	0.049	0.012	4.248	0.010	*
	5 70 MAE	-0.220	0.012	-19.113	< 0.001	***
	10 70 MAE	-0.085	0.012	-7.399	< 0.001	***
	15 70 MAE	-0.039	0.012	-3.410	0.155	
	30 70 MAE	0.020	0.012	1.773	0.996	
30 70 CSE	1 50 MAE	-0.148	0.012	-12.794	< 0.001	***
	5 50 MAE	-0.274	0.012	-23.795	< 0.001	***

Post Hoc Comparisons - Time ✻ Temperature ✻ Heating method

		Mean Difference	SE	t	p_{tukey}	
	10 50 MAE	-0.206	0.012	-17.861	< 0.001	***
	15 50 MAE	-0.169	0.012	-14.634	< 0.001	***
	30 50 MAE	-0.102	0.012	-8.882	< 0.001	***
	1 60 MAE	-0.142	0.012	-12.283	< 0.001	***
	5 60 MAE	-0.250	0.012	-21.666	< 0.001	***
	10 60 MAE	-0.124	0.012	-10.742	< 0.001	***
	15 60 MAE	-0.112	0.012	-9.730	< 0.001	***
	30 60 MAE	0.002	0.012	0.164	1.000	
	1 70 MAE	0.034	0.012	2.919	0.454	
	5 70 MAE	-0.236	0.012	-20.443	< 0.001	***
	10 70 MAE	-0.101	0.012	-8.728	< 0.001	***
	15 70 MAE	-0.055	0.012	-4.740	0.001	**
	30 70 MAE	0.005	0.012	0.443	1.000	
1 50 MAE	5 50 MAE	-0.127	0.012	-11.002	< 0.001	***
	10 50 MAE	-0.058	0.012	-5.067	< 0.001	***
	15 50 MAE	-0.021	0.012	-1.840	0.992	
	30 50 MAE	0.045	0.012	3.911	0.034	*
	1 60 MAE	0.006	0.012	0.511	1.000	
	5 60 MAE	-0.102	0.012	-8.873	< 0.001	***
	10 60 MAE	0.024	0.012	2.052	0.968	
	15 60 MAE	0.035	0.012	3.064	0.347	
	30 60 MAE	0.149	0.012	12.957	< 0.001	***
	1 70 MAE	0.181	0.012	15.713	< 0.001	***
	5 70 MAE	-0.088	0.012	-7.649	< 0.001	***
	10 70 MAE	0.047	0.012	4.065	0.020	*
	15 70 MAE	0.093	0.012	8.054	< 0.001	***
	30 70 MAE	0.153	0.012	13.237	< 0.001	***
5 50 MAE	10 50 MAE	0.068	0.012	5.934	< 0.001	***
	15 50 MAE	0.106	0.012	9.162	< 0.001	***
	30 50 MAE	0.172	0.012	14.913	< 0.001	***
	1 60 MAE	0.133	0.012	11.512	< 0.001	***
	5 60 MAE	0.025	0.012	2.129	0.950	
	10 60 MAE	0.151	0.012	13.054	< 0.001	***
	15 60 MAE	0.162	0.012	14.065	< 0.001	***
	30 60 MAE	0.276	0.012	23.959	< 0.001	***
	1 70 MAE	0.308	0.012	26.714	< 0.001	***
	5 70 MAE	0.039	0.012	3.353	0.180	
	10 70 MAE	0.174	0.012	15.067	< 0.001	***
	15 70 MAE	0.220	0.012	19.056	< 0.001	***
	30 70 MAE	0.280	0.012	24.239	< 0.001	***
10 50 MAE	15 50 MAE	0.037	0.012	3.227	0.244	
	30 50 MAE	0.104	0.012	8.979	< 0.001	***
	1 60 MAE	0.064	0.012	5.578	< 0.001	***
	5 60 MAE	-0.044	0.012	-3.805	0.048	*
	10 60 MAE	0.082	0.012	7.119	< 0.001	***
	15 60 MAE	0.094	0.012	8.131	< 0.001	***
	30 60 MAE	0.208	0.012	18.025	< 0.001	***
	1 70 MAE	0.240	0.012	20.780	< 0.001	***
	5 70 MAE	-0.030	0.012	-2.582	0.719	
	10 70 MAE	0.105	0.012	9.133	< 0.001	***
	15 70 MAE	0.151	0.012	13.121	< 0.001	***

Post Hoc Comparisons - Time ✻ Temperature ✻ Heating method

		Mean Difference	SE	t	p_{tukey}	
	30 70 MAE	0.211	0.012	18.304	< 0.001	***
15 50 MAE	30 50 MAE	0.066	0.012	5.751	< 0.001	***
	1 60 MAE	0.027	0.012	2.351	0.865	
	5 60 MAE	-0.081	0.012	-7.033	< 0.001	***
	10 60 MAE	0.045	0.012	3.892	0.036	*
	15 60 MAE	0.057	0.012	4.904	< 0.001	***
	30 60 MAE	0.171	0.012	14.797	< 0.001	***
	1 70 MAE	0.202	0.012	17.553	< 0.001	***
	5 70 MAE	-0.067	0.012	-5.809	< 0.001	***
	10 70 MAE	0.068	0.012	5.905	< 0.001	***
	15 70 MAE	0.114	0.012	9.894	< 0.001	***
	30 70 MAE	0.174	0.012	15.077	< 0.001	***
30 50 MAE	1 60 MAE	-0.039	0.012	-3.401	0.159	
	5 60 MAE	-0.147	0.012	-12.784	< 0.001	***
	10 60 MAE	-0.021	0.012	-1.859	0.991	
	15 60 MAE	-0.010	0.012	-0.848	1.000	
	30 60 MAE	0.104	0.012	9.046	< 0.001	***
	1 70 MAE	0.136	0.012	11.801	< 0.001	***
	5 70 MAE	-0.133	0.012	-11.561	< 0.001	***
	10 70 MAE	0.002	0.012	0.154	1.000	
	15 70 MAE	0.048	0.012	4.143	0.015	*
	30 70 MAE	0.108	0.012	9.325	< 0.001	***
1 60 MAE	5 60 MAE	-0.108	0.012	-9.383	< 0.001	***
	10 60 MAE	0.018	0.012	1.541	1.000	
	15 60 MAE	0.029	0.012	2.553	0.740	
	30 60 MAE	0.144	0.012	12.447	< 0.001	***
	1 70 MAE	0.175	0.012	15.202	< 0.001	***
	5 70 MAE	-0.094	0.012	-8.160	< 0.001	***
	10 70 MAE	0.041	0.012	3.555	0.104	
	15 70 MAE	0.087	0.012	7.543	< 0.001	***
	30 70 MAE	0.147	0.012	12.726	< 0.001	***
5 60 MAE	10 60 MAE	0.126	0.012	10.925	< 0.001	***
	15 60 MAE	0.138	0.012	11.936	< 0.001	***
	30 60 MAE	0.252	0.012	21.830	< 0.001	***
	1 70 MAE	0.284	0.012	24.585	< 0.001	***
	5 70 MAE	0.014	0.012	1.223	1.000	
	10 70 MAE	0.149	0.012	12.938	< 0.001	***
	15 70 MAE	0.195	0.012	16.927	< 0.001	***
	30 70 MAE	0.255	0.012	22.109	< 0.001	***
10 60 MAE	15 60 MAE	0.012	0.012	1.012	1.000	
	30 60 MAE	0.126	0.012	10.905	< 0.001	***
	1 70 MAE	0.158	0.012	13.661	< 0.001	***
	5 70 MAE	-0.112	0.012	-9.701	< 0.001	***
	10 70 MAE	0.023	0.012	2.013	0.974	
	15 70 MAE	0.069	0.012	6.002	< 0.001	***
	30 70 MAE	0.129	0.012	11.185	< 0.001	***
15 60 MAE	30 60 MAE	0.114	0.012	9.894	< 0.001	***
	1 70 MAE	0.146	0.012	12.649	< 0.001	***
	5 70 MAE	-0.124	0.012	-10.713	< 0.001	***
	10 70 MAE	0.012	0.012	1.002	1.000	
	15 70 MAE	0.058	0.012	4.990	< 0.001	***

Post Hoc Comparisons - Time ✻ Temperature ✻ Heating method

		Mean Difference	SE	t	p_{tukey}	
	30 70 MAE	0.117	0.012	10.173	< 0.001	***
30 60 MAE	1 70 MAE	0.032	0.012	2.755	0.584	
	5 70 MAE	-0.238	0.012	-20.607	< 0.001	***
	10 70 MAE	-0.103	0.012	-8.892	< 0.001	***
	15 70 MAE	-0.057	0.012	-4.904	< 0.001	***
	30 70 MAE	0.003	0.012	0.279	1.000	
1 70 MAE	5 70 MAE	-0.269	0.012	-23.362	< 0.001	***
	10 70 MAE	-0.134	0.012	-11.647	< 0.001	***
	15 70 MAE	-0.088	0.012	-7.659	< 0.001	***
	30 70 MAE	-0.029	0.012	-2.476	0.792	
5 70 MAE	10 70 MAE	0.135	0.012	11.715	< 0.001	***
	15 70 MAE	0.181	0.012	15.703	< 0.001	***
	30 70 MAE	0.241	0.012	20.886	< 0.001	***
10 70 MAE	15 70 MAE	0.046	0.012	3.988	0.026	*
	30 70 MAE	0.106	0.012	9.171	< 0.001	***
15 70 MAE	30 70 MAE	0.060	0.012	5.183	< 0.001	***

Note. P-value adjusted for comparing a family of 30

F.5.3 Antioxidant Activity (AOA)

ANOVA - Antioxidant Activity

Cases	Sum of Squares	df	Mean Square	F	p	η^2
Time	4.518	4	1.130	151.293	< 0.001***	0.212
Temperature	12.856	2	6.428	860.925	< 0.001***	0.604
Heating method	0.199	1	0.199	26.676	< 0.001***	0.009
Time ✻ Temperature	0.998	8	0.125	16.709	< 0.001***	0.047
Time ✻ Heating method	0.466	4	0.116	15.599	< 0.001***	0.022
Temperature ✻ Heating method	0.155	2	0.077	10.355	< 0.001***	0.007
Time ✻ Temperature ✻ Heating method	0.314	8	0.039	5.254	< 0.001***	0.015
Residuals	1.792	240	0.007			

Note. Type III Sum of Squares

Post Hoc Comparisons - Time

		Mean Difference	SE	t	p_{tukey}
1	5	-0.358	0.017	-21.525	< 0.001 ***
	10	-0.124	0.017	-7.438	< 0.001 ***
	15	-0.074	0.017	-4.455	< 0.001 ***
	30	-0.017	0.017	-1.000	0.855
5	10	0.234	0.017	14.087	< 0.001 ***
	15	0.284	0.017	17.070	< 0.001 ***
	30	0.341	0.017	20.526	< 0.001 ***
10	15	0.050	0.017	2.983	0.026 *
	30	0.107	0.017	6.439	< 0.001 ***
15	30	0.057	0.017	3.456	0.006 **

* p < 0.05, ** p < 0.01, *** p < 0.001

Note. P-value adjusted for comparing a family of 5

Note. Results are averaged over the levels of: Temperature, Heating method

Post Hoc Comparisons - Temperature

		Mean Difference	SE	t	p_{tukey}
50	60	0.354	0.013	27.483	< 0.001 ***
	70	0.524	0.013	40.666	< 0.001 ***
60	70	0.170	0.013	13.183	< 0.001 ***

*** p < 0.001

Note. P-value adjusted for comparing a family of 3

Note. Results are averaged over the levels of: Time, Heating method

Post Hoc Comparisons - Heating method

		Mean Difference	SE	t	p_{tukey}
CSE	MAE	0.054	0.011	5.165	< 0.001 ***

*** p < 0.001

Note. Results are averaged over the levels of: Time, Temperature

Post Hoc Comparisons - Time ✻ Temperature

		Mean Difference	SE	t	p_{tukey}	
1 50	5 50	-0.409	0.029	-14.185	< 0.001	***
	10 50	-0.332	0.029	-11.513	< 0.001	***
	15 50	-0.279	0.029	-9.693	< 0.001	***
	30 50	-0.164	0.029	-5.700	< 0.001	***
	1 60	0.179	0.029	6.229	< 0.001	***
	5 60	-0.142	0.029	-4.928	< 0.001	***
	10 60	0.143	0.029	4.969	< 0.001	***
	15 60	0.169	0.029	5.866	< 0.001	***
	30 60	0.237	0.029	8.226	< 0.001	***
	1 70	0.332	0.029	11.516	< 0.001	***
	5 70	-0.012	0.029	-0.425	1.000	
	10 70	0.329	0.029	11.406	< 0.001	***
	15 70	0.399	0.029	13.855	< 0.001	***
	30 70	0.388	0.029	13.487	< 0.001	***
5 50	10 50	0.077	0.029	2.671	0.329	
	15 50	0.129	0.029	4.492	0.001	**
	30 50	0.244	0.029	8.485	< 0.001	***
	1 60	0.588	0.029	20.414	< 0.001	***
	5 60	0.267	0.029	9.257	< 0.001	***
	10 60	0.552	0.029	19.153	< 0.001	***
	15 60	0.578	0.029	20.051	< 0.001	***
	30 60	0.646	0.029	22.411	< 0.001	***
	1 70	0.740	0.029	25.700	< 0.001	***
	5 70	0.396	0.029	13.760	< 0.001	***
	10 70	0.737	0.029	25.591	< 0.001	***
	15 70	0.808	0.029	28.040	< 0.001	***
	30 70	0.797	0.029	27.672	< 0.001	***
10 50	15 50	0.052	0.029	1.821	0.888	
	30 50	0.167	0.029	5.813	< 0.001	***
	1 60	0.511	0.029	17.743	< 0.001	***
	5 60	0.190	0.029	6.586	< 0.001	***
	10 60	0.475	0.029	16.482	< 0.001	***
	15 60	0.501	0.029	17.379	< 0.001	***
	30 60	0.569	0.029	19.740	< 0.001	***
	1 70	0.663	0.029	23.029	< 0.001	***
	5 70	0.319	0.029	11.088	< 0.001	***
	10 70	0.660	0.029	22.920	< 0.001	***
	15 70	0.731	0.029	25.369	< 0.001	***
	30 70	0.720	0.029	25.001	< 0.001	***
15 50	30 50	0.115	0.029	3.993	0.007	**
	1 60	0.459	0.029	15.922	< 0.001	***
	5 60	0.137	0.029	4.765	< 0.001	***
	10 60	0.422	0.029	14.661	< 0.001	***
	15 60	0.448	0.029	15.559	< 0.001	***
	30 60	0.516	0.029	17.919	< 0.001	***
	1 70	0.611	0.029	21.208	< 0.001	***
	5 70	0.267	0.029	9.268	< 0.001	***
	10 70	0.608	0.029	21.099	< 0.001	***
	15 70	0.678	0.029	23.548	< 0.001	***
	30 70	0.668	0.029	23.180	< 0.001	***
30 50	1 60	0.344	0.029	11.930	< 0.001	***

Post Hoc Comparisons - Time ✻ Temperature

		Mean Difference	SE	t	p_{tukey}	
	5 60	0.022	0.029	0.772	1.000	
	10 60	0.307	0.029	10.669	< 0.001	***
	15 60	0.333	0.029	11.566	< 0.001	***
	30 60	0.401	0.029	13.927	< 0.001	***
	1 70	0.496	0.029	17.216	< 0.001	***
	5 70	0.152	0.029	5.275	< 0.001	***
	10 70	0.493	0.029	17.107	< 0.001	***
	15 70	0.563	0.029	19.556	< 0.001	***
	30 70	0.553	0.029	19.188	< 0.001	***
1 60	5 60	-0.321	0.029	-11.157	< 0.001	***
	10 60	-0.036	0.029	-1.261	0.995	
	15 60	-0.010	0.029	-0.364	1.000	
	30 60	0.058	0.029	1.997	0.798	
	1 70	0.152	0.029	5.286	< 0.001	***
	5 70	-0.192	0.029	-6.655	< 0.001	***
	10 70	0.149	0.029	5.177	< 0.001	***
	15 70	0.220	0.029	7.626	< 0.001	***
	30 70	0.209	0.029	7.258	< 0.001	***
5 60	10 60	0.285	0.029	9.896	< 0.001	***
	15 60	0.311	0.029	10.794	< 0.001	***
	30 60	0.379	0.029	13.154	< 0.001	***
	1 70	0.474	0.029	16.443	< 0.001	***
	5 70	0.130	0.029	4.503	< 0.001	***
	10 70	0.470	0.029	16.334	< 0.001	***
	15 70	0.541	0.029	18.783	< 0.001	***
	30 70	0.530	0.029	18.415	< 0.001	***
10 60	15 60	0.026	0.029	0.897	1.000	
	30 60	0.094	0.029	3.258	0.082	
	1 70	0.189	0.029	6.547	< 0.001	***
	5 70	-0.155	0.029	-5.394	< 0.001	***
	10 70	0.185	0.029	6.438	< 0.001	***
	15 70	0.256	0.029	8.887	< 0.001	***
	30 70	0.245	0.029	8.519	< 0.001	***
15 60	30 60	0.068	0.029	2.361	0.547	
	1 70	0.163	0.029	5.650	< 0.001	***
	5 70	-0.181	0.029	-6.291	< 0.001	***
	10 70	0.160	0.029	5.541	< 0.001	***
	15 70	0.230	0.029	7.989	< 0.001	***
	30 70	0.220	0.029	7.622	< 0.001	***
30 60	1 70	0.095	0.029	3.289	0.075	
	5 70	-0.249	0.029	-8.652	< 0.001	***
	10 70	0.092	0.029	3.180	0.102	
	15 70	0.162	0.029	5.629	< 0.001	***
	30 70	0.152	0.029	5.261	< 0.001	***
1 70	5 70	-0.344	0.029	-11.941	< 0.001	***
	10 70	-0.003	0.029	-0.109	1.000	
	15 70	0.067	0.029	2.340	0.562	
	30 70	0.057	0.029	1.972	0.813	
5 70	10 70	0.341	0.029	11.832	< 0.001	***
	15 70	0.411	0.029	14.280	< 0.001	***
	30 70	0.401	0.029	13.913	< 0.001	***

Post Hoc Comparisons - Time ✻ Temperature

		Mean Difference	SE	t	p_{tukey}
10 70	15 70	0.071	0.029	2.449	0.481
	30 70	0.060	0.029	2.081	0.746
15 70	30 70	-0.011	0.029	-0.368	1.000

* p < 0.05, ** p < 0.01, *** p < 0.001

Note. P-value adjusted for comparing a family of 15

Note. Results are averaged over the levels of: Heating method

Post Hoc Comparisons - Time ✻ Heating method

		Mean Difference	SE	t	p_{tukey}
1 CSE	5 CSE	-0.467	0.024	-19.869	< 0.001 ***
	10 CSE	-0.234	0.024	-9.949	< 0.001 ***
	15 CSE	-0.175	0.024	-7.451	< 0.001 ***
	30 CSE	-0.099	0.024	-4.201	0.002 **
	1 MAE	-0.107	0.024	-4.544	< 0.001 ***
	5 MAE	-0.355	0.024	-15.116	< 0.001 ***
	10 MAE	-0.120	0.024	-5.114	< 0.001 ***
	15 MAE	-0.080	0.024	-3.393	0.027 *
	30 MAE	-0.041	0.024	-1.756	0.762
5 CSE	10 CSE	0.233	0.024	9.920	< 0.001 ***
	15 CSE	0.292	0.024	12.418	< 0.001 ***
	30 CSE	0.368	0.024	15.668	< 0.001 ***
	1 MAE	0.360	0.024	15.325	< 0.001 ***
	5 MAE	0.112	0.024	4.753	< 0.001 ***
	10 MAE	0.347	0.024	14.756	< 0.001 ***
	15 MAE	0.387	0.024	16.476	< 0.001 ***
	30 MAE	0.426	0.024	18.113	< 0.001 ***
10 CSE	15 CSE	0.059	0.024	2.498	0.275
	30 CSE	0.135	0.024	5.748	< 0.001 ***
	1 MAE	0.127	0.024	5.406	< 0.001 ***
	5 MAE	-0.122	0.024	-5.167	< 0.001 ***
	10 MAE	0.114	0.024	4.836	< 0.001 ***
	15 MAE	0.154	0.024	6.556	< 0.001 ***
	30 MAE	0.193	0.024	8.193	< 0.001 ***
15 CSE	30 CSE	0.076	0.024	3.250	0.042 *
	1 MAE	0.068	0.024	2.908	0.109
	5 MAE	-0.180	0.024	-7.665	< 0.001 ***
	10 MAE	0.055	0.024	2.338	0.369
	15 MAE	0.095	0.024	4.058	0.003 **
	30 MAE	0.134	0.024	5.695	< 0.001 ***
30 CSE	1 MAE	-0.008	0.024	-0.342	1.000
	5 MAE	-0.257	0.024	-10.914	< 0.001 ***
	10 MAE	-0.021	0.024	-0.912	0.996
	15 MAE	0.019	0.024	0.808	0.998
	30 MAE	0.058	0.024	2.446	0.304
1 MAE	5 MAE	-0.249	0.024	-10.572	< 0.001 ***
	10 MAE	-0.013	0.024	-0.570	1.000
	15 MAE	0.027	0.024	1.151	0.979
	30 MAE	0.066	0.024	2.788	0.147
5 MAE	10 MAE	0.235	0.024	10.002	< 0.001 ***
	15 MAE	0.276	0.024	11.723	< 0.001 ***

Post Hoc Comparisons - Time ✻ Heating method

		Mean Difference	SE	t	p_{tukey}
	30 MAE	0.314	0.024	13.360	< 0.001 ***
10 MAE	15 MAE	0.040	0.024	1.721	0.783
	30 MAE	0.079	0.024	3.358	0.031 *
15 MAE	30 MAE	0.039	0.024	1.637	0.829

Note. Results are averaged over the levels of: Temperature

Note. P-value adjusted for comparing a family of 10

* p < 0.05, ** p < 0.01, *** p < 0.001

Post Hoc Comparisons - Temperature ✻ Heating method

		Mean Difference	SE	t	p_{tukey}
50 CSE	60 CSE	0.297	0.018	16.291	< 0.001 ***
	70 CSE	0.506	0.018	27.786	< 0.001 ***
	50 MAE	0.004	0.018	0.242	1.000
	60 MAE	0.416	0.018	22.817	< 0.001 ***
	70 MAE	0.546	0.018	29.965	< 0.001 ***
60 CSE	70 CSE	0.209	0.018	11.495	< 0.001 ***
	50 MAE	-0.292	0.018	-16.050	< 0.001 ***
	60 MAE	0.119	0.018	6.525	< 0.001 ***
	70 MAE	0.249	0.018	13.674	< 0.001 ***
70 CSE	50 MAE	-0.502	0.018	-27.545	< 0.001 ***
	60 MAE	-0.091	0.018	-4.969	< 0.001 ***
	70 MAE	0.040	0.018	2.179	0.251
50 MAE	60 MAE	0.411	0.018	22.575	< 0.001 ***
	70 MAE	0.541	0.018	29.724	< 0.001 ***
60 MAE	70 MAE	0.130	0.018	7.148	< 0.001 ***

*** p < 0.001

Note. P-value adjusted for comparing a family of 6

Note. Results are averaged over the levels of: Time

Post Hoc Comparisons - Time ✻ Temperature ✻ Heating method

		Mean Difference	SE	t	p_{tukey}
1 50 CSE	5 50 CSE	-0.575	0.041	-14.118	< 0.001 ***
	10 50 CSE	-0.520	0.041	-12.755	< 0.001 ***
	15 50 CSE	-0.430	0.041	-10.560	< 0.001 ***
	30 50 CSE	-0.296	0.041	-7.273	< 0.001 ***
	1 60 CSE	0.044	0.041	1.069	1.000
	5 60 CSE	-0.288	0.041	-7.061	< 0.001 ***
	10 60 CSE	-0.045	0.041	-1.108	1.000
	15 60 CSE	-0.063	0.041	-1.547	1.000
	30 60 CSE	0.015	0.041	0.372	1.000
	1 70 CSE	0.252	0.041	6.186	< 0.001 ***
	5 70 CSE	-0.244	0.041	-5.980	< 0.001 ***
	10 70 CSE	0.158	0.041	3.885	0.037 *
	15 70 CSE	0.263	0.041	6.456	< 0.001 ***
	30 70 CSE	0.280	0.041	6.879	< 0.001 ***
	1 50 MAE	-0.251	0.041	-6.152	< 0.001 ***
	5 50 MAE	-0.493	0.041	-12.094	< 0.001 ***
	10 50 MAE	-0.394	0.041	-9.679	< 0.001 ***
	15 50 MAE	-0.379	0.041	-9.300	< 0.001 ***

Post Hoc Comparisons - Time ✻ Temperature ✻ Heating method

		Mean Difference	SE	t	p_{tukey}	
	30 50 MAE	-0.283	0.041	-6.941	< 0.001	***
	1 60 MAE	0.065	0.041	1.589	0.999	
	5 60 MAE	-0.247	0.041	-6.060	< 0.001	***
	10 60 MAE	0.081	0.041	1.983	0.979	
	15 60 MAE	0.150	0.041	3.691	0.069	
	30 60 MAE	0.208	0.041	5.111	< 0.001	***
	1 70 MAE	0.161	0.041	3.947	0.030	*
	5 70 MAE	-0.031	0.041	-0.773	1.000	
	10 70 MAE	0.248	0.041	6.094	< 0.001	***
	15 70 MAE	0.285	0.041	6.987	< 0.001	***
	30 70 MAE	0.246	0.041	6.044	< 0.001	***
5 50 CSE	10 50 CSE	0.056	0.041	1.364	1.000	
	15 50 CSE	0.145	0.041	3.559	0.103	
	30 50 CSE	0.279	0.041	6.846	< 0.001	***
	1 60 CSE	0.619	0.041	15.187	< 0.001	***
	5 60 CSE	0.287	0.041	7.057	< 0.001	***
	10 60 CSE	0.530	0.041	13.010	< 0.001	***
	15 60 CSE	0.512	0.041	12.571	< 0.001	***
	30 60 CSE	0.590	0.041	14.490	< 0.001	***
	1 70 CSE	0.827	0.041	20.305	< 0.001	***
	5 70 CSE	0.331	0.041	8.138	< 0.001	***
	10 70 CSE	0.733	0.041	18.003	< 0.001	***
	15 70 CSE	0.838	0.041	20.575	< 0.001	***
	30 70 CSE	0.855	0.041	20.997	< 0.001	***
	1 50 MAE	0.325	0.041	7.967	< 0.001	***
	5 50 MAE	0.082	0.041	2.025	0.972	
	10 50 MAE	0.181	0.041	4.439	0.005	**
	15 50 MAE	0.196	0.041	4.819	< 0.001	***
	30 50 MAE	0.292	0.041	7.178	< 0.001	***
	1 60 MAE	0.640	0.041	15.708	< 0.001	***
	5 60 MAE	0.328	0.041	8.059	< 0.001	***
	10 60 MAE	0.656	0.041	16.101	< 0.001	***
	15 60 MAE	0.725	0.041	17.810	< 0.001	***
	30 60 MAE	0.783	0.041	19.229	< 0.001	***
	1 70 MAE	0.736	0.041	18.066	< 0.001	***
	5 70 MAE	0.544	0.041	13.345	< 0.001	***
	10 70 MAE	0.823	0.041	20.213	< 0.001	***
	15 70 MAE	0.860	0.041	21.105	< 0.001	***
	30 70 MAE	0.821	0.041	20.162	< 0.001	***
10 50 CSE	15 50 CSE	0.089	0.041	2.195	0.931	
	30 50 CSE	0.223	0.041	5.482	< 0.001	***
	1 60 CSE	0.563	0.041	13.823	< 0.001	***
	5 60 CSE	0.232	0.041	5.694	< 0.001	***
	10 60 CSE	0.474	0.041	11.647	< 0.001	***
	15 60 CSE	0.457	0.041	11.207	< 0.001	***
	30 60 CSE	0.535	0.041	13.126	< 0.001	***
	1 70 CSE	0.772	0.041	18.941	< 0.001	***
	5 70 CSE	0.276	0.041	6.775	< 0.001	***
	10 70 CSE	0.678	0.041	16.640	< 0.001	***
	15 70 CSE	0.783	0.041	19.211	< 0.001	***
	30 70 CSE	0.800	0.041	19.634	< 0.001	***

Post Hoc Comparisons - Time ✻ Temperature ✻ Heating method

		Mean Difference	SE	t	p_{tukey}	
	1 50 MAE	0.269	0.041	6.603	< 0.001	***
	5 50 MAE	0.027	0.041	0.661	1.000	
	10 50 MAE	0.125	0.041	3.075	0.339	
	15 50 MAE	0.141	0.041	3.455	0.138	
	30 50 MAE	0.237	0.041	5.814	< 0.001	***
	1 60 MAE	0.584	0.041	14.344	< 0.001	***
	5 60 MAE	0.273	0.041	6.695	< 0.001	***
	10 60 MAE	0.600	0.041	14.738	< 0.001	***
	15 60 MAE	0.670	0.041	16.446	< 0.001	***
	30 60 MAE	0.728	0.041	17.865	< 0.001	***
	1 70 MAE	0.680	0.041	16.702	< 0.001	***
	5 70 MAE	0.488	0.041	11.982	< 0.001	***
	10 70 MAE	0.768	0.041	18.849	< 0.001	***
	15 70 MAE	0.804	0.041	19.741	< 0.001	***
	30 70 MAE	0.766	0.041	18.798	< 0.001	***
15 50 CSE	30 50 CSE	0.134	0.041	3.287	0.212	
	1 60 CSE	0.474	0.041	11.628	< 0.001	***
	5 60 CSE	0.143	0.041	3.499	0.122	
	10 60 CSE	0.385	0.041	9.452	< 0.001	***
	15 60 CSE	0.367	0.041	9.012	< 0.001	***
	30 60 CSE	0.445	0.041	10.931	< 0.001	***
	1 70 CSE	0.682	0.041	16.746	< 0.001	***
	5 70 CSE	0.187	0.041	4.580	0.003	**
	10 70 CSE	0.588	0.041	14.445	< 0.001	***
	15 70 CSE	0.693	0.041	17.016	< 0.001	***
	30 70 CSE	0.710	0.041	17.438	< 0.001	***
	1 50 MAE	0.180	0.041	4.408	0.005	**
	5 50 MAE	-0.062	0.041	-1.534	1.000	
	10 50 MAE	0.036	0.041	0.880	1.000	
	15 50 MAE	0.051	0.041	1.260	1.000	
	30 50 MAE	0.147	0.041	3.619	0.086	
	1 60 MAE	0.495	0.041	12.149	< 0.001	***
	5 60 MAE	0.183	0.041	4.500	0.004	**
	10 60 MAE	0.511	0.041	12.543	< 0.001	***
	15 60 MAE	0.580	0.041	14.251	< 0.001	***
	30 60 MAE	0.638	0.041	15.670	< 0.001	***
	1 70 MAE	0.591	0.041	14.507	< 0.001	***
	5 70 MAE	0.399	0.041	9.787	< 0.001	***
	10 70 MAE	0.678	0.041	16.654	< 0.001	***
	15 70 MAE	0.715	0.041	17.546	< 0.001	***
	30 70 MAE	0.676	0.041	16.603	< 0.001	***
30 50 CSE	1 60 CSE	0.340	0.041	8.341	< 0.001	***
	5 60 CSE	0.009	0.041	0.211	1.000	
	10 60 CSE	0.251	0.041	6.164	< 0.001	***
	15 60 CSE	0.233	0.041	5.725	< 0.001	***
	30 60 CSE	0.311	0.041	7.644	< 0.001	***
	1 70 CSE	0.548	0.041	13.459	< 0.001	***
	5 70 CSE	0.053	0.041	1.293	1.000	
	10 70 CSE	0.454	0.041	11.158	< 0.001	***
	15 70 CSE	0.559	0.041	13.729	< 0.001	***
	30 70 CSE	0.576	0.041	14.151	< 0.001	***

Post Hoc Comparisons - Time ✻ Temperature ✻ Heating method

		Mean Difference	SE	t	p_{tukey}	
	1 50 MAE	0.046	0.041	1.121	1.000	
	5 50 MAE	-0.196	0.041	-4.821	< 0.001	***
	10 50 MAE	-0.098	0.041	-2.407	0.835	
	15 50 MAE	-0.083	0.041	-2.027	0.972	
	30 50 MAE	0.014	0.041	0.332	1.000	
	1 60 MAE	0.361	0.041	8.862	< 0.001	***
	5 60 MAE	0.049	0.041	1.213	1.000	
	10 60 MAE	0.377	0.041	9.255	< 0.001	***
	15 60 MAE	0.447	0.041	10.964	< 0.001	***
	30 60 MAE	0.504	0.041	12.383	< 0.001	***
	1 70 MAE	0.457	0.041	11.220	< 0.001	***
	5 70 MAE	0.265	0.041	6.500	< 0.001	***
	10 70 MAE	0.544	0.041	13.367	< 0.001	***
	15 70 MAE	0.581	0.041	14.259	< 0.001	***
	30 70 MAE	0.542	0.041	13.316	< 0.001	***
1 60 CSE	5 60 CSE	-0.331	0.041	-8.130	< 0.001	***
	10 60 CSE	-0.089	0.041	-2.177	0.936	
	15 60 CSE	-0.107	0.041	-2.616	0.694	
	30 60 CSE	-0.028	0.041	-0.697	1.000	
	1 70 CSE	0.208	0.041	5.118	< 0.001	***
	5 70 CSE	-0.287	0.041	-7.049	< 0.001	***
	10 70 CSE	0.115	0.041	2.817	0.535	
	15 70 CSE	0.219	0.041	5.388	< 0.001	***
	30 70 CSE	0.237	0.041	5.810	< 0.001	***
	1 50 MAE	-0.294	0.041	-7.220	< 0.001	***
	5 50 MAE	-0.536	0.041	-13.162	< 0.001	***
	10 50 MAE	-0.438	0.041	-10.748	< 0.001	***
	15 50 MAE	-0.422	0.041	-10.368	< 0.001	***
	30 50 MAE	-0.326	0.041	-8.009	< 0.001	***
	1 60 MAE	0.021	0.041	0.521	1.000	
	5 60 MAE	-0.290	0.041	-7.128	< 0.001	***
	10 60 MAE	0.037	0.041	0.914	1.000	
	15 60 MAE	0.107	0.041	2.623	0.689	
	30 60 MAE	0.165	0.041	4.042	0.022	*
	1 70 MAE	0.117	0.041	2.879	0.485	
	5 70 MAE	-0.075	0.041	-1.842	0.992	
	10 70 MAE	0.205	0.041	5.026	< 0.001	***
	15 70 MAE	0.241	0.041	5.918	< 0.001	***
	30 70 MAE	0.203	0.041	4.975	< 0.001	***
5 60 CSE	10 60 CSE	0.242	0.041	5.953	< 0.001	***
	15 60 CSE	0.225	0.041	5.514	< 0.001	***
	30 60 CSE	0.303	0.041	7.433	< 0.001	***
	1 70 CSE	0.540	0.041	13.247	< 0.001	***
	5 70 CSE	0.044	0.041	1.081	1.000	
	10 70 CSE	0.446	0.041	10.946	< 0.001	***
	15 70 CSE	0.551	0.041	13.517	< 0.001	***
	30 70 CSE	0.568	0.041	13.940	< 0.001	***
	1 50 MAE	0.037	0.041	0.909	1.000	
	5 50 MAE	-0.205	0.041	-5.033	< 0.001	***
	10 50 MAE	-0.107	0.041	-2.618	0.692	
	15 50 MAE	-0.091	0.041	-2.239	0.915	

Post Hoc Comparisons - Time ✻ Temperature ✻ Heating method

		Mean Difference	SE	t	p_{tukey}	
	30 50 MAE	0.005	0.041	0.121	1.000	
	1 60 MAE	0.352	0.041	8.650	< 0.001	***
	5 60 MAE	0.041	0.041	1.001	1.000	
	10 60 MAE	0.368	0.041	9.044	< 0.001	***
	15 60 MAE	0.438	0.041	10.752	< 0.001	***
	30 60 MAE	0.496	0.041	12.172	< 0.001	***
	1 70 MAE	0.448	0.041	11.009	< 0.001	***
	5 70 MAE	0.256	0.041	6.288	< 0.001	***
	10 70 MAE	0.536	0.041	13.155	< 0.001	***
	15 70 MAE	0.572	0.041	14.048	< 0.001	***
	30 70 MAE	0.534	0.041	13.105	< 0.001	***
10 60 CSE	15 60 CSE	-0.018	0.041	-0.439	1.000	
	30 60 CSE	0.060	0.041	1.480	1.000	
	1 70 CSE	0.297	0.041	7.294	< 0.001	***
	5 70 CSE	-0.198	0.041	-4.872	< 0.001	***
	10 70 CSE	0.203	0.041	4.993	< 0.001	***
	15 70 CSE	0.308	0.041	7.564	< 0.001	***
	30 70 CSE	0.325	0.041	7.987	< 0.001	***
	1 50 MAE	-0.205	0.041	-5.044	< 0.001	***
	5 50 MAE	-0.447	0.041	-10.986	< 0.001	***
	10 50 MAE	-0.349	0.041	-8.571	< 0.001	***
	15 50 MAE	-0.334	0.041	-8.192	< 0.001	***
	30 50 MAE	-0.238	0.041	-5.832	< 0.001	***
	1 60 MAE	0.110	0.041	2.697	0.630	
	5 60 MAE	-0.202	0.041	-4.952	< 0.001	***
	10 60 MAE	0.126	0.041	3.091	0.328	
	15 60 MAE	0.195	0.041	4.799	0.001	**
	30 60 MAE	0.253	0.041	6.219	< 0.001	***
	1 70 MAE	0.206	0.041	5.056	< 0.001	***
	5 70 MAE	0.014	0.041	0.335	1.000	
	10 70 MAE	0.293	0.041	7.202	< 0.001	***
	15 70 MAE	0.330	0.041	8.095	< 0.001	***
	30 70 MAE	0.291	0.041	7.152	< 0.001	***
15 60 CSE	30 60 CSE	0.078	0.041	1.919	0.986	
	1 70 CSE	0.315	0.041	7.734	< 0.001	***
	5 70 CSE	-0.181	0.041	-4.433	0.005	**
	10 70 CSE	0.221	0.041	5.433	< 0.001	***
	15 70 CSE	0.326	0.041	8.004	< 0.001	***
	30 70 CSE	0.343	0.041	8.426	< 0.001	***
	1 50 MAE	-0.188	0.041	-4.604	0.002	**
	5 50 MAE	-0.430	0.041	-10.546	< 0.001	***
	10 50 MAE	-0.331	0.041	-8.132	< 0.001	***
	15 50 MAE	-0.316	0.041	-7.752	< 0.001	***
	30 50 MAE	-0.220	0.041	-5.393	< 0.001	***
	1 60 MAE	0.128	0.041	3.137	0.298	
	5 60 MAE	-0.184	0.041	-4.512	0.004	**
	10 60 MAE	0.144	0.041	3.530	0.112	
	15 60 MAE	0.213	0.041	5.239	< 0.001	***
	30 60 MAE	0.271	0.041	6.658	< 0.001	***
	1 70 MAE	0.224	0.041	5.495	< 0.001	***
	5 70 MAE	0.032	0.041	0.774	1.000	

Post Hoc Comparisons - Time ✻ Temperature ✻ Heating method

		Mean Difference	SE	t	p_{tukey}	
	10 70 MAE	0.311	0.041	7.642	< 0.001	***
	15 70 MAE	0.348	0.041	8.534	< 0.001	***
	30 70 MAE	0.309	0.041	7.591	< 0.001	***
30 60 CSE	1 70 CSE	0.237	0.041	5.815	< 0.001	***
	5 70 CSE	-0.259	0.041	-6.352	< 0.001	***
	10 70 CSE	0.143	0.041	3.514	0.117	
	15 70 CSE	0.248	0.041	6.085	< 0.001	***
	30 70 CSE	0.265	0.041	6.507	< 0.001	***
	1 50 MAE	-0.266	0.041	-6.523	< 0.001	***
	5 50 MAE	-0.508	0.041	-12.465	< 0.001	***
	10 50 MAE	-0.409	0.041	-10.051	< 0.001	***
	15 50 MAE	-0.394	0.041	-9.671	< 0.001	***
	30 50 MAE	-0.298	0.041	-7.312	< 0.001	***
	1 60 MAE	0.050	0.041	1.218	1.000	
	5 60 MAE	-0.262	0.041	-6.431	< 0.001	***
	10 60 MAE	0.066	0.041	1.611	0.999	
	15 60 MAE	0.135	0.041	3.320	0.195	
	30 60 MAE	0.193	0.041	4.739	0.001	**
	1 70 MAE	0.146	0.041	3.576	0.098	
	5 70 MAE	-0.047	0.041	-1.144	1.000	
	10 70 MAE	0.233	0.041	5.723	< 0.001	***
	15 70 MAE	0.269	0.041	6.615	< 0.001	***
	30 70 MAE	0.231	0.041	5.672	< 0.001	***
1 70 CSE	5 70 CSE	-0.496	0.041	-12.166	< 0.001	***
	10 70 CSE	-0.094	0.041	-2.301	0.889	
	15 70 CSE	0.011	0.041	0.270	1.000	
	30 70 CSE	0.028	0.041	0.693	1.000	
	1 50 MAE	-0.503	0.041	-12.338	< 0.001	***
	5 50 MAE	-0.745	0.041	-18.280	< 0.001	***
	10 50 MAE	-0.646	0.041	-15.866	< 0.001	***
	15 50 MAE	-0.631	0.041	-15.486	< 0.001	***
	30 50 MAE	-0.535	0.041	-13.127	< 0.001	***
	1 60 MAE	-0.187	0.041	-4.597	0.003	**
	5 60 MAE	-0.499	0.041	-12.246	< 0.001	***
	10 60 MAE	-0.171	0.041	-4.203	0.012	*
	15 60 MAE	-0.102	0.041	-2.495	0.780	
	30 60 MAE	-0.044	0.041	-1.076	1.000	
	1 70 MAE	-0.091	0.041	-2.239	0.915	
	5 70 MAE	-0.283	0.041	-6.959	< 0.001	***
	10 70 MAE	-0.004	0.041	-0.092	1.000	
	15 70 MAE	0.033	0.041	0.800	1.000	
	30 70 MAE	-0.006	0.041	-0.143	1.000	
5 70 CSE	10 70 CSE	0.402	0.041	9.865	< 0.001	***
	15 70 CSE	0.507	0.041	12.436	< 0.001	***
	30 70 CSE	0.524	0.041	12.859	< 0.001	***
	1 50 MAE	-0.007	0.041	-0.172	1.000	
	5 50 MAE	-0.249	0.041	-6.114	< 0.001	***
	10 50 MAE	-0.151	0.041	-3.699	0.068	
	15 50 MAE	-0.135	0.041	-3.320	0.195	
	30 50 MAE	-0.039	0.041	-0.961	1.000	
	1 60 MAE	0.308	0.041	7.569	< 0.001	***

Post Hoc Comparisons - Time ✻ Temperature ✻ Heating method

		Mean Difference	SE	t	p_{tukey}	
	5 60 MAE	-0.003	0.041	-0.080	1.000	
	10 60 MAE	0.324	0.041	7.963	< 0.001	***
	15 60 MAE	0.394	0.041	9.671	< 0.001	***
	30 60 MAE	0.452	0.041	11.091	< 0.001	***
	1 70 MAE	0.404	0.041	9.927	< 0.001	***
	5 70 MAE	0.212	0.041	5.207	< 0.001	***
	10 70 MAE	0.492	0.041	12.074	< 0.001	***
	15 70 MAE	0.528	0.041	12.967	< 0.001	***
	30 70 MAE	0.490	0.041	12.024	< 0.001	***
10 70 CSE	15 70 CSE	0.105	0.041	2.571	0.727	
	30 70 CSE	0.122	0.041	2.994	0.397	
	1 50 MAE	-0.409	0.041	-10.037	< 0.001	***
	5 50 MAE	-0.651	0.041	-15.979	< 0.001	***
	10 50 MAE	-0.553	0.041	-13.564	< 0.001	***
	15 50 MAE	-0.537	0.041	-13.185	< 0.001	***
	30 50 MAE	-0.441	0.041	-10.826	< 0.001	***
	1 60 MAE	-0.094	0.041	-2.296	0.891	
	5 60 MAE	-0.405	0.041	-9.945	< 0.001	***
	10 60 MAE	-0.077	0.041	-1.902	0.988	
	15 60 MAE	-0.008	0.041	-0.194	1.000	
	30 60 MAE	0.050	0.041	1.225	1.000	
	1 70 MAE	0.003	0.041	0.062	1.000	
	5 70 MAE	-0.190	0.041	-4.658	0.002	**
	10 70 MAE	0.090	0.041	2.209	0.926	
	15 70 MAE	0.126	0.041	3.101	0.321	
	30 70 MAE	0.088	0.041	2.158	0.942	
15 70 CSE	30 70 CSE	0.017	0.041	0.423	1.000	
	1 50 MAE	-0.514	0.041	-12.608	< 0.001	***
	5 50 MAE	-0.756	0.041	-18.550	< 0.001	***
	10 50 MAE	-0.657	0.041	-16.136	< 0.001	***
	15 50 MAE	-0.642	0.041	-15.756	< 0.001	***
	30 50 MAE	-0.546	0.041	-13.397	< 0.001	***
	1 60 MAE	-0.198	0.041	-4.867	< 0.001	***
	5 60 MAE	-0.510	0.041	-12.516	< 0.001	***
	10 60 MAE	-0.182	0.041	-4.473	0.004	**
	15 60 MAE	-0.113	0.041	-2.765	0.576	
	30 60 MAE	-0.055	0.041	-1.346	1.000	
	1 70 MAE	-0.102	0.041	-2.509	0.771	
	5 70 MAE	-0.294	0.041	-7.229	< 0.001	***
	10 70 MAE	-0.015	0.041	-0.362	1.000	
	15 70 MAE	0.022	0.041	0.530	1.000	
	30 70 MAE	-0.017	0.041	-0.413	1.000	
30 70 CSE	1 50 MAE	-0.531	0.041	-13.031	< 0.001	***
	5 50 MAE	-0.773	0.041	-18.973	< 0.001	***
	10 50 MAE	-0.674	0.041	-16.558	< 0.001	***
	15 50 MAE	-0.659	0.041	-16.179	< 0.001	***
	30 50 MAE	-0.563	0.041	-13.819	< 0.001	***
	1 60 MAE	-0.215	0.041	-5.289	< 0.001	***
	5 60 MAE	-0.527	0.041	-12.938	< 0.001	***
	10 60 MAE	-0.199	0.041	-4.896	< 0.001	***
	15 60 MAE	-0.130	0.041	-3.188	0.267	

Post Hoc Comparisons - Time ✻ Temperature ✻ Heating method

		Mean Difference	SE	t	p_{tukey}	
	30 60 MAE	-0.072	0.041	-1.768	0.996	
	1 70 MAE	-0.119	0.041	-2.931	0.444	
	5 70 MAE	-0.312	0.041	-7.652	< 0.001	***
	10 70 MAE	-0.032	0.041	-0.785	1.000	
	15 70 MAE	0.004	0.041	0.108	1.000	
	30 70 MAE	-0.034	0.041	-0.835	1.000	
1 50 MAE	5 50 MAE	-0.242	0.041	-5.942	< 0.001	***
	10 50 MAE	-0.144	0.041	-3.528	0.113	
	15 50 MAE	-0.128	0.041	-3.148	0.291	
	30 50 MAE	-0.032	0.041	-0.789	1.000	
	1 60 MAE	0.315	0.041	7.741	< 0.001	***
	5 60 MAE	0.004	0.041	0.092	1.000	
	10 60 MAE	0.331	0.041	8.135	< 0.001	***
	15 60 MAE	0.401	0.041	9.843	< 0.001	***
	30 60 MAE	0.459	0.041	11.262	< 0.001	***
	1 70 MAE	0.411	0.041	10.099	< 0.001	***
	5 70 MAE	0.219	0.041	5.379	< 0.001	***
	10 70 MAE	0.499	0.041	12.246	< 0.001	***
	15 70 MAE	0.535	0.041	13.138	< 0.001	***
	30 70 MAE	0.497	0.041	12.195	< 0.001	***
5 50 MAE	10 50 MAE	0.098	0.041	2.414	0.830	
	15 50 MAE	0.114	0.041	2.794	0.553	
	30 50 MAE	0.210	0.041	5.153	< 0.001	***
	1 60 MAE	0.557	0.041	13.683	< 0.001	***
	5 60 MAE	0.246	0.041	6.034	< 0.001	***
	10 60 MAE	0.573	0.041	14.077	< 0.001	***
	15 60 MAE	0.643	0.041	15.785	< 0.001	***
	30 60 MAE	0.701	0.041	17.204	< 0.001	***
	1 70 MAE	0.653	0.041	16.041	< 0.001	***
	5 70 MAE	0.461	0.041	11.321	< 0.001	***
	10 70 MAE	0.741	0.041	18.188	< 0.001	***
	15 70 MAE	0.777	0.041	19.080	< 0.001	***
	30 70 MAE	0.739	0.041	18.137	< 0.001	***
10 50 MAE	15 50 MAE	0.015	0.041	0.380	1.000	
	30 50 MAE	0.112	0.041	2.739	0.597	
	1 60 MAE	0.459	0.041	11.269	< 0.001	***
	5 60 MAE	0.147	0.041	3.620	0.086	
	10 60 MAE	0.475	0.041	11.662	< 0.001	***
	15 60 MAE	0.545	0.041	13.371	< 0.001	***
	30 60 MAE	0.602	0.041	14.790	< 0.001	***
	1 70 MAE	0.555	0.041	13.627	< 0.001	***
	5 70 MAE	0.363	0.041	8.906	< 0.001	***
	10 70 MAE	0.643	0.041	15.774	< 0.001	***
	15 70 MAE	0.679	0.041	16.666	< 0.001	***
	30 70 MAE	0.640	0.041	15.723	< 0.001	***
15 50 MAE	30 50 MAE	0.096	0.041	2.359	0.861	
	1 60 MAE	0.444	0.041	10.889	< 0.001	***
	5 60 MAE	0.132	0.041	3.240	0.237	
	10 60 MAE	0.460	0.041	11.283	< 0.001	***
	15 60 MAE	0.529	0.041	12.991	< 0.001	***
	30 60 MAE	0.587	0.041	14.410	< 0.001	***

Post Hoc Comparisons - Time ✻ Temperature ✻ Heating method

		Mean Difference	SE	t	p_{tukey}	
	1 70 MAE	0.540	0.041	13.247	< 0.001	***
	5 70 MAE	0.347	0.041	8.527	< 0.001	***
	10 70 MAE	0.627	0.041	15.394	< 0.001	***
	15 70 MAE	0.663	0.041	16.286	< 0.001	***
	30 70 MAE	0.625	0.041	15.343	< 0.001	***
30 50 MAE	1 60 MAE	0.347	0.041	8.530	< 0.001	***
	5 60 MAE	0.036	0.041	0.881	1.000	
	10 60 MAE	0.363	0.041	8.924	< 0.001	***
	15 60 MAE	0.433	0.041	10.632	< 0.001	***
	30 60 MAE	0.491	0.041	12.051	< 0.001	***
	1 70 MAE	0.444	0.041	10.888	< 0.001	***
	5 70 MAE	0.251	0.041	6.168	< 0.001	***
	10 70 MAE	0.531	0.041	13.035	< 0.001	***
	15 70 MAE	0.567	0.041	13.927	< 0.001	***
	30 70 MAE	0.529	0.041	12.984	< 0.001	***
1 60 MAE	5 60 MAE	-0.312	0.041	-7.649	< 0.001	***
	10 60 MAE	0.016	0.041	0.394	1.000	
	15 60 MAE	0.086	0.041	2.102	0.957	
	30 60 MAE	0.143	0.041	3.521	0.115	
	1 70 MAE	0.096	0.041	2.358	0.861	
	5 70 MAE	-0.096	0.041	-2.362	0.859	
	10 70 MAE	0.183	0.041	4.505	0.004	**
	15 70 MAE	0.220	0.041	5.397	< 0.001	***
	30 70 MAE	0.181	0.041	4.454	0.005	**
5 60 MAE	10 60 MAE	0.328	0.041	8.043	< 0.001	***
	15 60 MAE	0.397	0.041	9.751	< 0.001	***
	30 60 MAE	0.455	0.041	11.170	< 0.001	***
	1 70 MAE	0.408	0.041	10.007	< 0.001	***
	5 70 MAE	0.215	0.041	5.287	< 0.001	***
	10 70 MAE	0.495	0.041	12.154	< 0.001	***
	15 70 MAE	0.531	0.041	13.046	< 0.001	***
	30 70 MAE	0.493	0.041	12.103	< 0.001	***
10 60 MAE	15 60 MAE	0.070	0.041	1.708	0.998	
	30 60 MAE	0.127	0.041	3.128	0.304	
	1 70 MAE	0.080	0.041	1.965	0.981	
	5 70 MAE	-0.112	0.041	-2.756	0.584	
	10 70 MAE	0.167	0.041	4.111	0.017	*
	15 70 MAE	0.204	0.041	5.004	< 0.001	***
	30 70 MAE	0.165	0.041	4.061	0.020	*
15 60 MAE	30 60 MAE	0.058	0.041	1.419	1.000	
	1 70 MAE	0.010	0.041	0.256	1.000	
	5 70 MAE	-0.182	0.041	-4.464	0.004	**
	10 70 MAE	0.098	0.041	2.403	0.837	
	15 70 MAE	0.134	0.041	3.295	0.207	
	30 70 MAE	0.096	0.041	2.352	0.864	
30 60 MAE	1 70 MAE	-0.047	0.041	-1.163	1.000	
	5 70 MAE	-0.240	0.041	-5.884	< 0.001	***
	10 70 MAE	0.040	0.041	0.984	1.000	
	15 70 MAE	0.076	0.041	1.876	0.990	
	30 70 MAE	0.038	0.041	0.933	1.000	
1 70 MAE	5 70 MAE	-0.192	0.041	-4.720	0.001	**

Post Hoc Comparisons - Time ✻ Temperature ✻ Heating method

		Mean Difference	SE	t	p_{tukey}	
	10 70 MAE	0.087	0.041	2.147	0.945	
	15 70 MAE	0.124	0.041	3.039	0.364	
	30 70 MAE	0.085	0.041	2.096	0.958	
5 70 MAE	10 70 MAE	0.280	0.041	6.867	< 0.001	***
	15 70 MAE	0.316	0.041	7.759	< 0.001	***
	30 70 MAE	0.278	0.041	6.817	< 0.001	***
10 70 MAE	15 70 MAE	0.036	0.041	0.892	1.000	
	30 70 MAE	-0.002	0.041	-0.051	1.000	
15 70 MAE	30 70 MAE	-0.038	0.041	-0.943	1.000	

Note. P-value adjusted for comparing a family of 30
* p < 0.05, ** p < 0.01, *** p < 0.001

F.5.4 Correlation between TPC, TMA, and AOA on extraction time, temperature and heating methods (MAE and CSE) effects

Pearson's Correlations

Variable		Total Phenolic Content	Total Monomeric Anthocyanin	Antioxidant Activity
1. Total Phenolic Content	Pearson's r	—		
	p-value	—		
2. Total Monomeric Anthocyanin	Pearson's r	0.369***	—	
	p-value	< 0.001	—	
3. Antioxidant Activity	Pearson's r	-0.130*	0.694***	—
	p-value	0.033	< 0.001	—

Appendix F.6 Test of Significance the effect of ethanol concentration (0 – 100% v/v) and heating method (MAE at 60 °C and CSE at 70 °C) on Total Phenolic Content (TPC), Total Monomeric Anthocyanin (TMA), and Antioxidant Activity (AOA)

F.6.1 Total Phenolic Content (TPC)

ANOVA - Total Phenolic Content

Cases	Sum of Squares	df	Mean Square	F	p	η^2
Ethanol Concentration	102089.058	8	12761.132	2454.745	< 0.001***	0.978
Extraction method	352.002	1	352.002	67.712	< 0.001***	0.003
Ethanol Concentration ✻ Extraction method	1201.407	8	150.176	28.888	< 0.001***	0.012
Residuals	748.592	144	5.199			

Note. Type III Sum of Squares

Post Hoc Tests

Post Hoc Comparisons - Ethanol Concentration

		Mean Difference	SE	t	p_{tukey}	
0	10	6.088	0.760	8.011	< 0.001	***
	30	-32.743	0.760	-43.083	< 0.001	***
	40	-48.452	0.760	-63.752	< 0.001	***
	50	-52.965	0.760	-69.689	< 0.001	***
	60	-42.065	0.760	-55.348	< 0.001	***
	70	-35.823	0.760	-47.135	< 0.001	***
	90	-32.115	0.760	-42.256	< 0.001	***
	100	22.315	0.760	29.361	< 0.001	***
10	30	-38.831	0.760	-51.093	< 0.001	***
	40	-54.540	0.760	-71.762	< 0.001	***
	50	-59.053	0.760	-77.700	< 0.001	***
	60	-48.153	0.760	-63.358	< 0.001	***
	70	-41.911	0.760	-55.145	< 0.001	***
	90	-38.203	0.760	-50.266	< 0.001	***
	100	16.227	0.760	21.351	< 0.001	***
30	40	-15.709	0.760	-20.669	< 0.001	***
	50	-20.221	0.760	-26.607	< 0.001	***
	60	-9.322	0.760	-12.265	< 0.001	***
	70	-3.080	0.760	-4.052	0.003	**
	90	0.629	0.760	0.827	0.996	
	100	55.058	0.760	72.444	< 0.001	***
40	50	-4.512	0.760	-5.937	< 0.001	***
	60	6.387	0.760	8.404	< 0.001	***
	70	12.629	0.760	16.617	< 0.001	***
	90	16.338	0.760	21.496	< 0.001	***
	100	70.767	0.760	93.113	< 0.001	***
50	60	10.900	0.760	14.341	< 0.001	***
	70	17.142	0.760	22.554	< 0.001	***
	90	20.850	0.760	27.434	< 0.001	***

Post Hoc Comparisons - Ethanol Concentration

		Mean Difference	SE	t	p_{tukey}	
	100	75.279	0.760	99.050	< 0.001	***
60	70	6.242	0.760	8.213	< 0.001	***
	90	9.950	0.760	13.092	< 0.001	***
	100	64.380	0.760	84.709	< 0.001	***
70	90	3.708	0.760	4.879	< 0.001	***
	100	58.138	0.760	76.496	< 0.001	***
90	100	54.429	0.760	71.617	< 0.001	***

* p < 0.05, ** p < .01, *** p < 0.001

Note. P-value adjusted for comparing a family of 9

Note. Results are averaged over the levels of: Extraction method

Post Hoc Comparisons - Extraction method

		Mean Difference	SE	t	p_{tukey}	
CSE	MAE	2.948	0.358	8.229	< 0.001	***

*** p < 0.001

Note. Results are averaged over the levels of: Ethanol Concentration

Post Hoc Comparisons - Ethanol Concentration ✻ Extraction method

		Mean Difference	SE	t	p_{tukey}	
0 CSE	10 CSE	4.507	1.075	4.194	0.006	**
	30 CSE	-36.039	1.075	-33.530	< 0.001	***
	40 CSE	-50.295	1.075	-46.794	< 0.001	***
	50 CSE	-53.333	1.075	-49.621	< 0.001	***
	60 CSE	-45.266	1.075	-42.115	< 0.001	***
	70 CSE	-38.692	1.075	-35.999	< 0.001	***
	90 CSE	-33.504	1.075	-31.172	< 0.001	***
	100 CSE	12.566	1.075	11.691	< 0.001	***
	0 MAE	-2.451	1.075	-2.280	0.696	
	10 MAE	5.218	1.075	4.855	< 0.001	***
	30 MAE	-31.899	1.075	-29.678	< 0.001	***
	40 MAE	-49.060	1.075	-45.645	< 0.001	***
	50 MAE	-55.047	1.075	-51.215	< 0.001	***
	60 MAE	-41.316	1.075	-38.440	< 0.001	***
	70 MAE	-35.404	1.075	-32.940	< 0.001	***
	90 MAE	-33.177	1.075	-30.867	< 0.001	***
	100 MAE	29.612	1.075	27.551	< 0.001	***
10 CSE	30 CSE	-40.546	1.075	-37.724	< 0.001	***
	40 CSE	-54.803	1.075	-50.988	< 0.001	***
	50 CSE	-57.841	1.075	-53.814	< 0.001	***
	60 CSE	-49.773	1.075	-46.308	< 0.001	***
	70 CSE	-43.200	1.075	-40.193	< 0.001	***
	90 CSE	-38.011	1.075	-35.365	< 0.001	***
	100 CSE	8.059	1.075	7.498	< 0.001	***
	0 MAE	-6.958	1.075	-6.474	< 0.001	***
	10 MAE	0.711	1.075	0.661	1.000	
	30 MAE	-36.406	1.075	-33.872	< 0.001	***

Post Hoc Comparisons - Ethanol Concentration ✻ Extraction method

		Mean Difference	SE	t	p_{tukey}	
	40 MAE	-53.567	1.075	-49.838	< 0.001	***
	50 MAE	-59.554	1.075	-55.408	< 0.001	***
	60 MAE	-45.823	1.075	-42.633	< 0.001	***
	70 MAE	-39.912	1.075	-37.133	< 0.001	***
	90 MAE	-37.684	1.075	-35.061	< 0.001	***
	100 MAE	25.105	1.075	23.357	< 0.001	***
30 CSE	40 CSE	-14.257	1.075	-13.264	< 0.001	***
	50 CSE	-17.295	1.075	-16.091	< 0.001	***
	60 CSE	-9.227	1.075	-8.585	< 0.001	***
	70 CSE	-2.654	1.075	-2.469	0.558	
	90 CSE	2.535	1.075	2.358	0.640	
	100 CSE	48.605	1.075	45.222	< 0.001	***
	0 MAE	33.588	1.075	31.250	< 0.001	***
	10 MAE	41.257	1.075	38.385	< 0.001	***
	30 MAE	4.140	1.075	3.852	0.019	*
	40 MAE	-13.021	1.075	-12.115	< 0.001	***
	50 MAE	-19.008	1.075	-17.685	< 0.001	***
	60 MAE	-5.277	1.075	-4.909	< 0.001	***
	70 MAE	0.634	1.075	0.590	1.000	
	90 MAE	2.862	1.075	2.663	0.416	
	100 MAE	65.651	1.075	61.081	< 0.001	***
40 CSE	50 CSE	-3.038	1.075	-2.826	0.309	
	60 CSE	5.030	1.075	4.680	< 0.001	***
	70 CSE	11.603	1.075	10.795	< 0.001	***
	90 CSE	16.792	1.075	15.623	< 0.001	***
	100 CSE	62.862	1.075	58.486	< 0.001	***
	0 MAE	47.844	1.075	44.514	< 0.001	***
	10 MAE	55.513	1.075	51.649	< 0.001	***
	30 MAE	18.397	1.075	17.116	< 0.001	***
	40 MAE	1.236	1.075	1.150	1.000	
	50 MAE	-4.751	1.075	-4.420	0.002	**
	60 MAE	8.980	1.075	8.355	< 0.001	***
	70 MAE	14.891	1.075	13.855	< 0.001	***
	90 MAE	17.119	1.075	15.927	< 0.001	***
	100 MAE	79.908	1.075	74.345	< 0.001	***
50 CSE	60 CSE	8.068	1.075	7.506	< 0.001	***
	70 CSE	14.641	1.075	13.622	< 0.001	***
	90 CSE	19.830	1.075	18.449	< 0.001	***
	100 CSE	65.900	1.075	61.312	< 0.001	***
	0 MAE	50.882	1.075	47.340	< 0.001	***
	10 MAE	58.551	1.075	54.476	< 0.001	***
	30 MAE	21.435	1.075	19.943	< 0.001	***
	40 MAE	4.274	1.075	3.976	0.013	*
	50 MAE	-1.713	1.075	-1.594	0.980	
	60 MAE	12.018	1.075	11.181	< 0.001	***
	70 MAE	17.929	1.075	16.681	< 0.001	***
	90 MAE	20.157	1.075	18.754	< 0.001	***

Post Hoc Comparisons - Ethanol Concentration ✻ Extraction method

		Mean Difference	SE	t	p_{tukey}	
	100 MAE	82.946	1.075	77.172	< 0.001	***
60 CSE	70 CSE	6.573	1.075	6.116	< 0.001	***
	90 CSE	11.762	1.075	10.943	< 0.001	***
	100 CSE	57.832	1.075	53.806	< 0.001	***
	0 MAE	42.814	1.075	39.834	< 0.001	***
	10 MAE	50.483	1.075	46.969	< 0.001	***
	30 MAE	13.367	1.075	12.436	< 0.001	***
	40 MAE	-3.794	1.075	-3.530	0.054	
	50 MAE	-9.781	1.075	-9.100	< 0.001	***
	60 MAE	3.950	1.075	3.675	0.034	*
	70 MAE	9.861	1.075	9.175	< 0.001	***
	90 MAE	12.089	1.075	11.247	< 0.001	***
	100 MAE	74.878	1.075	69.666	< 0.001	***
70 CSE	90 CSE	5.189	1.075	4.827	< 0.001	***
	100 CSE	51.259	1.075	47.691	< 0.001	***
	0 MAE	36.241	1.075	33.719	< 0.001	***
	10 MAE	43.910	1.075	40.854	< 0.001	***
	30 MAE	6.794	1.075	6.321	< 0.001	***
	40 MAE	-10.367	1.075	-9.646	< 0.001	***
	50 MAE	-16.354	1.075	-15.216	< 0.001	***
	60 MAE	-2.623	1.075	-2.440	0.579	
	70 MAE	3.288	1.075	3.059	0.187	
	90 MAE	5.516	1.075	5.132	< 0.001	***
	100 MAE	68.305	1.075	63.550	< 0.001	***
90 CSE	100 CSE	46.070	1.075	42.863	< 0.001	***
	0 MAE	31.053	1.075	28.891	< 0.001	***
	10 MAE	38.722	1.075	36.026	< 0.001	***
	30 MAE	1.605	1.075	1.493	0.990	
	40 MAE	-15.556	1.075	-14.473	< 0.001	***
	50 MAE	-21.543	1.075	-20.043	< 0.001	***
	60 MAE	-7.812	1.075	-7.268	< 0.001	***
	70 MAE	-1.901	1.075	-1.768	0.948	
	90 MAE	0.327	1.075	0.304	1.000	
	100 MAE	63.116	1.075	58.723	< 0.001	***
100 CSE	0 MAE	-15.017	1.075	-13.972	< 0.001	***
	10 MAE	-7.348	1.075	-6.837	< 0.001	***
	30 MAE	-44.465	1.075	-41.370	< 0.001	***
	40 MAE	-61.626	1.075	-57.336	< 0.001	***
	50 MAE	-67.613	1.075	-62.906	< 0.001	***
	60 MAE	-53.882	1.075	-50.131	< 0.001	***
	70 MAE	-47.971	1.075	-44.631	< 0.001	***
	90 MAE	-45.743	1.075	-42.559	< 0.001	***
	100 MAE	17.046	1.075	15.859	< 0.001	***
0 MAE	10 MAE	7.669	1.075	7.135	< 0.001	***
	30 MAE	-29.448	1.075	-27.398	< 0.001	***
	40 MAE	-46.609	1.075	-43.364	< 0.001	***
	50 MAE	-52.596	1.075	-48.934	< 0.001	***

Post Hoc Comparisons - Ethanol Concentration ✻ Extraction method

		Mean Difference	SE	t	p_{tukey}
	60 MAE	-38.864	1.075	-36.159	< 0.001 ***
	70 MAE	-32.953	1.075	-30.659	< 0.001 ***
	90 MAE	-30.726	1.075	-28.587	< 0.001 ***
	100 MAE	32.063	1.075	29.831	< 0.001 ***
10 MAE	30 MAE	-37.117	1.075	-34.533	< 0.001 ***
	40 MAE	-54.278	1.075	-50.499	< 0.001 ***
	50 MAE	-60.264	1.075	-56.069	< 0.001 ***
	60 MAE	-46.533	1.075	-43.294	< 0.001 ***
	70 MAE	-40.622	1.075	-37.794	< 0.001 ***
	90 MAE	-38.394	1.075	-35.722	< 0.001 ***
	100 MAE	24.394	1.075	22.696	< 0.001 ***
30 MAE	40 MAE	-17.161	1.075	-15.967	< 0.001 ***
	50 MAE	-23.148	1.075	-21.536	< 0.001 ***
	60 MAE	-9.417	1.075	-8.761	< 0.001 ***
	70 MAE	-3.506	1.075	-3.262	0.114
	90 MAE	-1.278	1.075	-1.189	0.999
	100 MAE	61.511	1.075	57.229	< 0.001 ***
40 MAE	50 MAE	-5.987	1.075	-5.570	< 0.001 ***
	60 MAE	7.744	1.075	7.205	< 0.001 ***
	70 MAE	13.656	1.075	12.705	< 0.001 ***
	90 MAE	15.883	1.075	14.778	< 0.001 ***
	100 MAE	78.672	1.075	73.196	< 0.001 ***
50 MAE	60 MAE	13.731	1.075	12.775	< 0.001 ***
	70 MAE	19.642	1.075	18.275	< 0.001 ***
	90 MAE	21.870	1.075	20.348	< 0.001 ***
	100 MAE	84.659	1.075	78.766	< 0.001 ***
60 MAE	70 MAE	5.911	1.075	5.500	< 0.001 ***
	90 MAE	8.139	1.075	7.572	< 0.001 ***
	100 MAE	70.928	1.075	65.990	< 0.001 ***
70 MAE	90 MAE	2.228	1.075	2.073	0.827
	100 MAE	65.017	1.075	60.491	< 0.001 ***
90 MAE	100 MAE	62.789	1.075	58.418	< 0.001 ***

Note. P-value adjusted for comparing a family of 18

* p < 0.05, ** p < .01, *** p < 0.001

F.6.2 Total Monomeric Anthocyanin (TMA)

ANOVA - Total Monomeric Anthocyanin

Cases	Sum of Squares	df	Mean Square	F	p	η^2
Ethanol Concentration	1.881	8	0.235	1053.867	< 0.001***	0.968
Extraction method	5.454×10^{-5}	1	5.454×10^{-5}	0.245	0.622	2.808×10^{-5}
Ethanol Concentration ✻ Extraction method	0.030	8	0.004	16.538	< 0.001***	0.015
Residuals	0.032	144	2.231×10^{-4}			

Note. Type III Sum of Squares

Post Hoc Tests

Post Hoc Comparisons - Ethanol Concentration

		Mean Difference	SE	t	p_{tukey}
0	10	0.083	0.005	16.616	< 0.001***
	30	-0.044	0.005	-8.838	< 0.001***
	40	-0.182	0.005	-36.469	< 0.001***
	50	-0.204	0.005	-41.022	< 0.001***
	60	0.015	0.005	3.035	0.068
	70	0.060	0.005	11.974	< 0.001***
	90	0.082	0.005	16.516	< 0.001***
	100	0.106	0.005	21.325	< 0.001***
10	30	-0.127	0.005	-25.454	< 0.001***
	40	-0.264	0.005	-53.085	< 0.001***
	50	-0.287	0.005	-57.638	< 0.001***
	60	-0.068	0.005	-13.581	< 0.001***
	70	-0.023	0.005	-4.642	< 0.001***
	90	-5.000×10^{-4}	0.005	-0.100	1.000
	100	0.023	0.005	4.709	< 0.001***
30	40	-0.138	0.005	-27.630	< 0.001***
	50	-0.160	0.005	-32.183	< 0.001***
	60	0.059	0.005	11.873	< 0.001***
	70	0.104	0.005	20.812	< 0.001***
	90	0.126	0.005	25.354	< 0.001***
	100	0.150	0.005	30.164	< 0.001***
40	50	-0.023	0.005	-4.553	< 0.001***
	60	0.197	0.005	39.504	< 0.001***
	70	0.241	0.005	48.442	< 0.001***
	90	0.264	0.005	52.984	< 0.001***
	100	0.288	0.005	57.794	< 0.001***
50	60	0.219	0.005	44.057	< 0.001***
	70	0.264	0.005	52.995	< 0.001***
	90	0.286	0.005	57.537	< 0.001***
	100	0.310	0.005	62.347	< 0.001***
60	70	0.045	0.005	8.939	< 0.001***
	90	0.067	0.005	13.480	< 0.001***
	100	0.091	0.005	18.290	< 0.001***
70	90	0.023	0.005	4.542	< 0.001***

Post Hoc Comparisons - Ethanol Concentration

		Mean Difference	SE	t	p_{tukey}	
	100	0.047	0.005	9.351	< 0.001	***
90	100	0.024	0.005	4.810	< 0.001	***

* p < 0.05, ** p < 0.01, *** p < 0.001

Note. P-value adjusted for comparing a family of 9

Note. Results are averaged over the levels of: Extraction method

Post Hoc Comparisons - Extraction method

		Mean Difference	SE	t	p_{tukey}
CSE	MAE	-0.001	0.002	-0.494	0.622

Note. Results are averaged over the levels of: Ethanol Concentration

Post Hoc Comparisons - Ethanol Concentration ✻ Extraction method

		Mean Difference	SE	t	p_{tukey}	
0 CSE	10 CSE	0.078	0.007	11.079	< 0.001	***
	30 CSE	-0.045	0.007	-6.392	< 0.001	***
	40 CSE	-0.169	0.007	-23.988	< 0.001	***
	50 CSE	-0.195	0.007	-27.713	< 0.001	***
	60 CSE	-0.019	0.007	-2.714	0.380	
	70 CSE	0.044	0.007	6.250	< 0.001	***
	90 CSE	0.089	0.007	12.657	< 0.001	***
	100 CSE	0.104	0.007	14.740	< 0.001	***
	0 MAE	-0.008	0.007	-1.089	1.000	
	10 MAE	0.080	0.007	11.331	< 0.001	***
	30 MAE	-0.051	0.007	-7.196	< 0.001	***
	40 MAE	-0.202	0.007	-28.675	< 0.001	***
	50 MAE	-0.221	0.007	-31.390	< 0.001	***
	60 MAE	0.042	0.007	5.918	< 0.001	***
	70 MAE	0.068	0.007	9.595	< 0.001	***
	90 MAE	0.068	0.007	9.611	< 0.001	***
	100 MAE	0.101	0.007	14.330	< 0.001	***
10 CSE	30 CSE	-0.123	0.007	-17.470	< 0.001	***
	40 CSE	-0.247	0.007	-35.067	< 0.001	***
	50 CSE	-0.273	0.007	-38.791	< 0.001	***
	60 CSE	-0.097	0.007	-13.793	< 0.001	***
	70 CSE	-0.034	0.007	-4.829	< 0.001	***
	90 CSE	0.011	0.007	1.578	0.982	
	100 CSE	0.026	0.007	3.661	0.036	*
	0 MAE	-0.086	0.007	-12.168	< 0.001	***
	10 MAE	0.002	0.007	0.253	1.000	
	30 MAE	-0.129	0.007	-18.275	< 0.001	***
	40 MAE	-0.280	0.007	-39.754	< 0.001	***
	50 MAE	-0.299	0.007	-42.468	< 0.001	***
	60 MAE	-0.036	0.007	-5.161	< 0.001	***
	70 MAE	-0.010	0.007	-1.483	0.991	
	90 MAE	-0.010	0.007	-1.468	0.992	
	100 MAE	0.023	0.007	3.251	0.117	

Post Hoc Comparisons - Ethanol Concentration ✻ Extraction method

		Mean Difference	SE	t	p_{tukey}	
30 CSE	40 CSE	-0.124	0.007	-17.596	< 0.001	***
	50 CSE	-0.150	0.007	-21.321	< 0.001	***
	60 CSE	0.026	0.007	3.677	0.034	*
	70 CSE	0.089	0.007	12.641	< 0.001	***
	90 CSE	0.134	0.007	19.048	< 0.001	***
	100 CSE	0.149	0.007	21.132	< 0.001	***
	0 MAE	0.037	0.007	5.303	< 0.001	***
	10 MAE	0.125	0.007	17.723	< 0.001	***
	30 MAE	-0.006	0.007	-0.805	1.000	
	40 MAE	-0.157	0.007	-22.284	< 0.001	***
	50 MAE	-0.176	0.007	-24.998	< 0.001	***
	60 MAE	0.087	0.007	12.310	< 0.001	***
	70 MAE	0.113	0.007	15.987	< 0.001	***
	90 MAE	0.113	0.007	16.003	< 0.001	***
	100 MAE	0.146	0.007	20.721	< 0.001	***
40 CSE	50 CSE	-0.026	0.007	-3.724	0.029	*
	60 CSE	0.150	0.007	21.274	< 0.001	***
	70 CSE	0.213	0.007	30.238	< 0.001	***
	90 CSE	0.258	0.007	36.645	< 0.001	***
	100 CSE	0.273	0.007	38.728	< 0.001	***
	0 MAE	0.161	0.007	22.899	< 0.001	***
	10 MAE	0.249	0.007	35.319	< 0.001	***
	30 MAE	0.118	0.007	16.792	< 0.001	***
	40 MAE	-0.033	0.007	-4.687	< 0.001	***
	50 MAE	-0.052	0.007	-7.402	< 0.001	***
	60 MAE	0.211	0.007	29.906	< 0.001	***
	70 MAE	0.236	0.007	33.583	< 0.001	***
	90 MAE	0.237	0.007	33.599	< 0.001	***
	100 MAE	0.270	0.007	38.318	< 0.001	***
50 CSE	60 CSE	0.176	0.007	24.998	< 0.001	***
	70 CSE	0.239	0.007	33.962	< 0.001	***
	90 CSE	0.284	0.007	40.369	< 0.001	***
	100 CSE	0.299	0.007	42.453	< 0.001	***
	0 MAE	0.187	0.007	26.624	< 0.001	***
	10 MAE	0.275	0.007	39.044	< 0.001	***
	30 MAE	0.144	0.007	20.516	< 0.001	***
	40 MAE	-0.007	0.007	-0.963	1.000	
	50 MAE	-0.026	0.007	-3.677	0.034	*
	60 MAE	0.237	0.007	33.631	< 0.001	***
	70 MAE	0.263	0.007	37.308	< 0.001	***
	90 MAE	0.263	0.007	37.323	< 0.001	***
	100 MAE	0.296	0.007	42.042	< 0.001	***
60 CSE	70 CSE	0.063	0.007	8.964	< 0.001	***
	90 CSE	0.108	0.007	15.371	< 0.001	***
	100 CSE	0.123	0.007	17.454	< 0.001	***
	0 MAE	0.011	0.007	1.626	0.976	
	10 MAE	0.099	0.007	14.046	< 0.001	***

Post Hoc Comparisons - Ethanol Concentration ✻ Extraction method

		Mean Difference	SE	t	p_{tukey}	
	30 MAE	-0.032	0.007	-4.482	0.002	**
	40 MAE	-0.183	0.007	-25.961	< 0.001	***
	50 MAE	-0.202	0.007	-28.675	< 0.001	***
	60 MAE	0.061	0.007	8.633	< 0.001	***
	70 MAE	0.087	0.007	12.310	< 0.001	***
	90 MAE	0.087	0.007	12.325	< 0.001	***
	100 MAE	0.120	0.007	17.044	< 0.001	***
70 CSE	90 CSE	0.045	0.007	6.407	< 0.001	***
	100 CSE	0.060	0.007	8.491	< 0.001	***
	0 MAE	-0.052	0.007	-7.338	< 0.001	***
	10 MAE	0.036	0.007	5.082	< 0.001	***
	30 MAE	-0.095	0.007	-13.446	< 0.001	***
	40 MAE	-0.246	0.007	-34.925	< 0.001	***
	50 MAE	-0.265	0.007	-37.639	< 0.001	***
	60 MAE	-0.002	0.007	-0.331	1.000	
	70 MAE	0.024	0.007	3.346	0.091	
	90 MAE	0.024	0.007	3.361	0.087	
	100 MAE	0.057	0.007	8.080	< 0.001	***
90 CSE	100 CSE	0.015	0.007	2.083	0.821	
	0 MAE	-0.097	0.007	-13.746	< 0.001	***
	10 MAE	-0.009	0.007	-1.326	0.997	
	30 MAE	-0.140	0.007	-19.853	< 0.001	***
	40 MAE	-0.291	0.007	-41.332	< 0.001	***
	50 MAE	-0.310	0.007	-44.046	< 0.001	***
	60 MAE	-0.047	0.007	-6.739	< 0.001	***
	70 MAE	-0.022	0.007	-3.062	0.186	
	90 MAE	-0.021	0.007	-3.046	0.193	
	100 MAE	0.012	0.007	1.673	0.969	
100 CSE	0 MAE	-0.111	0.007	-15.829	< 0.001	***
	10 MAE	-0.024	0.007	-3.409	0.076	
	30 MAE	-0.154	0.007	-21.936	< 0.001	***
	40 MAE	-0.306	0.007	-43.415	< 0.001	***
	50 MAE	-0.325	0.007	-46.130	< 0.001	***
	60 MAE	-0.062	0.007	-8.822	< 0.001	***
	70 MAE	-0.036	0.007	-5.145	< 0.001	***
	90 MAE	-0.036	0.007	-5.129	< 0.001	***
	100 MAE	-0.003	0.007	-0.410	1.000	
0 MAE	10 MAE	0.087	0.007	12.420	< 0.001	***
	30 MAE	-0.043	0.007	-6.107	< 0.001	***
	40 MAE	-0.194	0.007	-27.586	< 0.001	***
	50 MAE	-0.213	0.007	-30.301	< 0.001	***
	60 MAE	0.049	0.007	7.007	< 0.001	***
	70 MAE	0.075	0.007	10.684	< 0.001	***
	90 MAE	0.075	0.007	10.700	< 0.001	***
	100 MAE	0.109	0.007	15.419	< 0.001	***
10 MAE	30 MAE	-0.130	0.007	-18.528	< 0.001	***
	40 MAE	-0.282	0.007	-40.006	< 0.001	***

Post Hoc Comparisons - Ethanol Concentration ✻ Extraction method

		Mean Difference	SE	t	p_{tukey}
	50 MAE	-0.301	0.007	-42.721	< 0.001***
	60 MAE	-0.038	0.007	-5.413	< 0.001***
	70 MAE	-0.012	0.007	-1.736	0.956
	90 MAE	-0.012	0.007	-1.720	0.960
	100 MAE	0.021	0.007	2.999	0.215
30 MAE	40 MAE	-0.151	0.007	-21.479	< 0.001***
	50 MAE	-0.170	0.007	-24.193	< 0.001***
	60 MAE	0.092	0.007	13.115	< 0.001***
	70 MAE	0.118	0.007	16.792	< 0.001***
	90 MAE	0.118	0.007	16.807	< 0.001***
	100 MAE	0.152	0.007	21.526	< 0.001***
40 MAE	50 MAE	-0.019	0.007	-2.714	0.380
	60 MAE	0.244	0.007	34.593	< 0.001***
	70 MAE	0.269	0.007	38.270	< 0.001***
	90 MAE	0.270	0.007	38.286	< 0.001***
	100 MAE	0.303	0.007	43.005	< 0.001***
50 MAE	60 MAE	0.263	0.007	37.308	< 0.001***
	70 MAE	0.289	0.007	40.985	< 0.001***
	90 MAE	0.289	0.007	41.001	< 0.001***
	100 MAE	0.322	0.007	45.719	< 0.001***
60 MAE	70 MAE	0.026	0.007	3.677	0.034*
	90 MAE	0.026	0.007	3.693	0.033*
	100 MAE	0.059	0.007	8.412	< 0.001***
70 MAE	90 MAE	1.111×10^{-4}	0.007	0.016	1.000
	100 MAE	0.033	0.007	4.734	< 0.001***
90 MAE	100 MAE	0.033	0.007	4.719	< 0.001***

Note. P-value adjusted for comparing a family of 18

* p < 0.05, ** p < 0.01, *** p < 0.001

F.6.3 Antioxidant Activity (AOA)

ANOVA - Antioxidant Activity

Cases	Sum of Squares	df	Mean Square	F	p	η^2
Ethanol Concentration	7161.093	8	895.137	339.488	< 0.001***	0.036
Extraction method	182880.768	1	182880.768	69359.076	< 0.001***	0.931
Ethanol Concentration ✻ Extraction method	6000.668	8	750.083	284.475	< 0.001***	0.031
Residuals	379.688	144	2.637			

Note. Type III Sum of Squares

Post Hoc Tests

Post Hoc Comparisons - Ethanol Concentration

		Mean Difference	SE	t	p_{tukey}	
0	10	9.521	0.541	17.590	< 0.001	***
	30	9.171	0.541	16.943	< 0.001	***
	40	9.038	0.541	16.698	< 0.001	***
	50	4.453	0.541	8.227	< 0.001	***
	60	3.572	0.541	6.599	< 0.001	***
	70	0.473	0.541	0.874	0.994	
	90	-5.456	0.541	-10.079	< 0.001	***
	100	-11.048	0.541	-20.411	< 0.001	***
10	30	-0.351	0.541	-0.648	0.999	
	40	-0.483	0.541	-0.892	0.993	
	50	-5.068	0.541	-9.364	< 0.001	***
	60	-5.949	0.541	-10.992	< 0.001	***
	70	-9.048	0.541	-16.716	< 0.001	***
	90	-14.977	0.541	-27.670	< 0.001	***
	100	-20.569	0.541	-38.001	< 0.001	***
30	40	-0.132	0.541	-0.244	1.000	
	50	-4.718	0.541	-8.716	< 0.001	***
	60	-5.599	0.541	-10.344	< 0.001	***
	70	-8.697	0.541	-16.068	< 0.001	***
	90	-14.626	0.541	-27.022	< 0.001	***
	100	-20.218	0.541	-37.354	< 0.001	***
40	50	-4.586	0.541	-8.472	< 0.001	***
	60	-5.467	0.541	-10.100	< 0.001	***
	70	-8.565	0.541	-15.824	< 0.001	***
	90	-14.494	0.541	-26.778	< 0.001	***
	100	-20.086	0.541	-37.109	< 0.001	***
50	60	-0.881	0.541	-1.628	0.788	
	70	-3.979	0.541	-7.352	< 0.001	***
	90	-9.908	0.541	-18.306	< 0.001	***
	100	-15.501	0.541	-28.638	< 0.001	***
60	70	-3.098	0.541	-5.724	< 0.001	***
	90	-9.027	0.541	-16.678	< 0.001	***
	100	-14.619	0.541	-27.010	< 0.001	***

Post Hoc Comparisons - Ethanol Concentration

		Mean Difference	SE	t	p_{tukey}
70	90	-5.929	0.541	-10.954	< 0.001 ***
	100	-11.521	0.541	-21.285	< 0.001 ***
90	100	-5.592	0.541	-10.332	< 0.001 ***

*** p < 0.001

Note. P-value adjusted for comparing a family of 9

Note. Results are averaged over the levels of: Extraction method

Post Hoc Comparisons - Extraction method

		Mean Difference	SE	t	p_{tukey}
CSE	MAE	-67.198	0.255	-263.361	< 0.001 ***

*** p < 0.001

Note. Results are averaged over the levels of: Ethanol Concentration

Post Hoc Comparisons - Ethanol Concentration ✻ Extraction method

		Mean Difference	SE	t	p_{tukey}
0 CSE	10 CSE	0.410	0.765	0.536	1.000
	30 CSE	0.324	0.765	0.424	1.000
	40 CSE	-0.250	0.765	-0.327	1.000
	50 CSE	-0.449	0.765	-0.586	1.000
	60 CSE	-0.608	0.765	-0.794	1.000
	70 CSE	-1.070	0.765	-1.398	0.995
	90 CSE	-1.362	0.765	-1.780	0.946
	100 CSE	-1.530	0.765	-1.999	0.865
	0 MAE	-72.589	0.765	-94.830	< 0.001 ***
	10 MAE	-53.957	0.765	-70.489	< 0.001 ***
	30 MAE	-54.572	0.765	-71.293	< 0.001 ***
	40 MAE	-54.262	0.765	-70.888	< 0.001 ***
	50 MAE	-63.234	0.765	-82.609	< 0.001 ***
	60 MAE	-64.838	0.765	-84.704	< 0.001 ***
	70 MAE	-70.572	0.765	-92.195	< 0.001 ***
	90 MAE	-82.138	0.765	-107.304	< 0.001 ***
	100 MAE	-93.154	0.765	-121.696	< 0.001 ***
10 CSE	30 CSE	-0.086	0.765	-0.112	1.000
	40 CSE	-0.660	0.765	-0.862	1.000
	50 CSE	-0.859	0.765	-1.122	1.000
	60 CSE	-1.018	0.765	-1.330	0.997
	70 CSE	-1.480	0.765	-1.933	0.894
	90 CSE	-1.772	0.765	-2.315	0.671
	100 CSE	-1.940	0.765	-2.534	0.509
	0 MAE	-72.999	0.765	-95.365	< 0.001 ***
	10 MAE	-54.367	0.765	-71.024	< 0.001 ***
	30 MAE	-54.982	0.765	-71.828	< 0.001 ***
	40 MAE	-54.672	0.765	-71.423	< 0.001 ***
	50 MAE	-63.644	0.765	-83.145	< 0.001 ***

Post Hoc Comparisons - Ethanol Concentration ✻ Extraction method

		Mean Difference	SE	t	p_{tukey}
	60 MAE	-65.248	0.765	-85.239	< 0.001 ***
	70 MAE	-70.982	0.765	-92.731	< 0.001 ***
	90 MAE	-82.548	0.765	-107.840	< 0.001 ***
	100 MAE	-93.564	0.765	-122.232	< 0.001 ***
30 CSE	40 CSE	-0.574	0.765	-0.750	1.000
	50 CSE	-0.773	0.765	-1.010	1.000
	60 CSE	-0.932	0.765	-1.218	0.999
	70 CSE	-1.394	0.765	-1.822	0.934
	90 CSE	-1.687	0.765	-2.203	0.748
	100 CSE	-1.854	0.765	-2.423	0.592
	0 MAE	-72.913	0.765	-95.253	< 0.001 ***
	10 MAE	-54.281	0.765	-70.912	< 0.001 ***
	30 MAE	-54.897	0.765	-71.717	< 0.001 ***
	40 MAE	-54.587	0.765	-71.312	< 0.001 ***
	50 MAE	-63.559	0.765	-83.033	< 0.001 ***
	60 MAE	-65.162	0.765	-85.127	< 0.001 ***
	70 MAE	-70.897	0.765	-92.619	< 0.001 ***
	90 MAE	-82.462	0.765	-107.728	< 0.001 ***
	100 MAE	-93.479	0.765	-122.120	< 0.001 ***
40 CSE	50 CSE	-0.199	0.765	-0.260	1.000
	60 CSE	-0.358	0.765	-0.467	1.000
	70 CSE	-0.820	0.765	-1.071	1.000
	90 CSE	-1.112	0.765	-1.453	0.992
	100 CSE	-1.280	0.765	-1.672	0.969
	0 MAE	-72.339	0.765	-94.503	< 0.001 ***
	10 MAE	-53.707	0.765	-70.162	< 0.001 ***
	30 MAE	-54.322	0.765	-70.966	< 0.001 ***
	40 MAE	-54.012	0.765	-70.561	< 0.001 ***
	50 MAE	-62.984	0.765	-82.282	< 0.001 ***
	60 MAE	-64.588	0.765	-84.377	< 0.001 ***
	70 MAE	-70.322	0.765	-91.868	< 0.001 ***
	90 MAE	-81.888	0.765	-106.978	< 0.001 ***
	100 MAE	-92.904	0.765	-121.370	< 0.001 ***
50 CSE	60 CSE	-0.159	0.765	-0.208	1.000
	70 CSE	-0.621	0.765	-0.811	1.000
	90 CSE	-0.913	0.765	-1.193	0.999
	100 CSE	-1.081	0.765	-1.412	0.994
	0 MAE	-72.140	0.765	-94.243	< 0.001 ***
	10 MAE	-53.508	0.765	-69.902	< 0.001 ***
	30 MAE	-54.123	0.765	-70.706	< 0.001 ***
	40 MAE	-53.813	0.765	-70.301	< 0.001 ***
	50 MAE	-62.786	0.765	-82.023	< 0.001 ***
	60 MAE	-64.389	0.765	-84.117	< 0.001 ***
	70 MAE	-70.123	0.765	-91.609	< 0.001 ***
	90 MAE	-81.689	0.765	-106.718	< 0.001 ***
	100 MAE	-92.706	0.765	-121.110	< 0.001 ***
60 CSE	70 CSE	-0.462	0.765	-0.604	1.000

Post Hoc Comparisons - Ethanol Concentration ✻ Extraction method

		Mean Difference	SE	t	p_{tukey}
	90 CSE	-0.754	0.765	-0.986	1.000
	100 CSE	-0.922	0.765	-1.205	0.999
	0 MAE	-71.981	0.765	-94.036	< 0.001 ***
	10 MAE	-53.349	0.765	-69.695	< 0.001 ***
	30 MAE	-53.964	0.765	-70.499	< 0.001 ***
	40 MAE	-53.654	0.765	-70.094	< 0.001 ***
	50 MAE	-62.627	0.765	-81.815	< 0.001 ***
	60 MAE	-64.230	0.765	-83.910	< 0.001 ***
	70 MAE	-69.964	0.765	-91.401	< 0.001 ***
	90 MAE	-81.530	0.765	-106.510	< 0.001 ***
	100 MAE	-92.547	0.765	-120.902	< 0.001 ***
70 CSE	90 CSE	-0.292	0.765	-0.382	1.000
	100 CSE	-0.460	0.765	-0.601	1.000
	0 MAE	-71.519	0.765	-93.432	< 0.001 ***
	10 MAE	-52.887	0.765	-69.091	< 0.001 ***
	30 MAE	-53.502	0.765	-69.895	< 0.001 ***
	40 MAE	-53.192	0.765	-69.490	< 0.001 ***
	50 MAE	-62.164	0.765	-81.211	< 0.001 ***
	60 MAE	-63.768	0.765	-83.306	< 0.001 ***
	70 MAE	-69.502	0.765	-90.797	< 0.001 ***
	90 MAE	-81.068	0.765	-105.906	< 0.001 ***
	100 MAE	-92.084	0.765	-120.298	< 0.001 ***
90 CSE	100 CSE	-0.168	0.765	-0.219	1.000
	0 MAE	-71.227	0.765	-93.050	< 0.001 ***
	10 MAE	-52.594	0.765	-68.709	< 0.001 ***
	30 MAE	-53.210	0.765	-69.513	< 0.001 ***
	40 MAE	-52.900	0.765	-69.108	< 0.001 ***
	50 MAE	-61.872	0.765	-80.829	< 0.001 ***
	60 MAE	-63.476	0.765	-82.924	< 0.001 ***
	70 MAE	-69.210	0.765	-90.415	< 0.001 ***
	90 MAE	-80.776	0.765	-105.525	< 0.001 ***
	100 MAE	-91.792	0.765	-119.917	< 0.001 ***
100 CSE	0 MAE	-71.059	0.765	-92.831	< 0.001 ***
	10 MAE	-52.427	0.765	-68.490	< 0.001 ***
	30 MAE	-53.042	0.765	-69.294	< 0.001 ***
	40 MAE	-52.732	0.765	-68.889	< 0.001 ***
	50 MAE	-61.704	0.765	-80.610	< 0.001 ***
	60 MAE	-63.308	0.765	-82.705	< 0.001 ***
	70 MAE	-69.042	0.765	-90.196	< 0.001 ***
	90 MAE	-80.608	0.765	-105.305	< 0.001 ***
	100 MAE	-91.624	0.765	-119.698	< 0.001 ***
0 MAE	10 MAE	18.632	0.765	24.341	< 0.001 ***
	30 MAE	18.017	0.765	23.537	< 0.001 ***
	40 MAE	18.327	0.765	23.942	< 0.001 ***
	50 MAE	9.354	0.765	12.221	< 0.001 ***
	60 MAE	7.751	0.765	10.126	< 0.001 ***
	70 MAE	2.017	0.765	2.635	0.436

Post Hoc Comparisons - Ethanol Concentration ✻ Extraction method

		Mean Difference	SE	t	p_{tukey}
	90 MAE	-9.549	0.765	-12.475	< 0.001 ***
	100 MAE	-20.566	0.765	-26.867	< 0.001 ***
10 MAE	30 MAE	-0.616	0.765	-0.804	1.000
	40 MAE	-0.306	0.765	-0.399	1.000
	50 MAE	-9.278	0.765	-12.120	< 0.001 ***
	60 MAE	-10.881	0.765	-14.215	< 0.001 ***
	70 MAE	-16.616	0.765	-21.706	< 0.001 ***
	90 MAE	-28.181	0.765	-36.816	< 0.001 ***
	100 MAE	-39.198	0.765	-51.208	< 0.001 ***
30 MAE	40 MAE	0.310	0.765	0.405	1.000
	50 MAE	-8.662	0.765	-11.316	< 0.001 ***
	60 MAE	-10.266	0.765	-13.411	< 0.001 ***
	70 MAE	-16.000	0.765	-20.902	< 0.001 ***
	90 MAE	-27.566	0.765	-36.011	< 0.001 ***
	100 MAE	-38.582	0.765	-50.404	< 0.001 ***
40 MAE	50 MAE	-8.972	0.765	-11.721	< 0.001 ***
	60 MAE	-10.576	0.765	-13.816	< 0.001 ***
	70 MAE	-16.310	0.765	-21.307	< 0.001 ***
	90 MAE	-27.876	0.765	-36.416	< 0.001 ***
	100 MAE	-38.892	0.765	-50.809	< 0.001 ***
50 MAE	60 MAE	-1.603	0.765	-2.095	0.815
	70 MAE	-7.338	0.765	-9.586	< 0.001 ***
	90 MAE	-18.903	0.765	-24.695	< 0.001 ***
	100 MAE	-29.920	0.765	-39.087	< 0.001 ***
60 MAE	70 MAE	-5.734	0.765	-7.491	< 0.001 ***
	90 MAE	-17.300	0.765	-22.601	< 0.001 ***
	100 MAE	-28.317	0.765	-36.993	< 0.001 ***
70 MAE	90 MAE	-11.566	0.765	-15.109	< 0.001 ***
	100 MAE	-22.582	0.765	-29.501	< 0.001 ***
90 MAE	100 MAE	-11.017	0.765	-14.392	< 0.001 ***

*** $p < 0.001$

Note. P-value adjusted for comparing a family of 18

F.6.4 Correlation between TPC, TMA, and AOA on ethanol concentration and heating method (MAE and CSE at 50 °C) effects

Pearson's Correlations

Variable		Total Phenolic Content	Total Monomeric Anthocyanin	Antioxidant Activity
1. Total Phenolic Content	Pearson's r	—		
	p-value	—		
2. Total Monomeric Anthocyanin	Pearson's r	0.663 ***	—	
	p-value	< 0.001	—	
3. Antioxidant Activity	Pearson's r	-0.153	-0.099	—
	p-value	0.053	0.211	—

* p < 0.05, ** p < 0.01, *** p < 0.001

Appendix F.7 Test of Significance the effect of solvent-to-feed (S/F) ratio and heating method (MAE at 60 °C and CSE at 70 °C) on Total Phenolic Content (TPC), Total Monomeric Anthocyanin (TMA), and Antioxidant Activity (AOA) – without Post Hoc Tests

ANOVA - Total Phenolic Content

Cases	Sum of Squares	df	Mean Square	F	p	η^2
S/F Ratio	14962.389	4	3740.597	664.075	< 0.001***	0.965
Heating Method	12.559	1	12.559	2.230	0.139	8.102×10^{-4}
S/F Ratio ✻ Heating Method	75.189	4	18.797	3.337	0.014	0.005
Residuals	450.623	80	5.633			

Note. Type III Sum of Squares

ANOVA - Total Monomeric Anthocyanin

Cases	Sum of Squares	df	Mean Square	F	p	η^2
S/F Ratio	0.018	4	0.004	7.394	< 0.001***	0.148
Heating Method	0.047	1	0.047	78.810	< 0.001***	0.395
S/F Ratio ✻ Heating Method	0.007	4	0.002	2.802	0.031	0.056
Residuals	0.048	80	5.989×10^{-4}			

ANOVA - Antioxidant Activity

Cases	Sum of Squares	df	Mean Square	F	p	η^2
S/F Ratio	118.346	4	29.586	2108.381	< 0.001***	0.976
Heating Method	1.124	1	1.124	80.133	< 0.001***	0.009
S/F Ratio ✻ Heating Method	0.612	4	0.153	10.909	< 0.001***	0.005
Residuals	1.123	80	0.014			

Note. Type III Sum of Squares

Pearson's Correlations

Variable		Total Phenolic Content	Total Monomeric Anthocyanin	Antioxidant Activity
1. Total Phenolic Content	Pearson's r	—		
	p-value	—		
2. Total Monomeric Anthocyanin	Pearson's r	0.062	—	
	p-value	0.564	—	
3. Antioxidant Activity	Pearson's r	0.955***	0.014	—
	p-value	< 0.001	0.896	—

* p < 0.05, ** p < 0.01, *** p < 0.001

Appendix F.8 Test of Significance the effect of ethanol concentration (0 – 100% v/v) and heating method (MAE and CSE at 50 °C) on Total Phenolic Content (TPC), Total Monomeric Anthocyanin (TMA), and Antioxidant Activity (AOA)

F.8.1 Total Phenolic Content (TPC)

ANOVA - Total Phenolic Content

Cases	Sum of Squares	df	Mean Square	F	p	η^2
Ethanol Concentration	86281.145	8	10785.143	2550.677	< 0.001***	0.982
Heating method	571.867	1	571.867	135.246	< 0.001***	0.007
Ethanol Concentration ✻ Heating method	368.208	8	46.026	10.885	< 0.001***	0.004
Residuals	608.882	144	4.228			

Note. Type III Sum of Squares

Post Hoc Tests

Post Hoc Comparisons - Ethanol Concentration

		Mean Difference	SE	t	p_{tukey}
0	10	-11.640	0.685	-16.982	< 0.001***
	30	-29.112	0.685	-42.472	< 0.001***
	40	-37.890	0.685	-55.279	< 0.001***
	50	-54.113	0.685	-78.947	< 0.001***
	60	-33.226	0.685	-48.474	< 0.001***
	70	-26.631	0.685	-38.853	< 0.001***
	90	-10.086	0.685	-14.714	< 0.001***
	100	29.443	0.685	42.955	< 0.001***
10	30	-17.472	0.685	-25.490	< 0.001***
	40	-26.250	0.685	-38.297	< 0.001***
	50	-42.473	0.685	-61.965	< 0.001***
	60	-21.586	0.685	-31.492	< 0.001***
	70	-14.991	0.685	-21.871	< 0.001***
	90	1.555	0.685	2.268	0.369
	100	41.083	0.685	59.938	< 0.001***
30	40	-8.778	0.685	-12.807	< 0.001***
	50	-25.001	0.685	-36.475	< 0.001***
	60	-4.114	0.685	-6.002	< 0.001***
	70	2.481	0.685	3.619	0.012*
	90	19.026	0.685	27.758	< 0.001***
	100	58.555	0.685	85.428	< 0.001***
40	50	-16.223	0.685	-23.668	< 0.001***
	60	4.664	0.685	6.805	< 0.001***
	70	11.259	0.685	16.426	< 0.001***
	90	27.805	0.685	40.565	< 0.001***
	100	67.333	0.685	98.235	< 0.001***
50	60	20.887	0.685	30.473	< 0.001***

Post Hoc Comparisons - Ethanol Concentration

		Mean Difference	SE	t	p_{tukey}
	70	27.482	0.685	40.094	< 0.001 ***
	90	44.027	0.685	64.233	< 0.001 ***
	100	83.556	0.685	121.903	< 0.001 ***
60	70	6.595	0.685	9.621	< 0.001 ***
	90	23.140	0.685	33.760	< 0.001 ***
	100	62.669	0.685	91.429	< 0.001 ***
70	90	16.546	0.685	24.139	< 0.001 ***
	100	56.074	0.685	81.808	< 0.001 ***
90	100	39.529	0.685	57.670	< 0.001 ***

* p < 0.05, *** p < 0.001

Note. P-value adjusted for comparing a family of 9

Note. Results are averaged over the levels of: Heating method

Post Hoc Comparisons - Heating method

		Mean Difference	SE	t	p_{tukey}
CSE	MAE	-3.758	0.323	-11.630	< 0.001 ***

*** p < 0.001

Note. Results are averaged over the levels of: Ethanol Concentration

Post Hoc Comparisons - Ethanol Concentration ✻ Heating method

		Mean Difference	SE	t	p_{tukey}
0 CSE	10 CSE	-9.162	0.969	-9.452	< 0.001 ***
	30 CSE	-25.342	0.969	-26.144	< 0.001 ***
	40 CSE	-34.991	0.969	-36.098	< 0.001 ***
	50 CSE	-54.912	0.969	-56.649	< 0.001 ***
	60 CSE	-32.744	0.969	-33.780	< 0.001 ***
	70 CSE	-26.281	0.969	-27.112	< 0.001 ***
	90 CSE	-9.307	0.969	-9.601	< 0.001 ***
	100 CSE	29.012	0.969	29.930	< 0.001 ***
	0 MAE	-1.641	0.969	-1.692	0.965
	10 MAE	-15.759	0.969	-16.257	< 0.001 ***
	30 MAE	-34.522	0.969	-35.614	< 0.001 ***
	40 MAE	-42.430	0.969	-43.771	< 0.001 ***
	50 MAE	-54.954	0.969	-56.692	< 0.001 ***
	60 MAE	-35.348	0.969	-36.465	< 0.001 ***
	70 MAE	-28.622	0.969	-29.527	< 0.001 ***
	90 MAE	-12.505	0.969	-12.901	< 0.001 ***
	100 MAE	28.233	0.969	29.126	< 0.001 ***
10 CSE	30 CSE	-16.180	0.969	-16.692	< 0.001 ***
	40 CSE	-25.829	0.969	-26.646	< 0.001 ***
	50 CSE	-45.750	0.969	-47.197	< 0.001 ***
	60 CSE	-23.582	0.969	-24.328	< 0.001 ***
	70 CSE	-17.119	0.969	-17.660	< 0.001 ***
	90 CSE	-0.144	0.969	-0.149	1.000

Post Hoc Comparisons - Ethanol Concentration ✻ Heating method

		Mean Difference	SE	t	p_{tukey}
	100 CSE	38.174	0.969	39.382	< 0.001 ***
	0 MAE	7.522	0.969	7.759	< 0.001 ***
	10 MAE	-6.596	0.969	-6.805	< 0.001 ***
	30 MAE	-25.360	0.969	-26.162	< 0.001 ***
	40 MAE	-33.267	0.969	-34.319	< 0.001 ***
	50 MAE	-45.792	0.969	-47.240	< 0.001 ***
	60 MAE	-26.185	0.969	-27.013	< 0.001 ***
	70 MAE	-19.460	0.969	-20.075	< 0.001 ***
	90 MAE	-3.343	0.969	-3.449	0.068
	100 MAE	37.395	0.969	38.578	< 0.001 ***
30 CSE	40 CSE	-9.649	0.969	-9.954	< 0.001 ***
	50 CSE	-29.570	0.969	-30.505	< 0.001 ***
	60 CSE	-7.402	0.969	-7.636	< 0.001 ***
	70 CSE	-0.939	0.969	-0.969	1.000
	90 CSE	16.036	0.969	16.543	< 0.001 ***
	100 CSE	54.354	0.969	56.073	< 0.001 ***
	0 MAE	23.702	0.969	24.451	< 0.001 ***
	10 MAE	9.584	0.969	9.887	< 0.001 ***
	30 MAE	-9.180	0.969	-9.470	< 0.001 ***
	40 MAE	-17.087	0.969	-17.628	< 0.001 ***
	50 MAE	-29.612	0.969	-30.548	< 0.001 ***
	60 MAE	-10.005	0.969	-10.322	< 0.001 ***
	70 MAE	-3.280	0.969	-3.383	0.082
	90 MAE	12.837	0.969	13.243	< 0.001 ***
	100 MAE	53.575	0.969	55.269	< 0.001 ***
40 CSE	50 CSE	-19.921	0.969	-20.551	< 0.001 ***
	60 CSE	2.247	0.969	2.318	0.669
	70 CSE	8.710	0.969	8.985	< 0.001 ***
	90 CSE	25.684	0.969	26.497	< 0.001 ***
	100 CSE	64.003	0.969	66.027	< 0.001 ***
	0 MAE	33.350	0.969	34.405	< 0.001 ***
	10 MAE	19.232	0.969	19.841	< 0.001 ***
	30 MAE	0.469	0.969	0.484	1.000
	40 MAE	-7.439	0.969	-7.674	< 0.001 ***
	50 MAE	-19.963	0.969	-20.594	< 0.001 ***
	60 MAE	-0.356	0.969	-0.368	1.000
	70 MAE	6.369	0.969	6.571	< 0.001 ***
	90 MAE	22.486	0.969	23.197	< 0.001 ***
	100 MAE	63.224	0.969	65.223	< 0.001 ***
50 CSE	60 CSE	22.168	0.969	22.869	< 0.001 ***
	70 CSE	28.631	0.969	29.537	< 0.001 ***
	90 CSE	45.606	0.969	47.048	< 0.001 ***
	100 CSE	83.924	0.969	86.578	< 0.001 ***
	0 MAE	53.272	0.969	54.956	< 0.001 ***
	10 MAE	39.154	0.969	40.392	< 0.001 ***
	30 MAE	20.390	0.969	21.035	< 0.001 ***
	40 MAE	12.483	0.969	12.877	< 0.001 ***
	50 MAE	-0.042	0.969	-0.043	1.000
	60 MAE	19.565	0.969	20.183	< 0.001 ***
	70 MAE	26.290	0.969	27.122	< 0.001 ***

Post Hoc Comparisons - Ethanol Concentration ✻ Heating method

		Mean Difference	SE	t	p_{tukey}
	90 MAE	42.407	0.969	43.748	< 0.001 ***
	100 MAE	83.145	0.969	85.775	< 0.001 ***
60 CSE	70 CSE	6.463	0.969	6.668	< 0.001 ***
	90 CSE	23.438	0.969	24.179	< 0.001 ***
	100 CSE	61.757	0.969	63.710	< 0.001 ***
	0 MAE	31.104	0.969	32.087	< 0.001 ***
	10 MAE	16.986	0.969	17.523	< 0.001 ***
	30 MAE	-1.778	0.969	-1.834	0.930
	40 MAE	-9.685	0.969	-9.992	< 0.001 ***
	50 MAE	-22.210	0.969	-22.912	< 0.001 ***
	60 MAE	-2.603	0.969	-2.685	0.400
	70 MAE	4.123	0.969	4.253	0.005 **
	90 MAE	20.239	0.969	20.879	< 0.001 ***
	100 MAE	60.977	0.969	62.906	< 0.001 ***
70 CSE	90 CSE	16.974	0.969	17.511	< 0.001 ***
	100 CSE	55.293	0.969	57.042	< 0.001 ***
	0 MAE	24.640	0.969	25.420	< 0.001 ***
	10 MAE	10.522	0.969	10.855	< 0.001 ***
	30 MAE	-8.241	0.969	-8.502	< 0.001 ***
	40 MAE	-16.149	0.969	-16.659	< 0.001 ***
	50 MAE	-28.673	0.969	-29.580	< 0.001 ***
	60 MAE	-9.066	0.969	-9.353	< 0.001 ***
	70 MAE	-2.341	0.969	-2.415	0.598
	90 MAE	13.776	0.969	14.212	< 0.001 ***
	100 MAE	54.514	0.969	56.238	< 0.001 ***
90 CSE	100 CSE	38.319	0.969	39.531	< 0.001 ***
	0 MAE	7.666	0.969	7.908	< 0.001 ***
	10 MAE	-6.452	0.969	-6.656	< 0.001 ***
	30 MAE	-25.216	0.969	-26.013	< 0.001 ***
	40 MAE	-33.123	0.969	-34.170	< 0.001 ***
	50 MAE	-45.648	0.969	-47.091	< 0.001 ***
	60 MAE	-26.041	0.969	-26.864	< 0.001 ***
	70 MAE	-19.315	0.969	-19.926	< 0.001 ***
	90 MAE	-3.198	0.969	-3.300	0.103
	100 MAE	37.540	0.969	38.727	< 0.001 ***
100 CSE	0 MAE	-30.653	0.969	-31.622	< 0.001 ***
	10 MAE	-44.771	0.969	-46.187	< 0.001 ***
	30 MAE	-63.535	0.969	-65.544	< 0.001 ***
	40 MAE	-71.442	0.969	-73.701	< 0.001 ***
	50 MAE	-83.966	0.969	-86.622	< 0.001 ***
	60 MAE	-64.360	0.969	-66.395	< 0.001 ***
	70 MAE	-57.634	0.969	-59.457	< 0.001 ***
	90 MAE	-41.517	0.969	-42.830	< 0.001 ***
	100 MAE	-0.779	0.969	-0.804	1.000
0 MAE	10 MAE	-14.118	0.969	-14.564	< 0.001 ***
	30 MAE	-32.882	0.969	-33.922	< 0.001 ***
	40 MAE	-40.789	0.969	-42.079	< 0.001 ***
	50 MAE	-53.314	0.969	-55.000	< 0.001 ***
	60 MAE	-33.707	0.969	-34.773	< 0.001 ***
	70 MAE	-26.981	0.969	-27.834	< 0.001 ***

Post Hoc Comparisons - Ethanol Concentration ✻ Heating method

		Mean Difference	SE	t	p_{tukey}
	90 MAE	-10.865	0.969	-11.208	< 0.001 ***
	100 MAE	29.874	0.969	30.818	< 0.001 ***
10 MAE	30 MAE	-18.764	0.969	-19.357	< 0.001 ***
	40 MAE	-26.671	0.969	-27.515	< 0.001 ***
	50 MAE	-39.196	0.969	-40.435	< 0.001 ***
	60 MAE	-19.589	0.969	-20.208	< 0.001 ***
	70 MAE	-12.863	0.969	-13.270	< 0.001 ***
	90 MAE	3.253	0.969	3.356	0.088
	100 MAE	43.992	0.969	45.383	< 0.001 ***
30 MAE	40 MAE	-7.907	0.969	-8.157	< 0.001 ***
	50 MAE	-20.432	0.969	-21.078	< 0.001 ***
	60 MAE	-0.825	0.969	-0.851	1.000
	70 MAE	5.901	0.969	6.087	< 0.001 ***
	90 MAE	22.017	0.969	22.713	< 0.001 ***
	100 MAE	62.755	0.969	64.740	< 0.001 ***
40 MAE	50 MAE	-12.524	0.969	-12.921	< 0.001 ***
	60 MAE	7.082	0.969	7.306	< 0.001 ***
	70 MAE	13.808	0.969	14.245	< 0.001 ***
	90 MAE	29.925	0.969	30.871	< 0.001 ***
	100 MAE	70.663	0.969	72.897	< 0.001 ***
50 MAE	60 MAE	19.607	0.969	20.227	< 0.001 ***
	70 MAE	26.332	0.969	27.165	< 0.001 ***
	90 MAE	42.449	0.969	43.791	< 0.001 ***
	100 MAE	83.187	0.969	85.818	< 0.001 ***
60 MAE	70 MAE	6.726	0.969	6.939	< 0.001 ***
	90 MAE	22.842	0.969	23.565	< 0.001 ***
	100 MAE	63.581	0.969	65.591	< 0.001 ***
70 MAE	90 MAE	16.117	0.969	16.626	< 0.001 ***
	100 MAE	56.855	0.969	58.653	< 0.001 ***
90 MAE	100 MAE	40.738	0.969	42.026	< 0.001 ***

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Note. P-value adjusted for comparing a family of 18

F.8.2 Total Monomeric Anthocyanin (TMA)

ANOVA - Total Monomeric Anthocyanin

Cases	Sum of Squares	df	Mean Square	F	p	η^2
Ethanol Concentration	1.448	8	0.181	932.525	< 0.001***	0.924
Heating Method	0.079	1	0.079	406.594	< 0.001***	0.050
Ethanol Concentration ✻ Heating Method	0.012	8	0.001	7.593	< 0.001***	0.008
Residuals	0.028	144	1.941×10^{-4}			

Note. Type III Sum of Squares

Post Hoc Tests

Post Hoc Comparisons - Ethanol Concentration

		Mean Difference	SE	t	p_{tukey}	
0	10	-0.038	0.005	-8.206	< 0.001	***
	30	-0.062	0.005	-13.445	< 0.001	***
	40	-0.174	0.005	-37.464	< 0.001	***
	50	-0.297	0.005	-63.935	< 0.001	***
	60	-0.091	0.005	-19.629	< 0.001	***
	70	-0.047	0.005	-10.167	< 0.001	***
	90	-0.010	0.005	-2.081	0.490	
	100	0.020	0.005	4.306	< 0.001	***
10	30	-0.024	0.005	-5.239	< 0.001	***
	40	-0.136	0.005	-29.258	< 0.001	***
	50	-0.259	0.005	-55.729	< 0.001	***
	60	-0.053	0.005	-11.423	< 0.001	***
	70	-0.009	0.005	-1.962	0.572	
	90	0.028	0.005	6.124	< 0.001	***
	100	0.058	0.005	12.512	< 0.001	***
30	40	-0.112	0.005	-24.019	< 0.001	***
	50	-0.235	0.005	-50.490	< 0.001	***
	60	-0.029	0.005	-6.184	< 0.001	***
	70	0.015	0.005	3.277	0.035	*
	90	0.053	0.005	11.364	< 0.001	***
	100	0.082	0.005	17.751	< 0.001	***
40	50	-0.123	0.005	-26.471	< 0.001	***
	60	0.083	0.005	17.835	< 0.001	***
	70	0.127	0.005	27.296	< 0.001	***
	90	0.164	0.005	35.382	< 0.001	***
	100	0.194	0.005	41.770	< 0.001	***
50	60	0.206	0.005	44.306	< 0.001	***
	70	0.250	0.005	53.767	< 0.001	***
	90	0.287	0.005	61.853	< 0.001	***
	100	0.317	0.005	68.241	< 0.001	***
60	70	0.044	0.005	9.462	< 0.001	***
	90	0.082	0.005	17.548	< 0.001	***
	100	0.111	0.005	23.935	< 0.001	***
70	90	0.038	0.005	8.086	< 0.001	***

Post Hoc Comparisons - Ethanol Concentration

		Mean Difference	SE	t	p_{tukey}
	100	0.067	0.005	14.474	< 0.001 ***
90	100	0.030	0.005	6.387	< 0.001 ***

* p < 0.05, ** p < 0.01, *** p < 0.001

Note. P-value adjusted for comparing a family of 9

Note. Results are averaged over the levels of: Heating Method

Post Hoc Comparisons - Heating Method

		Mean Difference	SE	t	p_{tukey}
CSE	MAE	-0.044	0.002	-20.164	< 0.001 ***

*** p < 0.001

Note. Results are averaged over the levels of: Ethanol Concentration

Post Hoc Comparisons - Ethanol Concentration ✻ Heating Method

		Mean Difference	SE	t	p_{tukey}
0 CSE	10 CSE	-0.029	0.007	-4.364	0.003 **
	30 CSE	-0.049	0.007	-7.494	< 0.001 ***
	40 CSE	-0.170	0.007	-25.950	< 0.001 ***
	50 CSE	-0.301	0.007	-45.826	< 0.001 ***
	60 CSE	-0.093	0.007	-14.210	< 0.001 ***
	70 CSE	-0.064	0.007	-9.811	< 0.001 ***
	90 CSE	-0.018	0.007	-2.690	0.397
	100 CSE	0.019	0.007	2.876	0.280
	0 MAE	-0.046	0.007	-6.936	< 0.001 ***
	10 MAE	-0.093	0.007	-14.176	< 0.001 ***
	30 MAE	-0.121	0.007	-18.456	< 0.001 ***
	40 MAE	-0.223	0.007	-33.968	< 0.001 ***
	50 MAE	-0.338	0.007	-51.527	< 0.001 ***
	60 MAE	-0.135	0.007	-20.486	< 0.001 ***
	70 MAE	-0.076	0.007	-11.503	< 0.001 ***
	90 MAE	-0.047	0.007	-7.189	< 0.001 ***
	100 MAE	-0.024	0.007	-3.722	0.030 *
10 CSE	30 CSE	-0.021	0.007	-3.130	0.159
	40 CSE	-0.142	0.007	-21.585	< 0.001 ***
	50 CSE	-0.272	0.007	-41.462	< 0.001 ***
	60 CSE	-0.065	0.007	-9.845	< 0.001 ***
	70 CSE	-0.036	0.007	-5.447	< 0.001 ***
	90 CSE	0.011	0.007	1.675	0.968
	100 CSE	0.048	0.007	7.240	< 0.001 ***
	0 MAE	-0.017	0.007	-2.571	0.482
	10 MAE	-0.064	0.007	-9.811	< 0.001 ***
	30 MAE	-0.093	0.007	-14.091	< 0.001 ***
	40 MAE	-0.194	0.007	-29.603	< 0.001 ***
	50 MAE	-0.310	0.007	-47.162	< 0.001 ***
	60 MAE	-0.106	0.007	-16.121	< 0.001 ***
	70 MAE	-0.047	0.007	-7.139	< 0.001 ***
	90 MAE	-0.019	0.007	-2.825	0.310
	100 MAE	0.004	0.007	0.643	1.000
30 CSE	40 CSE	-0.121	0.007	-18.456	< 0.001 ***

Post Hoc Comparisons - Heating Method

		Mean Difference	SE	t	p_{tukey}	
	50 CSE	-0.252	0.007	-38.332	< 0.001	***
	60 CSE	-0.044	0.007	-6.716	< 0.001	***
	70 CSE	-0.015	0.007	-2.318	0.669	
	90 CSE	0.032	0.007	4.804	< 0.001	***
	100 CSE	0.068	0.007	10.370	< 0.001	***
	0 MAE	0.004	0.007	0.558	1.000	
	10 MAE	-0.044	0.007	-6.682	< 0.001	***
	30 MAE	-0.072	0.007	-10.962	< 0.001	***
	40 MAE	-0.174	0.007	-26.474	< 0.001	***
	50 MAE	-0.289	0.007	-44.033	< 0.001	***
	60 MAE	-0.085	0.007	-12.992	< 0.001	***
	70 MAE	-0.026	0.007	-4.009	0.011	*
	90 MAE	0.002	0.007	0.304	1.000	
	100 MAE	0.025	0.007	3.772	0.025	*
40 CSE	50 CSE	-0.131	0.007	-19.877	< 0.001	***
	60 CSE	0.077	0.007	11.740	< 0.001	***
	70 CSE	0.106	0.007	16.138	< 0.001	***
	90 CSE	0.153	0.007	23.260	< 0.001	***
	100 CSE	0.189	0.007	28.825	< 0.001	***
	0 MAE	0.125	0.007	19.014	< 0.001	***
	10 MAE	0.077	0.007	11.774	< 0.001	***
	30 MAE	0.049	0.007	7.494	< 0.001	***
	40 MAE	-0.053	0.007	-8.018	< 0.001	***
	50 MAE	-0.168	0.007	-25.577	< 0.001	***
	60 MAE	0.036	0.007	5.464	< 0.001	***
	70 MAE	0.095	0.007	14.446	< 0.001	***
	90 MAE	0.123	0.007	18.760	< 0.001	***
	100 MAE	0.146	0.007	22.228	< 0.001	***
50 CSE	60 CSE	0.208	0.007	31.616	< 0.001	***
	70 CSE	0.237	0.007	36.015	< 0.001	***
	90 CSE	0.283	0.007	43.136	< 0.001	***
	100 CSE	0.320	0.007	48.702	< 0.001	***
	0 MAE	0.255	0.007	38.890	< 0.001	***
	10 MAE	0.208	0.007	31.650	< 0.001	***
	30 MAE	0.180	0.007	27.370	< 0.001	***
	40 MAE	0.078	0.007	11.858	< 0.001	***
	50 MAE	-0.037	0.007	-5.701	< 0.001	***
	60 MAE	0.166	0.007	25.341	< 0.001	***
	70 MAE	0.225	0.007	34.323	< 0.001	***
	90 MAE	0.254	0.007	38.637	< 0.001	***
	100 MAE	0.277	0.007	42.105	< 0.001	***
60 CSE	70 CSE	0.029	0.007	4.398	0.003	**
	90 CSE	0.076	0.007	11.520	< 0.001	***
	100 CSE	0.112	0.007	17.085	< 0.001	***
	0 MAE	0.048	0.007	7.274	< 0.001	***
	10 MAE	2.222×10^{-4}	0.007	0.034	1.000	
	30 MAE	-0.028	0.007	-4.246	0.005	**
	40 MAE	-0.130	0.007	-19.758	< 0.001	***
	50 MAE	-0.245	0.007	-37.317	< 0.001	***
	60 MAE	-0.041	0.007	-6.276	< 0.001	***

Post Hoc Comparisons - Heating Method

		Mean Difference	SE	t	p_{tukey}	
	70 MAE	0.018	0.007	2.707	0.386	
	90 MAE	0.046	0.007	7.020	< 0.001	***
	100 MAE	0.069	0.007	10.488	< 0.001	***
70 CSE	90 CSE	0.047	0.007	7.122	< 0.001	***
	100 CSE	0.083	0.007	12.687	< 0.001	***
	0 MAE	0.019	0.007	2.876	0.280	
	10 MAE	-0.029	0.007	-4.364	0.003	**
	30 MAE	-0.057	0.007	-8.644	< 0.001	***
	40 MAE	-0.159	0.007	-24.156	< 0.001	***
	50 MAE	-0.274	0.007	-41.715	< 0.001	***
	60 MAE	-0.070	0.007	-10.674	< 0.001	***
	70 MAE	-0.011	0.007	-1.692	0.965	
	90 MAE	0.017	0.007	2.622	0.445	
	100 MAE	0.040	0.007	6.090	< 0.001	***
90 CSE	100 CSE	0.037	0.007	5.565	< 0.001	***
	0 MAE	-0.028	0.007	-4.246	0.005	**
	10 MAE	-0.075	0.007	-11.486	< 0.001	***
	30 MAE	-0.104	0.007	-15.766	< 0.001	***
	40 MAE	-0.205	0.007	-31.278	< 0.001	***
	50 MAE	-0.321	0.007	-48.837	< 0.001	***
	60 MAE	-0.117	0.007	-17.796	< 0.001	***
	70 MAE	-0.058	0.007	-8.813	< 0.001	***
	90 MAE	-0.030	0.007	-4.500	0.002	**
	100 MAE	-0.007	0.007	-1.032	1.000	
100 CSE	0 MAE	-0.064	0.007	-9.811	< 0.001	***
	10 MAE	-0.112	0.007	-17.052	< 0.001	***
	30 MAE	-0.140	0.007	-21.331	< 0.001	***
	40 MAE	-0.242	0.007	-36.844	< 0.001	***
	50 MAE	-0.357	0.007	-54.403	< 0.001	***
	60 MAE	-0.153	0.007	-23.361	< 0.001	***
	70 MAE	-0.094	0.007	-14.379	< 0.001	***
	90 MAE	-0.066	0.007	-10.065	< 0.001	***
	100 MAE	-0.043	0.007	-6.597	< 0.001	***
0 MAE	10 MAE	-0.048	0.007	-7.240	< 0.001	***
	30 MAE	-0.076	0.007	-11.520	< 0.001	***
	40 MAE	-0.178	0.007	-27.032	< 0.001	***
	50 MAE	-0.293	0.007	-44.591	< 0.001	***
	60 MAE	-0.089	0.007	-13.550	< 0.001	***
	70 MAE	-0.030	0.007	-4.567	0.001	**
	90 MAE	-0.002	0.007	-0.254	1.000	
	100 MAE	0.021	0.007	3.214	0.128	
10 MAE	30 MAE	-0.028	0.007	-4.280	0.004	**
	40 MAE	-0.130	0.007	-19.792	< 0.001	***
	50 MAE	-0.245	0.007	-37.351	< 0.001	***
	60 MAE	-0.041	0.007	-6.310	< 0.001	***
	70 MAE	0.018	0.007	2.673	0.409	
	90 MAE	0.046	0.007	6.986	< 0.001	***
	100 MAE	0.069	0.007	10.454	< 0.001	***
30 MAE	40 MAE	-0.102	0.007	-15.512	< 0.001	***
	50 MAE	-0.217	0.007	-33.071	< 0.001	***

Post Hoc Comparisons - Heating Method

		Mean Difference	SE	t	p_{tukey}	
	60 MAE	-0.013	0.007	-2.030	0.850	
	70 MAE	0.046	0.007	6.953	< 0.001	***
	90 MAE	0.074	0.007	11.266	< 0.001	***
	100 MAE	0.097	0.007	14.734	< 0.001	***
40 MAE	50 MAE	-0.115	0.007	-17.559	< 0.001	***
	60 MAE	0.089	0.007	13.482	< 0.001	***
	70 MAE	0.148	0.007	22.465	< 0.001	***
	90 MAE	0.176	0.007	26.778	< 0.001	***
	100 MAE	0.199	0.007	30.246	< 0.001	***
50 MAE	60 MAE	0.204	0.007	31.041	< 0.001	***
	70 MAE	0.263	0.007	40.024	< 0.001	***
	90 MAE	0.291	0.007	44.337	< 0.001	***
	100 MAE	0.314	0.007	47.805	< 0.001	***
60 MAE	70 MAE	0.059	0.007	8.983	< 0.001	***
	90 MAE	0.087	0.007	13.296	< 0.001	***
	100 MAE	0.110	0.007	16.764	< 0.001	***
70 MAE	90 MAE	0.028	0.007	4.314	0.004	**
	100 MAE	0.051	0.007	7.781	< 0.001	***
90 MAE	100 MAE	0.023	0.007	3.468	0.064	

Note. P-value adjusted for comparing a family of 18

* p < 0.05, ** p < 0.01, *** p < 0.001

F.8.3 Antioxidant Activity (AOA)

ANOVA - Antioxidant Activity

Cases	Sum of Squares	df	Mean Square	F	p	η^2
Ethanol Concentration	44.010	8	5.501	2317.054	< 0.001***	0.982
Heating Method	0.033	1	0.033	13.874	< 0.001***	7.352×10^{-4}
Ethanol Concentration ✻ Heating Method	0.419	8	0.052	22.077	< 0.001***	0.009
Residuals	0.342	144	0.002			

Note. Type III Sum of Squares

Post Hoc Tests

Post Hoc Comparisons - Ethanol Concentration

		Mean Difference	SE	t	p_{tukey}
0	10	-0.016	0.016	-0.992	0.986
	30	0.013	0.016	0.821	0.996
	40	-0.138	0.016	-8.517	< 0.001 ***
	50	-0.150	0.016	-9.235	< 0.001 ***
	60	-0.434	0.016	-26.714	< 0.001 ***
	70	-0.866	0.016	-53.291	< 0.001 ***
	90	-1.259	0.016	-77.542	< 0.001 ***
	100	-1.359	0.016	-83.699	< 0.001 ***
10	30	0.029	0.016	1.813	0.674
	40	-0.122	0.016	-7.525	< 0.001 ***
	50	-0.134	0.016	-8.243	< 0.001 ***
	60	-0.418	0.016	-25.722	< 0.001 ***
	70	-0.849	0.016	-52.299	< 0.001 ***
	90	-1.243	0.016	-76.550	< 0.001 ***
	100	-1.343	0.016	-82.707	< 0.001 ***
30	40	-0.152	0.016	-9.338	< 0.001 ***
	50	-0.163	0.016	-10.056	< 0.001 ***
	60	-0.447	0.016	-27.535	< 0.001 ***
	70	-0.879	0.016	-54.112	< 0.001 ***
	90	-1.273	0.016	-78.363	< 0.001 ***
	100	-1.373	0.016	-84.520	< 0.001 ***
40	50	-0.012	0.016	-0.718	0.998
	60	-0.296	0.016	-18.197	< 0.001 ***
	70	-0.727	0.016	-44.774	< 0.001 ***
	90	-1.121	0.016	-69.025	< 0.001 ***
	100	-1.221	0.016	-75.182	< 0.001 ***
50	60	-0.284	0.016	-17.479	< 0.001 ***
	70	-0.716	0.016	-44.056	< 0.001 ***
	90	-1.109	0.016	-68.307	< 0.001 ***
	100	-1.209	0.016	-74.464	< 0.001 ***
60	70	-0.432	0.016	-26.577	< 0.001 ***
	90	-0.826	0.016	-50.828	< 0.001 ***
	100	-0.926	0.016	-56.985	< 0.001 ***
70	90	-0.394	0.016	-24.251	< 0.001 ***
	100	-0.494	0.016	-30.408	< 0.001 ***
90	100	-0.100	0.016	-6.157	< 0.001 ***

*** $p < 0.001$

Post Hoc Comparisons - Ethanol Concentration

	Mean Difference	SE	t	p_{tukey}

Note. P-value adjusted for comparing a family of 9

Note. Results are averaged over the levels of: Heating Method

Post Hoc Comparisons - Heating Method

		Mean Difference	SE	t	p_{tukey}
CSE	MAE	0.029	0.008	3.725	< 0.001 ***

*** $p < 0.001$

Note. Results are averaged over the levels of: Ethanol Concentration

Post Hoc Comparisons - Ethanol Concentration ✻ Heating Method

		Mean Difference	SE	t	p_{tukey}
0 CSE	10 CSE	-0.032	0.023	-1.403	0.995
	30 CSE	-0.006	0.023	-0.242	1.000
	40 CSE	-0.247	0.023	-10.739	< 0.001 ***
	50 CSE	-0.248	0.023	-10.787	< 0.001 ***
	60 CSE	-0.602	0.023	-26.218	< 0.001 ***
	70 CSE	-0.966	0.023	-42.036	< 0.001 ***
	90 CSE	-1.321	0.023	-57.515	< 0.001 ***
	100 CSE	-1.427	0.023	-62.111	< 0.001 ***
	0 MAE	-0.113	0.023	-4.934	< 0.001 ***
	10 MAE	-0.113	0.023	-4.934	< 0.001 ***
	30 MAE	-0.081	0.023	-3.531	0.053
	40 MAE	-0.143	0.023	-6.240	< 0.001 ***
	50 MAE	-0.166	0.023	-7.208	< 0.001 ***
	60 MAE	-0.379	0.023	-16.495	< 0.001 ***
	70 MAE	-0.879	0.023	-38.263	< 0.001 ***
	90 MAE	-1.311	0.023	-57.080	< 0.001 ***
	100 MAE	-1.406	0.023	-61.192	< 0.001 ***
10 CSE	30 CSE	0.027	0.023	1.161	0.999
	40 CSE	-0.214	0.023	-9.336	< 0.001 ***
	50 CSE	-0.216	0.023	-9.384	< 0.001 ***
	60 CSE	-0.570	0.023	-24.815	< 0.001 ***
	70 CSE	-0.933	0.023	-40.633	< 0.001 ***
	90 CSE	-1.289	0.023	-56.113	< 0.001 ***
	100 CSE	-1.394	0.023	-60.708	< 0.001 ***
	0 MAE	-0.081	0.023	-3.531	0.053
	10 MAE	-0.081	0.023	-3.531	0.053
	30 MAE	-0.049	0.023	-2.128	0.795
	40 MAE	-0.111	0.023	-4.837	< 0.001 ***
	50 MAE	-0.133	0.023	-5.805	< 0.001 ***
	60 MAE	-0.347	0.023	-15.092	< 0.001 ***
	70 MAE	-0.847	0.023	-36.860	< 0.001 ***
	90 MAE	-1.279	0.023	-55.677	< 0.001 ***
	100 MAE	-1.373	0.023	-59.789	< 0.001 ***
30 CSE	40 CSE	-0.241	0.023	-10.497	< 0.001 ***
	50 CSE	-0.242	0.023	-10.545	< 0.001 ***
	60 CSE	-0.597	0.023	-25.976	< 0.001 ***
	70 CSE	-0.960	0.023	-41.794	< 0.001 ***
	90 CSE	-1.316	0.023	-57.274	< 0.001 ***

Post Hoc Comparisons - Ethanol Concentration ✻ Heating Method

		Mean Difference	SE	t	p_{tukey}
	100 CSE	-1.421	0.023	-61.869	< 0.001 ***
	0 MAE	-0.108	0.023	-4.692	< 0.001 ***
	10 MAE	-0.108	0.023	-4.692	< 0.001 ***
	30 MAE	-0.076	0.023	-3.289	0.106
	40 MAE	-0.138	0.023	-5.998	< 0.001 ***
	50 MAE	-0.160	0.023	-6.966	< 0.001 ***
	60 MAE	-0.373	0.023	-16.253	< 0.001 ***
	70 MAE	-0.873	0.023	-38.021	< 0.001 ***
	90 MAE	-1.306	0.023	-56.838	< 0.001 ***
	100 MAE	-1.400	0.023	-60.950	< 0.001 ***
40 CSE	50 CSE	-0.001	0.023	-0.048	1.000
	60 CSE	-0.356	0.023	-15.479	< 0.001 ***
	70 CSE	-0.719	0.023	-31.297	< 0.001 ***
	90 CSE	-1.074	0.023	-46.777	< 0.001 ***
	100 CSE	-1.180	0.023	-51.372	< 0.001 ***
	0 MAE	0.133	0.023	5.805	< 0.001 ***
	10 MAE	0.133	0.023	5.805	< 0.001 ***
	30 MAE	0.166	0.023	7.208	< 0.001 ***
	40 MAE	0.103	0.023	4.499	0.002 **
	50 MAE	0.081	0.023	3.531	0.053
	60 MAE	-0.132	0.023	-5.756	< 0.001 ***
	70 MAE	-0.632	0.023	-27.524	< 0.001 ***
	90 MAE	-1.064	0.023	-46.341	< 0.001 ***
	100 MAE	-1.159	0.023	-50.453	< 0.001 ***
50 CSE	60 CSE	-0.354	0.023	-15.431	< 0.001 ***
	70 CSE	-0.718	0.023	-31.249	< 0.001 ***
	90 CSE	-1.073	0.023	-46.728	< 0.001 ***
	100 CSE	-1.179	0.023	-51.324	< 0.001 ***
	0 MAE	0.134	0.023	5.853	< 0.001 ***
	10 MAE	0.134	0.023	5.853	< 0.001 ***
	30 MAE	0.167	0.023	7.256	< 0.001 ***
	40 MAE	0.104	0.023	4.547	0.002 **
	50 MAE	0.082	0.023	3.580	0.046 *
	60 MAE	-0.131	0.023	-5.708	< 0.001 ***
	70 MAE	-0.631	0.023	-27.476	< 0.001 ***
	90 MAE	-1.063	0.023	-46.293	< 0.001 ***
	100 MAE	-1.158	0.023	-50.405	< 0.001 ***
60 CSE	70 CSE	-0.363	0.023	-15.818	< 0.001 ***
	90 CSE	-0.719	0.023	-31.297	< 0.001 ***
	100 CSE	-0.824	0.023	-35.893	< 0.001 ***
	0 MAE	0.489	0.023	21.284	< 0.001 ***
	10 MAE	0.489	0.023	21.284	< 0.001 ***
	30 MAE	0.521	0.023	22.687	< 0.001 ***
	40 MAE	0.459	0.023	19.978	< 0.001 ***
	50 MAE	0.437	0.023	19.011	< 0.001 ***
	60 MAE	0.223	0.023	9.723	< 0.001 ***
	70 MAE	-0.277	0.023	-12.045	< 0.001 ***
	90 MAE	-0.709	0.023	-30.862	< 0.001 ***
	100 MAE	-0.803	0.023	-34.974	< 0.001 ***
70 CSE	90 CSE	-0.356	0.023	-15.479	< 0.001 ***
	100 CSE	-0.461	0.023	-20.075	< 0.001 ***

Post Hoc Comparisons - Ethanol Concentration ✻ Heating Method

		Mean Difference	SE	t	p_{tukey}
	0 MAE	0.852	0.023	37.102	< 0.001 ***
	10 MAE	0.852	0.023	37.102	< 0.001 ***
	30 MAE	0.884	0.023	38.505	< 0.001 ***
	40 MAE	0.822	0.023	35.796	< 0.001 ***
	50 MAE	0.800	0.023	34.829	< 0.001 ***
	60 MAE	0.587	0.023	25.541	< 0.001 ***
	70 MAE	0.087	0.023	3.773	0.025 *
	90 MAE	-0.346	0.023	-15.044	< 0.001 ***
	100 MAE	-0.440	0.023	-19.156	< 0.001 ***
90 CSE	100 CSE	-0.106	0.023	-4.595	0.001 **
	0 MAE	1.208	0.023	52.581	< 0.001 ***
	10 MAE	1.208	0.023	52.581	< 0.001 ***
	30 MAE	1.240	0.023	53.984	< 0.001 ***
	40 MAE	1.178	0.023	51.275	< 0.001 ***
	50 MAE	1.156	0.023	50.308	< 0.001 ***
	60 MAE	0.942	0.023	41.020	< 0.001 ***
	70 MAE	0.442	0.023	19.252	< 0.001 ***
	90 MAE	0.010	0.023	0.435	1.000
	100 MAE	-0.084	0.023	-3.676	0.034 *
100 CSE	0 MAE	1.313	0.023	57.177	< 0.001 ***
	10 MAE	1.313	0.023	57.177	< 0.001 ***
	30 MAE	1.346	0.023	58.580	< 0.001 ***
	40 MAE	1.283	0.023	55.871	< 0.001 ***
	50 MAE	1.261	0.023	54.903	< 0.001 ***
	60 MAE	1.048	0.023	45.616	< 0.001 ***
	70 MAE	0.548	0.023	23.848	< 0.001 ***
	90 MAE	0.116	0.023	5.031	< 0.001 ***
	100 MAE	0.021	0.023	0.919	1.000
0 MAE	10 MAE	-5.218×10^{-15}	0.023	-2.272×10^{-13}	1.000
	30 MAE	0.032	0.023	1.403	0.995
	40 MAE	-0.030	0.023	-1.306	0.998
	50 MAE	-0.052	0.023	-2.274	0.701
	60 MAE	-0.266	0.023	-11.561	< 0.001 ***
	70 MAE	-0.766	0.023	-33.329	< 0.001 ***
	90 MAE	-1.198	0.023	-52.146	< 0.001 ***
	100 MAE	-1.292	0.023	-56.258	< 0.001 ***
10 MAE	30 MAE	0.032	0.023	1.403	0.995
	40 MAE	-0.030	0.023	-1.306	0.998
	50 MAE	-0.052	0.023	-2.274	0.701
	60 MAE	-0.266	0.023	-11.561	< 0.001 ***
	70 MAE	-0.766	0.023	-33.329	< 0.001 ***
	90 MAE	-1.198	0.023	-52.146	< 0.001 ***
	100 MAE	-1.292	0.023	-56.258	< 0.001 ***
30 MAE	40 MAE	-0.062	0.023	-2.709	0.384
	50 MAE	-0.084	0.023	-3.676	0.034 *
	60 MAE	-0.298	0.023	-12.964	< 0.001 ***
	70 MAE	-0.798	0.023	-34.732	< 0.001 ***
	90 MAE	-1.230	0.023	-53.549	< 0.001 ***
	100 MAE	-1.324	0.023	-57.661	< 0.001 ***
40 MAE	50 MAE	-0.022	0.023	-0.967	1.000
	60 MAE	-0.236	0.023	-10.255	< 0.001 ***

Post Hoc Comparisons - Ethanol Concentration ✻ Heating Method

		Mean Difference	SE	t	p_{tukey}
	70 MAE	-0.736	0.023	-32.023	< 0.001 ***
	90 MAE	-1.168	0.023	-50.840	< 0.001 ***
	100 MAE	-1.262	0.023	-54.952	< 0.001 ***
50 MAE	60 MAE	-0.213	0.023	-9.288	< 0.001 ***
	70 MAE	-0.713	0.023	-31.055	< 0.001 ***
	90 MAE	-1.146	0.023	-49.873	< 0.001 ***
	100 MAE	-1.240	0.023	-53.984	< 0.001 ***
60 MAE	70 MAE	-0.500	0.023	-21.768	< 0.001 ***
	90 MAE	-0.932	0.023	-40.585	< 0.001 ***
	100 MAE	-1.027	0.023	-44.697	< 0.001 ***
70 MAE	90 MAE	-0.432	0.023	-18.817	< 0.001 ***
	100 MAE	-0.527	0.023	-22.929	< 0.001 ***
90 MAE	100 MAE	-0.094	0.023	-4.112	0.008 **

Note. P-value adjusted for comparing a family of 18

* p < 0.05, ** p < 0.01, *** p < 0.001

F.8.4 Correlation between TPC, TMA, and AOA on ethanol concentration and heating method (MAE and CSE at 50 °C) effects

Pearson's Correlations

Variable		Total Phenolic Content	Total Monomeric Anthocyanin	Antioxidant Activity
1. Total Phenolic Content	Pearson's r	—		
	p-value	—		
2. Total Monomeric Anthocyanin	Pearson's r	0.823 ***	—	
	p-value	< 0.001	—	
3. Antioxidant Activity	Pearson's r	-0.523 ***	-0.417***	—
	p-value	< 0.001	< 0.001	—

* p < 0.05, ** p < 0.01, *** p < 0.001

Appendix F.9 Test of Significance the effect of solvent-to-feed (S/F) ratio and heating method (MAE and CSE at 50 °C) on Total Phenolic Content (TPC), Total Monomeric Anthocyanin (TMA), and Antioxidant Activity (AOA) – without Post Hoc Tests

ANOVA - Total Phenolic Content

Cases	Sum of Squares	df	Mean Square	F	p	η^2
S/F Ratio	14263.877	3	4754.626	561.120	< 0.001***	0.927
Heating Method	210.809	1	210.809	24.879	< 0.001***	0.014
S/F Ratio ✻ Heating Method	364.812	3	121.604	14.351	< 0.001***	0.024
Residuals	542.302	64	8.473			

Note. Type III Sum of Squares

ANOVA - Total Monomeric Anthocyanin

Cases	Sum of Squares	df	Mean Square	F	p	η^2
S/F Ratio	0.149	3	0.050	265.767	< 0.001***	0.692
Heating Method	0.044	1	0.044	232.432	< 0.001***	0.202
S/F Ratio ✻ Heating Method	0.011	3	0.004	19.514	< 0.001***	0.051
Residuals	0.012	64	1.874×10^{-4}			

Note. Type III Sum of Squares

ANOVA - Antioxidant Activity

Cases	Sum of Squares	df	Mean Square	F	p	η^2
S/F Ratio	132.655	3	44.218	6358.527	< 0.001***	0.995
Heating Method	0.231	1	0.231	33.246	< 0.001***	0.002
S/F Ratio ✻ Heating Method	0.034	3	0.011	1.616	0.194	2.529×10^{-4}
Residuals	0.445	64	0.007			

Note. Type III Sum of Squares

Pearson's Correlations

Variable		Total Phenolic Content	Total Monomeric Anthocyanin	Antioxidant Activity
1. Total Phenolic Content	Pearson's r	—		
	p-value	—		
2. Total Monomeric Anthocyanin	Pearson's r	0.106	—	
	p-value	0.375	—	
3. Antioxidant Activity	Pearson's r	0.888***	0.195	—
	p-value	< 0.001	0.101	—

* p < 0.05, ** p < 0.01, *** p < 0.001

Appendix F.10 Test of Significance the effect of CPH particle size and heating method (MAE and CSE) on Total Phenolic Content (TPC), Total Monomeric Anthocyanin (TMA), and Antioxidant Activity (AOA) of CPH layer extracts (epicarp, mesocarp, endocarp)

F.10.1 Total Phenolic Content (TPC)

ANOVA - Total Phenolic Content

Cases	Sum of Squares	df	Mean Square	F	p	η^2
CPH Particle size	268728.234	4	67182.058	7292.967	< 0.001***	0.359
CPH layer	375274.383	2	187637.191	20369.006	< 0.001***	0.501
Heating method	997.646	1	997.646	108.300	< 0.001***	0.001
CPH Particle size ✻ CPH layer	96769.013	8	12096.127	1313.098	< 0.001***	0.129
CPH Particle size ✻ Heating method	1140.103	4	285.026	30.941	< 0.001***	0.002
CPH layer ✻ Heating method	1673.416	2	836.708	90.829	< 0.001***	0.002
CPH Particle size ✻ CPH layer ✻ Heating method	1728.241	8	216.030	23.451	< 0.001***	0.002
Residuals	2210.855	240	9.212			

Note. Type III Sum of Squares

Post Hoc Tests

Post Hoc Comparisons - CPH Particle size

		Mean Difference	SE	t	p_{tukey}
≤ 38micron	(125-150micron)	30.127	0.584	51.577	< 0.001***
	(38-63micron)	15.900	0.584	27.221	< 0.001***
	(63-90micron)	24.370	0.584	41.722	< 0.001***
	without grinding (0.5x0.5cm)	92.274	0.584	157.974	< 0.001***
(125-150micron)	(38-63micron)	-14.226	0.584	-24.356	< 0.001***
	(63-90micron)	-5.757	0.584	-9.856	< 0.001***
	without grinding (0.5x0.5cm)	62.147	0.584	106.397	< 0.001***
(38-63micron)	(63-90micron)	8.470	0.584	14.500	< 0.001***
	without grinding (0.5x0.5cm)	76.374	0.584	130.753	< 0.001***
(63-90micron)	without grinding (0.5x0.5cm)	67.904	0.584	116.253	< 0.001***

*** $p < 0.001$

Note. P-value adjusted for comparing a family of 5

Note. Results are averaged over the levels of: CPH layer, Heating method

Post Hoc Comparisons - CPH layer

		Mean Difference	SE	t	p_{tukey}
Endocarp	Epicarp	-87.152	0.452	-192.623	< 0.001 ***
	Mesocarp	-19.955	0.452	-44.104	< 0.001 ***
Epicarp	Mesocarp	67.197	0.452	148.520	< 0.001 ***

*** p < 0.001

Note. P-value adjusted for comparing a family of 3

Note. Results are averaged over the levels of: CPH Particle size, Heating method

Post Hoc Comparisons - Heating method

	Mean Difference	SE	t	p_{tukey}
CSE MAE	-3.844	0.369	-10.407	< 0.001 ***

*** p < 0.001

Note. Results are averaged over the levels of: CPH Particle size, CPH layer

F.10.2 Total Monomeric Anthocyanin (TMA)

ANOVA - Total Monomeric Anthocyanin

Cases	Sum of Squares	df	Mean Square	F	p	η^2
CPH Particle size	2.414	4	0.603	3077.289	< 0.001	0.639
CPH layer	0.869	2	0.434	2215.276	< 0.001	0.230
Heating method	0.059	1	0.059	301.306	< 0.001	0.016
CPH Particle size ✻ CPH layer	0.254	8	0.032	161.870	< 0.001	0.067
CPH Particle size ✻ Heating method	0.020	4	0.005	25.249	< 0.001	0.005
CPH layer ✻ Heating method	0.080	2	0.040	203.751	< 0.001	0.021
CPH Particle size ✻ CPH layer ✻ Heating method	0.037	8	0.005	23.612	< 0.001	0.010
Residuals	0.047	240	1.961×10^{-4}			

Note. Type III Sum of Squares

Post Hoc Tests

Post Hoc Comparisons - CPH Particle size

		Mean Difference	SE	t	p_{tukey}
≤ 38micron	(125-150micron)	0.154	0.003	57.176	< 0.001 ***
	(38-63micron)	0.051	0.003	18.900	< 0.001 ***
	(63-90micron)	0.101	0.003	37.473	< 0.001 ***
	without grinding (0.5x0.5cm)	0.275	0.003	102.105	< 0.001 ***
(125-150micron)	(38-63micron)	-0.103	0.003	-38.276	< 0.001 ***
	(63-90micron)	-0.053	0.003	-19.703	< 0.001 ***
	without grinding (0.5x0.5cm)	0.121	0.003	44.928	< 0.001 ***
(38-63micron)	(63-90micron)	0.050	0.003	18.573	< 0.001 ***
	without grinding (0.5x0.5cm)	0.224	0.003	83.204	< 0.001 ***
(63-90micron)	without grinding (0.5x0.5cm)	0.174	0.003	64.631	< 0.001 ***

*** p < 0.001

Note. P-value adjusted for comparing a family of 5

Note. Results are averaged over the levels of: CPH layer, Heating method

Post Hoc Comparisons - CPH layer

		Mean Difference	SE	t	p_{tukey}
Endocarp	Epicarp	-0.117	0.002	-56.134	< 0.001 ***
	Mesocarp	-0.123	0.002	-59.045	< 0.001 ***
Epicarp	Mesocarp	-0.006	0.002	-2.912	0.011 *

* p < 0.05, ** p < 0.01, *** p < 0.001

Note. P-value adjusted for comparing a family of 3

Note. Results are averaged over the levels of: CPH Particle size, Heating method

Post Hoc Comparisons - Heating method

		Mean Difference	SE	t	p_{tukey}
CSE	MAE	-0.030	0.002	-17.358	< 0.001 ***

*** $p < 0.001$

Note. Results are averaged over the levels of: CPH Particle size, CPH layer

F.10.3 Antioxidant Activity (AOA)

ANOVA - Antioxidant Activity

Cases	Sum of Squares	df	Mean Square	F	p	η^2
CPH Particle size	6.150	4	1.538	177.012	< 0.001***	0.044
CPH layer	71.499	2	35.749	4115.803	< 0.001***	0.517
Heating method	0.059	1	0.059	6.742	0.010*	4.236×10^{-4}
CPH Particle size ✻ CPH layer	55.847	8	6.981	803.709	< 0.001***	0.404
CPH Particle size ✻ Heating method	0.474	4	0.119	13.645	< 0.001***	0.003
CPH layer ✻ Heating method	2.010	2	1.005	115.728	< 0.001***	0.015
CPH Particle size ✻ CPH layer ✻ Heating method	0.104	8	0.013	1.498	0.159	7.529×10^{-4}
Residuals	2.085	240	0.009			

Note. Type III Sum of Squares

Post Hoc Tests

Post Hoc Comparisons - CPH Particle size

		Mean Difference	SE	t	p_{tukey}
≤ 38micron	(125-150micron)	-0.439	0.018	-24.488	< 0.001***
	(38-63micron)	-0.131	0.018	-7.295	< 0.001***
	(63-90micron)	-0.240	0.018	-13.372	< 0.001***
	without grinding (0.5x0.5cm)	-0.089	0.018	-4.958	< 0.001***
(125-150micron)	(38-63micron)	0.308	0.018	17.194	< 0.001***
	(63-90micron)	0.199	0.018	11.116	< 0.001***
	without grinding (0.5x0.5cm)	0.350	0.018	19.531	< 0.001***
(38-63micron)	(63-90micron)	-0.109	0.018	-6.077	< 0.001***
	without grinding (0.5x0.5cm)	0.042	0.018	2.337	0.137
(63-90micron)	without grinding (0.5x0.5cm)	0.151	0.018	8.415	< 0.001***

* $p < 0.05$, *** $p < 0.001$

Note. P-value adjusted for comparing a family of 5

Note. Results are averaged over the levels of: CPH layer, Heating method

Post Hoc Comparisons - CPH layer

		Mean Difference	SE	t	p_{tukey}
Endocarp	Epicarp	0.911	0.014	65.589	< 0.001***
	Mesocarp	1.210	0.014	87.083	< 0.001***
Epicarp	Mesocarp	0.299	0.014	21.494	< 0.001***

*** $p < 0.001$

Note. P-value adjusted for comparing a family of 3

Note. Results are averaged over the levels of: CPH Particle size, Heating method

Post Hoc Comparisons - Heating method

		Mean Difference	SE	t	p_{tukey}
CSE	MAE	0.029	0.011	2.597	0.010 **

** p < 0.01

Note. Results are averaged over the levels of: CPH Particle size, CPH layer

Post Hoc Comparisons - CPH layer ✻ Heating method

		Mean Difference	SE	t	p_{tukey}
Endocarp CSE	Epicarp CSE	0.991	0.020	50.454	< 0.001 ***
	Mesocarp CSE	1.080	0.020	54.993	< 0.001 ***
	Endocarp MAE	-0.003	0.020	-0.174	1.000
	Epicarp MAE	0.828	0.020	42.129	< 0.001 ***
	Mesocarp MAE	1.336	0.020	67.988	< 0.001 ***
Epicarp CSE	Mesocarp CSE	0.089	0.020	4.539	< 0.001 ***
	Endocarp MAE	-0.995	0.020	-50.627	< 0.001 ***
	Epicarp MAE	-0.164	0.020	-8.324	< 0.001 ***
	Mesocarp MAE	0.345	0.020	17.534	< 0.001 ***
Mesocarp CSE	Endocarp MAE	-1.084	0.020	-55.166	< 0.001 ***
	Epicarp MAE	-0.253	0.020	-12.863	< 0.001 ***
	Mesocarp MAE	0.255	0.020	12.995	< 0.001 ***
Endocarp MAE	Epicarp MAE	0.831	0.020	42.303	< 0.001 ***
	Mesocarp MAE	1.339	0.020	68.162	< 0.001 ***
Epicarp MAE	Mesocarp MAE	0.508	0.020	25.858	< 0.001 ***

** p < 0.01, *** p < 0.001

Note. P-value adjusted for comparing a family of 6

Note. Results are averaged over the levels of: CPH Particle size

F.10.4 Correlation between TPC, TMA, and AOA on CPH layer (epicarp, mesocarp, endocarp) extracts

Pearson's Correlations

Variable		Total Phenolic Content	Total Monomeric Anthocyanin	Antioxidant Activity
1. Total Phenolic Content	Pearson's r	—		
	p-value	—		
2. Total Monomeric Anthocyanin	Pearson's r	0.747***	—	
	p-value	< 0.001	—	
3. Antioxidant Activity	Pearson's r	-0.415***	-0.504***	—
	p-value	< 0.001	< 0.001	—

* p < 0.05, ** p < 0.01, *** p < 0.001

Appendix F.11 Test of Significance the effect of CPH particle size and heating method (MAE and CSE) on Proximate Contents (TGA analysis)

F.11.1 Moisture content

ANOVA - %Moisture Content

Cases	Sum of Squares	df	Mean Square	F	p	η^2
CPH Particle Size	1.937	3	0.646	1.533	0.257	0.066
Treatment	20.996	2	10.498	24.919	< 0.001***	0.716
CPH Particle Size ✻ Treatment	1.315	6	0.219	0.520	0.783	0.045
Residuals	5.055	12	0.421			

Note. Type III Sum of Squares

Post Hoc Tests

Post Hoc Comparisons - Treatment

		Mean Difference	SE	t	p_{tukey}
CSE	MAE	-0.860	0.325	-2.649	0.052
	Untreated	1.409	0.325	4.342	0.003**
MAE	Untreated	2.269	0.325	6.992	< 0.001***

* p < 0.05, ** p < 0.01, *** p < 0.001

Note. P-value adjusted for comparing a family of 3

Note. Results are averaged over the levels of: CPH Particle Size

F.11.2 Volatile Matter content

ANOVA - %Volatile Matter

Cases	Sum of Squares	df	Mean Square	F	P	η^2
CPH Particle Size	16.116	3	5.372	12.274	< 0.001***	0.427
Treatment	3.857	2	1.928	4.406	0.037*	0.102
CPH Particle Size ✻ Treatment	12.500	6	2.083	4.760	0.010*	0.331
Residuals	5.252	12	0.438			

Note. Type III Sum of Squares

Post Hoc Tests

Post Hoc Comparisons - CPH Particle Size

		Mean Difference	SE	t	p_{tukey}
≤ 38 micron	(125-150 micron)	1.412	0≤ 382	3.698	0.014*
	(38-63 micron)	-0.111	0≤ 382	-0.290	0.991
	(63-90 micron)	1.722	0≤ 382	4.508	0.003**
(125-150 micron)	(38-63 micron)	-1.523	0≤ 382	-3.987	0.008**
	(63-90 micron)	0.309	0≤ 382	0.810	0.849
(38-63 micron)	(63-90 micron)	1.832	0≤ 382	4.797	0.002**

* p < 0.05, ** p < 0.01

Note. P-value adjusted for comparing a family of 4

Note. Results are averaged over the levels of: Treatment

Post Hoc Comparisons - Treatment

		Mean Difference	SE	t	p_{tukey}
CSE	MAE	-0.966	0.331	-2.919	0.032 *
	Untreated	-0.637	0.331	-1.925	0.174
MAE	Untreated	0.329	0.331	0.994	0.594

* $p < 0.05$

Note. P-value adjusted for comparing a family of 3

Note. Results are averaged over the levels of: CPH Particle Size

F.11.3 Fixed Carbon content

ANOVA - %Fixed Carbon

Cases	Sum of Squares	df	Mean Square	F	p	η^2
CPH Particle Size	23.361	3	7.787	24.393	< 0.001***	0.518
Treatment	9.675	2	4.838	15.154	< 0.001***	0.215
CPH Particle Size ✻ Treatment	8.217	6	1.369	4.290	0.015*	0.182
Residuals	3.831	12	0.319			

Note. Type III Sum of Squares

Post Hoc Tests

Post Hoc Comparisons - CPH Particle Size

		Mean Difference	SE	t	p_{tukey}
≤ 38 micron	(125-150 micron)	-2.435	0.326	-7.465	< 0.001***
	(38-63 micron)	-0.701	0.326	-2.149	0.193
	(63-90 micron)	-2.046	0.326	-6.271	< 0.001***
(125-150 micron)	(38-63 micron)	1.734	0.326	5.317	< 0.001***
	(63-90 micron)	0.390	0.326	1.195	0.641
(38-63 micron)	(63-90 micron)	-1.345	0.326	-4.122	0.007**

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Note. P-value adjusted for comparing a family of 4

Note. Results are averaged over the levels of: Treatment

Post Hoc Comparisons - Treatment

		Mean Difference	SE	t	p_{tukey}
CSE	MAE	1.336	0.283	4.729	0.001**
	Untreated	1.358	0.283	4.806	0.001**
MAE	Untreated	0.022	0.283	0.077	0.997

** p < 0.01

Note. P-value adjusted for comparing a family of 3

Note. Results are averaged over the levels of: CPH Particle Size

F.11.4 Ash content

ANOVA - %Ash

Cases	Sum of Squares	df	Mean Square	F	p	η^2
CPH Particle Size	1.364	3	0.455	0.739	0.549	0.032
Treatment	31.038	2	15.519	25.215	< 0.001***	0.726
CPH Particle Size ✻ Treatment	2.944	6	0.491	0.797	0.590	0.069
Residuals	7.386	12	0.615			

Note. Type III Sum of Squares

Post Hoc Tests

Post Hoc Comparisons - Treatment

		Mean Difference	SE	t	p_{tukey}
CSE	MAE	0.489	0.392	1.247	0.450
	Untreated	-2.130	0.392	-5.431	< 0.001***
MAE	Untreated	-2.620	0.392	-6.678	< 0.001***

*** p < 0.001

Note. P-value adjusted for comparing a family of 3

Note. Results are averaged over the levels of: CPH Particle Size

Appendix F.12 Test of Significance the effect of CPH particle size and heating method (MAE and CSE) on BET analysis

F.12.1 BET Surface Area

ANOVA - BET Surface Area

Cases	Sum of Squares	df	Mean Square	F	p	η^2
CPH Particle size	3.646	3	1.215	131.290	< 0.001***	0.602
Heating method	2.036	2	1.018	109.957	< 0.001***	0.336
CPH Particle size ✻ Heating method	0.262	6	0.044	4.724	0.011*	0.043
Residuals	0.111	12	0.009			

Note. Type III Sum of Squares

Post Hoc Tests

Post Hoc Comparisons - CPH Particle size

		Mean Difference	SE	t	p_{tukey}	
≤ 38micron	(125-150micron)	0.970	0.056	17.465	< 0.001	***
	(38-63micron)	0.620	0.056	11.153	< 0.001	***
	(63-90micron)	0.938	0.056	16.890	< 0.001	***
(125-150 micron)	(38-63micron)	-0.351	0.056	-6.311	< 0.001	***
	(63-90micron)	-0.032	0.056	-0.575	0.938	
(38-63 micron)	(63-90micron)	0.319	0.056	5.737	< 0.001	***

*** p < 0.001

Note. P-value adjusted for comparing a family of 4

Note. Results are averaged over the levels of: Heating method

Post Hoc Comparisons - Heating method

		Mean Difference	SE	t	p_{tukey}	
CSE	MAE	-0.133	0.048	-2.755	0.043	*
	Untreated	-0.673	0.048	-13.997	< 0.001	***
MAE	Untreated	-0.541	0.048	-11.242	< 0.001	***

* p < 0.05, *** p < 0.001

Note. P-value adjusted for comparing a family of 3

Note. Results are averaged over the levels of: CPH Particle size

F.12.2 Micropore Volume

ANOVA - Micropore Volume

Cases	Sum of Squares	df	Mean Square	F	p	η²
CPH Particle size	0.279	3	0.093	266.646	< 0.001***	0.604
Heating method	0.154	2	0.077	220.721	< 0.001***	0.333
CPH Particle size ✻ Heating method	0.025	6	0.004	11.906	< 0.001***	0.054
Residuals	0.004	12	3.486×10^{-4}			

Note. Type III Sum of Squares

Post Hoc Tests

Post Hoc Comparisons - CPH Particle size

		Mean Difference	SE	t	p_{tukey}
≤ 38micron	(125-150micron)	0.264	0.011	24.476	< 0.001 ***
	(38-63micron)	0.180	0.011	16.668	< 0.001 ***
	(63-90micron)	0.264	0.011	24.507	< 0.001 ***
(125-150micron)	(38-63micron)	-0.084	0.011	-7.808	< 0.001 ***
	(63-90micron)	3.333×10^{-4}	0.011	0.031	1.000
(38-63micron)	(63-90micron)	0.085	0.011	7.839	< 0.001 ***

*** p < 0.001

Note. P-value adjusted for comparing a family of 4

Note. Results are averaged over the levels of: Heating method

Post Hoc Comparisons - Heating method

		Mean Difference	SE	t	p_{tukey}
CSE	MAE	-0.034	0.009	-3.669	0.008 **
	Untreated	-0.184	0.009	-19.751	< 0.001 ***
MAE	Untreated	-0.150	0.009	-16.082	< 0.001 ***

* p < 0.05, ** p < 0.01, *** p < 0.001

Note. P-value adjusted for comparing a family of 3

Note. Results are averaged over the levels of: CPH Particle size

F.12.3 Mesopore Volume

ANOVA - Mesopore Volume

Cases	Sum of Squares	df	Mean Square	F	p	η^2
CPH Particle size	5.737	3	1.912	24.043	< 0.001***	0.392
Heating method	6.736	2	3.368	42.349	< 0.001***	0.460
CPH Particle size ✻ Heating method	1.202	6	0.200	2.518	0.082	0.082
Residuals	0.954	12	0.080			

Note. Type III Sum of Squares

Post Hoc Tests

Post Hoc Comparisons - CPH Particle size

		Mean Difference	SE	t	p_{tukey}
≤ 38micron	(125-150micron)	1.241	0.163	7.620	< 0.001 ***
	(38-63micron)	0.845	0.163	5.191	0.001 **
	(63-90micron)	1.147	0.163	7.042	< 0.001 ***
(125-150micron)	(38-63micron)	-0.395	0.163	-2.429	0.124
	(63-90micron)	-0.094	0.163	-0.577	0.937
(38-63micron)	(63-90micron)	0.302	0.163	1.852	0.298

** p < 0.01, *** p < 0.001

Note. P-value adjusted for comparing a family of 4

Note. Results are averaged over the levels of: Heating method

Post Hoc Comparisons - Heating method

		Mean Difference	SE	t	p_{tukey}
CSE	MAE	-0.291	0.141	-2.064	0.140
	Untreated	-1.241	0.141	-8.799	< 0.001 ***
MAE	Untreated	-0.950	0.141	-6.735	< 0.001 ***

*** p < 0.001

Note. P-value adjusted for comparing a family of 3

Note. Results are averaged over the levels of: CPH Particle size

F.12.4 Total Pore Volume

ANOVA - Total pore volume

Cases	Sum of Squares	df	Mean Square	F	p	η^2
CPH Particle size	41.389	3	13.796	12.728	< 0.001***	0.285
Heating method	83.997	2	41.998	38.747	< 0.001***	0.577
CPH Particle size ✻ Heating method	7.084	6	1.181	1.089	0.422	0.049
Residuals	13.007	12	1.084			

Note. Type III Sum of Squares

Post Hoc Tests

Post Hoc Comparisons - CPH Particle size

		Mean Difference	SE	t	p_{tukey}
≤ 38micron	(125-150micron)	3.278	0.601	5.454	< 0.001***
	(38-63micron)	2.014	0.601	3.351	0.026*
	(63-90micron)	3.145	0.601	5.232	0.001**
(125-150micron)	(38-63micron)	-1.264	0.601	-2.103	0.207
	(63-90micron)	-0.133	0.601	-0.222	0.996
(38-63micron)	(63-90micron)	1.131	0.601	1.882	0.286

Note. Results are averaged over the levels of: Heating method

Note. P-value adjusted for comparing a family of 4

* p < 0.05, ** p < 0.01, *** p < 0.001

Post Hoc Comparisons - Heating method

		Mean Difference	SE	t	p_{tukey}
CSE	MAE	-0.988	0.521	-1.898	0.182
	Untreated	-4.369	0.521	-8.393	< 0.001***
MAE	Untreated	-3.381	0.521	-6.495	< 0.001***

*** p < 0.001

Note. P-value adjusted for comparing a family of 3

Note. Results are averaged over the levels of: CPH Particle size

F.12.5 Average pore diameter

ANOVA - Average pore diameter

Cases	Sum of Squares	df	Mean Square	F	p	η^2
CPH Particle size	118.404	3	39.468	4.724	0.021*	0.404
Heating method	27.660	2	13.830	1.655	0.232	0.094
CPH Particle size ✻ Heating method	46.475	6	7.746	0.927	0.509	0.159
Residuals	100.249	12	8.354			

Note. Type III Sum of Squares

Post Hoc Tests

Post Hoc Comparisons - CPH Particle size

		Mean Difference	SE	t	p_{tukey}
≤ 38micron	(125-150micron)	-5.507	1.669	-3.300	0.028 *
	(38-63micron)	-1.434	1.669	-0.859	0.825
	(63-90micron)	-4.445	1.669	-2.664	0.084
(125-150micron)	(38-63micron)	4.073	1.669	2.441	0.122
	(63-90micron)	1.062	1.669	0.637	0.918
(38-63micron)	(63-90micron)	-3.011	1.669	-1.804	0.318

* $p < 0.05$

Note. P-value adjusted for comparing a family of 4

Note. Results are averaged over the levels of: Heating method