OPTIMIZATION OF MICROWAVE-ASSISTED EXTRACTION OF BIOACTIVE COMPOUNDS FROM DIFFERENT VARIETIES OF COFFEE PULP

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MASTER OF SCIENCE
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Mengistu Fiseha Mekonnen

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ABSTRACT

Coffee, one of the most widely consumed beverages globally, generates large amounts of coffee pulp waste during processing. This Pulp, rich in bioactive compounds such as polyphenols, flavonoids, and sugars, presents an opportunity for valorization. This study aimed to optimize Microwave-Assisted Extraction (MAE) conditions for bioactive compounds from Geisha and Bourbon coffee pulp varieties using Response Surface Methodology (RSM). Microwave power (500–900 Watt), extraction time (45–80 min), and sample-to-solvent ratios (1:1 to 1:10 g/mL) were varied to evaluate their effects on extraction yield, total phenolic content (TPC), antioxidant activity (DPPH assay), and sugar content (fructose and galactose). The optimized conditions for microwave-assisted extraction (MAE) were 708.7 W for 72.3 min for the Geisha variety and 699.3 W for 71.05 min for the Bourbon variety, both utilizing a solvent-to-sample ratio of 10:1 (mL/g). Under these conditions, Geisha yielded 12.66%, with a total phenolic content (TPC) of 25.36 mg GAE/g, DPPH radical scavenging activity of 6.43 mg TE/g, fructose content of 6.65%, and galactose content of 1.46%. Bourbon yielded 10.74%, with a TPC of 23.57 mg GAE/g, DPPH activity of 6.18 mg TE/g, fructose content of 7.02%, and galactose content of 1.69%. These optimized MAE conditions for each variety were applied prior to comprehensive chemical characterization. Antioxidant activities, including ABTS and FRAP assays, as well as TPC and DPPH values, were subsequently evaluated and compared to results obtained using conventional hot water extraction methods. MAE significantly enhanced antioxidant compound recovery from coffee pulp compared to conventional extraction. TPC increased from 21.3 mg GAE/g to

25.36 mg GAE/g in Geisha and from 22.5 mg GAE/g to 24.50 mg GAE/g in Bourbon. DPPH rose from 3.16 mg TE/g to 7.51 mg TE/g in Geisha and from 3.61 mg TE/g to 7.56 mg TE/g in Bourbon. ABTS values improved from 21.25 mg TE/g to 33.78 mg TE/g in Geisha and from 20.89 mg TE/g to 32.43 mg TE/g in Bourbon. FRAP values increased from 2.44 mg TE/g to 4.56 mg TE/g in Geisha and from 2.32 mg TE/g to 4.12 mg TE/g in Bourbon. Mass spectrometry revealed a richer phenolic profile in MAE extracts, including flavonoids such as hibiscetin and alkaloids such as caffeine and trigonelline. Enzyme inhibition assays showed strong α-amylase (90%) and α-glucosidase (IC_{50} = 2.8 mg/mL) inhibition in Geisha MAE extracts. Overall, MAE proved to be a rapid, eco-friendly method for extracting valuable bioactive from coffee pulp, with promising applications in functional foods, nutraceuticals, and sustainable waste utilization.

Keywords: Coffee Pulp, Microwave-Assisted Extraction, Bioactive Compounds, Antioxidant Activity, Functional Foods

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ABBREVIATIONS AND SYMBOLS

ABTS	2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)
ANOVA	Analysis of Variance
BBD	BoxBehnken Design
DNS	Dinitrosalicylic Acid
DPPH	2,2-Diphenyl-1-picrylhydrazyl
FRAP	Ferric Reducing Antioxidant Power
GC	Gas Chromatography
HPLC	High Performance Liquid Chromatography
MAE	Microwave-Assisted Extraction
NADES	Natural Deep Eutectic Solvent
PLE	Pressurized Liquid Extraction
RSM	Response Surface Methodology
SFE	Supercritical Fluid Extraction
TEAC	Trolox Equivalent Antioxidant Capacity
TF	Total Flavonoid Content
TPC	Total Phenolic Content
UAE	Ultrasound-Assisted Extraction

CHAPTER 1

INTRODUCTION

1.1 Introduction to the Research Problem and Its Significance

Coffee (Coffea spp.) is one of the most important agricultural commodities globally, ranking second only to petroleum in terms of trade value. It is cultivated in over 70 countries and plays a vital role in the livelihoods of millions of people. Among the three primary coffee species— Arabica, Robusta, and Liberica—Arabica dominates global production due to its superior flavor and adaptability to high-altitude, cool climates. Robusta is favored for instant coffee because of its high soluble solids, while Liberica, although less common, thrives in hot, wet environments.

Coffee beans contain over 1,500 chemical constituents, including essential oils, carbohydrates, and various bioactive compounds. During coffee processing—particularly through wet and dry methods—a significant byproduct known as coffee pulp is generated. It accounts for nearly 29% of the dry weight of coffee cherries, with an estimated global output of 1.5 to 2 million metric tons annually. In Thailand, the leading coffee producer in Southeast Asia, production reached over 98,000 metric tons in 2019 (FAO, 2019). Robusta varieties dominate cultivation in the south (75%), while Arabica is mostly grown in northern regions. Despite the large volume of pulp produced, this byproduct remains largely underutilized—often left on farms or used as low-grade compost (Ketelaar & Leemans, 2019).

Recent studies have spotlighted coffee pulp for its rich composition of polyphenols, flavonoids, caffeine, chlorogenic acids, trigonelline, and dietary fiber—all of which contribute to its potential antioxidant, anti- inflammatory, antimicrobial, and prebiotic properties. For instance, chlorogenic acids aid glucose metabolism and reduce oxidative stress, while flavonoids such as quercetin and rutin offer cardiovascular benefits (González-Molina et al., 2018; Mussatto et al., 2011). These bioactives are typically identified using HPLC, LC-MS, GC, and FTIR, with

applications spanning food, nutraceuticals, and pharmaceuticals (Chemat et al., 2017).

Conventional extraction methods like Soxhlet extraction and maceration are often time-consuming and solvent-intensive. In contrast, Microwave-Assisted Extraction (MAE) is an efficient, eco-friendly method that reduces extraction time and solvent use while preserving heat-sensitive compounds (Routray & Orsat, 2012). Water, a non-toxic and sustainable solvent, is especially suitable in MAE for extracting hydrophilic compounds like polyphenols.

Several parameters influence MAE effectiveness, including microwave power, extraction time, and solvent-to-sample ratio. Studies show optimal results at 500–700 watts (González-González et al., 2022). Exceeding this range can cause localized overheating, degrading thermolabile bioactives (Wang & Weller, 2006). An extraction time of ~70 minutes and a 20:1 mL/g solvent-to-sample ratio have yielded high polyphenol recovery in previous studies (Tran et al., 2020). These findings underscore the need for careful optimization of MAE conditions to ensure maximum yield without compromising compound integrity.

Tran et al. (2020)'s MAE method (700 W, 70 min, 20:1, using 50% ethanol) can be further improved by switching to pure water, employing Response Surface Methodology (RSM) for multivariate optimization, and testing pulsed or shorter microwave cycles to enhance sustainability. Coupling total phenolic quantification with HPLC-MS/MS profiling of individual phenolics would better characterize extract composition. Future studies should also explore enzyme inhibition, anti-inflammatory activity, prebiotic effects, and perform shelf-life and cost–benefit analyses to support industrial application.

The chemical composition of coffee pulp varies with genotype and environmental conditions, especially among Arabica varieties like Bourbon and Geisha. While studies show compositional variability among these types, not all differences translate into variations in functional bioactivity (Menezes et al., 2020; Zanin et al., 2021). For example, Geisha pulp tends to contain higher caffeine levels, offering stronger antimicrobial effects, whereas Bourbon may differ in phenolic profile.

Coffee pulp contains minerals (9%), proteins (5–15%), lipids (2–7%), and carbohydrates (21–32%) (Crush, 2014; Venkatachalapathy, 2016). Other constituents include tannins, chlorogenic acid, and caffeine, with potential uses in citric acid production, natural fragrances, and as functional food ingredients (Aline et al., 2022). Its health-promoting potential is supported by preclinical studies. For instance, coffee pulp extract has shown benefits in reducing body weight, improving glucose tolerance, and enhancing cardiovascular and hepatic health in rats fed high-fat diets (Nemzer et al., 2021). Furthermore, its antioxidant activity helps combat oxidative stress, while anti-inflammatory effects may ease chronic inflammatory diseases. Preliminary findings also suggest anti-diabetic and even anti-cancer effects, likely due to its high antioxidant content (Ballesteros et al., 2019).

This study aims to optimize the MAE process for extracting bioactive compounds from Geisha and Bourbon coffee pulp, utilizing green solvents like water. Through a Box-Behnken experimental design, the study will systematically evaluate the effects of microwave power, time, and solvent ratio. Beyond total phenolic content, the research will characterize individual compounds, test biological activity, and assess prebiotic and toxicity profiles to support potential food or pharmaceutical applications. By improving extraction efficiency, reducing solvent and energy usage, and exploring new applications for coffee pulp, this study supports a sustainable, value-added approach to agricultural waste. In doing so, it contributes to circular bioeconomy goals, enhances resource utilization, and promotes the health potential of coffee-derived pulp.

1.2 Objectives

1.2.1 To optimize the extraction conditions for bioactive compounds from Bourbon and Geisha coffee variety pulp using microwave-assisted extraction

1.2.2 To characterize the composition and assess the bioactivity properties of the bioactive compounds extracted from coffee pulp.

1.3 Scope of the Study

This study focuses on optimizing Microwave-Assisted Extraction (MAE) for recovering bioactive compounds from Geisha and Bourbon coffee pulp varieties. It investigates the effects of microwave power, extraction time, and solvent-to-sample ratio on extraction yield, total phenolic content (TPC), antioxidant activity, and sugar content. The optimized extracts are further analyzed for antioxidant capacity using DPPH, ABTS, and FRAP assays, chemical composition via mass spectrometry, and enzyme inhibition potential. The scope includes a comparison with conventional extraction methods to highlight the advantages of MAE in enhancing the recovery of functional compounds from coffee pulp for potential use in food and nutraceutical applications.

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CHAPTER 2

LITRATURE REVIEW

2.1 Coffee Pulps

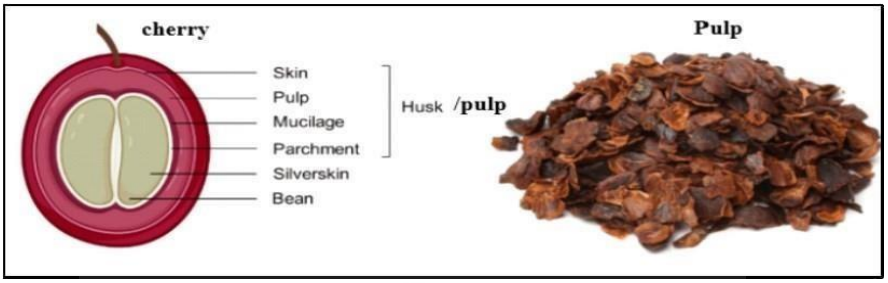
Coffee pulp, a byproduct of coffee processing, consists of the outer skin, mucilage, and parchment of the coffee bean (Figure 2.1). This pulp is not merely waste but a reservoir of valuable compounds such as phenolic compounds, chlorogenic acids, carbohydrates, lipids, and proteins. These components have been linked to health-promoting effects, making coffee pulp a significant resource in various industries (Gemechu, 2020). Due to its potential health benefits and versatile applications across various industries, it holds significant value as a resource with health-promoting properties. Significant amounts of coffee pulp, which is a byproduct of coffee processing, are produced within the coffee industry (Bondam et al., 2022).

The potential of coffee pulp in nutraceuticals is an area of growing interest. According to Ramalakshmi et al. (2009) in nutritional and functional significance of coffee pulp, the bioactive compounds extracted from coffee pulp can be used in the formulation of nutraceutical products due to their potential health benefits, such as anti-inflammatory and anti-carcinogenic effects. The study by Iriondo-DeHond et al. (2017) also supports the concept of utilizing coffee pulp in health-focused products, particularly highlighting the role of Maillard reaction products formed during processing. The antioxidant properties primarily antioxidants like phenolic compounds and chlorogenic acids, exhibits substantial antioxidant activity. This activity is crucial in mitigating oxidative stress and reducing inflammation in the human body (Akbari et al., 2022; Magoni et al., 2018). Extracts from coffee pulp can significantly reduce inflammation in both cellular and animal models.

Additionally, these extracts have demonstrated potential as antibacterial agents, effectively inhibiting the growth of various bacteria and fungi, which could revolutionize pharmaceutical applications and food preservation (Bouarab Chibane et al., 2018).

Economically, the valorization of coffee pulp presents vast opportunities, especially for coffee-producing countries. As Murthy and Madhava Naidu (2012) suggest, this valorization could improve the income of coffee farmers and reduce the environmental footprint of coffee production. Bitzer et al. (2012) observe a shift in the perception of waste, now viewed as a valuable resource. This change could lead to innovative economic models in the coffee industry. Bondam et al. (2022) highlight the potential applications of coffee pulp extracts in pharmaceuticals, suggesting their use in drug delivery systems and as excipients in drug formulations.

According to Murthy and Madhava Naidu (2012), the exploration of the potential value inherent in coffee pulp has advantageous economic prospects for countries involved in coffee production. According to Bitzer et al. (2012), there is a paradigm shift occurring in the perception of waste, wherein it is now being recognized as a valuable resource. This shift has the potential to increase the income of coffee farmers and also contribute to a reduction in the environmental impact of their operations. The study conducted by Bondam et al. (2022) reveals the promising prospects of utilizing coffee pulp extracts in the pharmaceutical sector. These extracts have the potential to be employed in drug delivery systems and can also function as excipients in pharmaceutical formulations. This presents novel and new applications for the bioactive compounds found in coffee pulp. The utilization of coffee pulp holds significant promise, yet there are substantial practical obstacles that need to be addressed. These concerns are with the extraction techniques employed, the establishment of consistent bioactive content, and the feasibility of large-scale manufacturing (Espro et al., 2021). The resolution of these difficulties is of utmost importance to fully use the inherent potential of coffee pulp in many sectors.



Source Rebollo-Hernanz et al. (2021)

Figure 2.1 Coffee cherry, from the outer layers

2.2 Chemical Composition of Coffee Pulp

Different varieties of coffee have different chemical compositions. The main chemical components of coffee include alkaloids, phenolic acids, flavonoids, terpenoids, sterols, and volatile components. (Saud & Salamatullah, 2021) The chemical complexity of coffee is emerging, especially due to observed physiological effects that cannot be related only to the presence of caffeine. Moreover, coffee contains an exceptionally substantial number of antioxidants, such as chlorogenic acids, hydroxycinnamic acids, caffeine, and Maillard reaction products such as melanoidins. (Perrone et al., 2012) The official chemical makeup of coffee is 2-3% caffeine, 13% protein, 3-5% tannin, and 10-15% fixed fat (Farah, 2019).

Different varieties of coffee, such as Catura, Catimor, Geisha, and Bourbon, may have different chemical compositions, but the exact differences are not specified in the research results. Phenolic compounds are prominent in coffee pulp, contributing to its antioxidant properties. Chlorogenic acids, in particular, are abundant and have been associated with various health benefits (Iriondo- DeHond et al., 2019). The presence of phenolic compounds makes coffee pulp an attractive source for natural antioxidants with potential applications in the food, pharmaceutical industries. Coffee pulp is a significant source of carbohydrates, primarily in the form of sugars, including glucose, fructose, and sucrose (Shen et al., 2022). These carbohydrates not only influence the taste and texture of coffee pulp but also have potential

as a renewable resource for bioethanol production and the development of sustainable materials (Blinová et al., 2017). Proteins in coffee pulp consist of various amino acids and peptides. These proteins, due to their antioxidant and antibacterial properties, have garnered interest for potential applications in the food industry (Zaky et al., 2020). They can also be used to enhance animal feed and as a source of sustenance for animals.

Coffee pulp contains a variety of organic acids, including citric acid, malic acid, and tartaric acid, which contribute to its characteristic acidic flavor (Osorio et al., 2023). These organic acids are valuable in the culinary world, finding use in the creation of sauces and beverages. Polyphenols are another class of bioactive compounds present in coffee pulp. These compounds exhibit antioxidant properties and are associated with potential health benefits, including anti-inflammatory and anti-cancer effects (Santos et al., 2021). The concentration of polyphenols may vary based on coffee pulp composition and processing methods.

2.2.1 Coffee Cherry Pulp Composition Across Different Countries

The coffee pulp and husk are the first by-products of wet and dry processing methods to extract the bean or seed in the coffee processing industry, accounting for about 29% and 12% of the overall coffee cherry by dry weight, respectively. According to Klingel et al. (2020), between 0.5 and 0.18 tons of coffee pulp and husk are produced from a single ton of fresh coffee cherries, and carbohydrates, minerals, and proteins, as well as organic compounds such as tannins, chlorogenic acid, caffeine, and other bioactive compounds, are contained therein (Janissen & Huynh, 2018). The composition of coffee pulp is presented in terms of protein, carbohydrate, phenolic compounds (tannin), and bioactive compounds (caffeine and chlorogenic acid). The coffee cherry by- products from Colombia had a high amount of fat, while the Mexican coffee cherry by-products had measured high amounts in terms of minerals and dietary fiber (Table 2.1).

Table 2.1 Chemical Composition of Bioactive Compound from Different Countries

Component	Content in Coffee Pulp
Ash (Total minerals)	10% on average. Examples: 10.7% in Colombian arabica pulp (Machado et al., 2023); ~10.6% in Azores (Portugal) pulp (Costa et al., 2025). Some regions report higher values (e.g. 16.7% in Thai coffee pulp) (Machado et al., 2023), likely due to soil differences.
Protein	Typically, 8–12%. For instance, 10.2% in Colombian pulp (Machado et al., 2023). An Azores study found ~6% total amino acids (Costa et al., 2025) (slightly lower, possibly due to analysis method), while literature reports a range of 4–12% (Hu et al., 2023)
Fat (Lipids)	Low, usually around 1–3%. e.g. 1.7% in coffee pulp flour (Machado et al., 2023); and 1.6% in Azores pulp (Costa et al., 2025). Surveys indicate 1–2% as a typical range (Hu et al., 2023)
Carbohydrates (Available)	Roughly 30–35% available carbohydrates (sugars and other non-fiber carbs). Colombian pulp contained 31.2% non-fiber carbs (Machado et al., 2023); If dietary fiber is included, total carbohydrate content can be 50% (Costa et al., 2025). Reducing sugars around 12% and nonreducing 2% have been noted in dry pulp (Murthy & Naidu, 2012)
Dietary Fiber	Very high – often 20–50% of dry pulp. Recent analyses show 46% total dietary fiber in Colombian pulp (Machado et al., 2023) and ~52% in Azores pulp (Costa et al., 2025). This highlights coffee pulp as an excellent source of fiber.
Total Phenolics	On the order of a few mg GAE/g. Values vary by variety and processing: roughly 4–9 mg gallic acid equivalents per g dry pulp have been reported (Ballesteros et al., 2019). These phenolic compounds contribute significantly to antioxidant activity.

Table 2.1 (continued)

Component	Content in Coffee Pulp
Flavonoids	Typically, lower than total phenolics. 7–8 mg catechin equivalents per g in pulp extracts under certain conditions (Hu et al., 2023). (Flavonoid content can vary with processing, and some studies report lower values.)
Chlorogenic Acid	0.3–0.9% of dry pulp (major phenolic acid in coffee pulp). Fresh wet-processed Arabica pulp showed ~2.6 mg/g (0.26%) chlorogenic acid (Murthy & Naidu, 2018), which increased to 5.4 mg/g after ensiling (Murthy & Naidu, 2018). An Azores study found 9.2 mg/g (0.92%) in their pulp sample (Costa et al., 2025). These chlorogenic acids (3-,4-,5-caffeoylquinic acids) are key antioxidants.
Tannins	A subset of phenolics (polyphenols). Fresh coffee pulp has around 3–4 mg/g of tannins (measured as tannic/catechin equivalents) (Murthy & Naidu, 2018) However, older analyses that include bound tannins indicate a wider range up to ~1.8–8.6% of pulp weight (Murthy & Naidu, 2012)
Caffeine	Typically, about 1% of dry pulp (as caffeine is a major methylxanthine in coffee). Measured values range from ~0.98% (Costa et al., 2025). up to ~1.86% (Murthy & Naidu, 2018)) depending on variety and processing. For instance, fresh Arabica pulp in Mexico contained 18.6 mg/g (1.86%) caffeine (Murthy & Naidu, 2018), while Azores Arabica pulp had ~9.8 mg/g (0.98%) (Costa et al., 2025). Robusta pulp generally would be on the higher end due to higher caffeine content.
Minerals (elements)	High mineral content (part of ash). Coffee pulp is notably rich in potassium – about 20–25 mg/g K in dried pulp (~2–2.5% K) (GilRamírez et al., 2024). Calcium and magnesium are also present in coffee pulp (Costa et al., 2025).

Table 2.1 (continued)

Component	Content in Coffee Pulp
Minerals (elements)	For example, study found total ash ~10% with K as the major mineral (~5% of pulp), followed by smaller fractions of Ca and Mg (Puerta, 2011). Trace elements like iron and zinc are present at tens of µg/g level (Machado et al., 2023).
Vitamins	Vitamin E ~72.7 µg/g (≈7.3 mg per 100 g), primarily α-tocopherol Costa et al., 2025)). Vitamin C: Fresh pulp likely contains some vitamin C (and other water-soluble vitamins), but most recent studies do not quantify these – any vitamin C is likely low and may degrade during drying. Bcomplex vitamins have not been well documented in coffee pulp in recent literature

2.2.2 Inhibitory Effects of Coffee Pulp and Cascara on Digestive Enzymes

Coffee pulp and cascara (dried husks of coffee cherries) are reemerging not only as waste but also as potential health-promoting sources of bioactive compounds. Several functional effects have been attributed to these compounds, including the inhibition of key digestive enzymes involved in carbohydrate and lipid metabolism, leading to their consideration as potential agents in managing metabolic diseases like type 2 diabetes, obesity, and hyperlipidemia (Cañas et al., 2021; Patil et al., 2022)

The enzyme-inhibitory activity of coffee pulp and cascara is largely attributed to phenolic compounds, including chlorogenic acids, procyanidins, and flavonoids (Kalita et al., 2021). Moreover, α-amylase and α-glucosidase, two key classes of enzymes that mediate enzyme hydrolysis for complex carbohydrates to generate glucose, have been inhibited by such kinds of compounds, preventing the absorption of glucose after food consumption and thus regulating postprandial blood sugar levels as well (Patil et al., 2022; Trân et al., 2024).Additionally, research using simulated digestion models has found that coffee pulp can slow down how quickly starch breaks down in the body, which helps keep blood sugar levels more stable (Aguilera et al., 2024). This suggests that coffee by-products might act as natural enzyme blockers something that's gaining attention as more people look to food-based ways to prevent chronic diseases. Along with being sustainably sourced, their health benefits make them a great fit for functional foods, supplements, and products aimed at supporting metabolic health.

2.3 Production and Processing of Coffee and Coffee Pulp

2.3.1 Global Production Trends, and Sustainable Practices

Various studies have delved into the characteristics of the Caturra, Catimor, Geisha, and Bourbon coffee varieties. In Southern Mexico, for instance, a study by González-Rivas et al. (2021) found that a substantial portion of coffee plantations in the region feature a blend of varietals, including hybrids like Catimor and Caturra. A comprehensive mapping study focusing on coffee quality along the production- to-consumer chain. Borém et al. (2022) revealed that Arabica coffee, particularly Bourbon and Geisha varieties, has been the primary focus of research. The study indicates a thorough exploration of these varietals within the coffee industry. Research on the flavor profiles of different coffee kinds, including Bourbon and Geisha, has been documented in a review paper on coffee flavor by Silva et al. (2021). The World Coffee Research coffee types catalog serves as a valuable resource containing information on 47 Robusta types and 55 Arabica variants, encompassing Bourbon and Geisha. This catalog contributes to a comprehensive understanding of

the diverse coffee varieties available. A systematic analysis of the effects of climate change on coffee agro-systems, conducted by Bunn et al. (2021), revealed that Arabica received the most attention in research, followed closely by publications dedicated to both Bourbon and Geisha variations. This reflects the prioritization of these particular coffee species in the context of climate change impacts on coffee cultivation. Latin America and the Caribbean regions stand out as the primary powerhouses in global coffee production. Brazil took over as the world's top coffee producer in 2018, according to Slavova and Georgieva (2019), accounting for more than one-third of global coffee production. Remarkably, during the same period, Brazil also earned the title of the third-largest consumer of coffee, underscoring its deep-rooted connection to this beloved beverage. In that noteworthy year, several Asian nations, including Vietnam, Indonesia, and India, joined hands with Ethiopia to collectively make a significant mark on the global coffee stage. According to Sisay (2018), Ethiopia established itself as a dominant force on the African continent by securing the fifth spot in global coffee production. Ethiopian coffee, renowned for its unique quality attributes, boasts enchanting fragrances and captivating flavor profiles. According to Abebe et al. (2020), this coffee's origins are in Arabica beans, and it exhibits a rich tapestry of quality diversity. Both in the coffee processing journey, whether it's the dry or wet method, the generation of by-products, notably pulp waste, is a crucial consideration. Proper management of this waste is essential, not only to minimize environmental impact but also to embrace sustainability in coffee production.

2.3.2 Coffee Processing and Pulp Production

There are two predominant coffee processing processes that are extensively employed in Thailand as well as globally. There are variations in the quality profiles among the three coffee production processes. Dry processing is a cost-effective and straightforward method used in coffee production, particularly in Western Africa and Brazil. It involves sorting and cleaning coffee berries to remove dirt, leaves, and twigs. After cleaning, the berries are spread under sunlight and agitated to hinder fermentation. This method is popular due to its ability to produce coffees with a flavor profile closely resembling naturally occurring coffee (Paudel & Parajuli, 2020).

Dry processing (Figure 2.2) involves encapsulating coffee beans within a

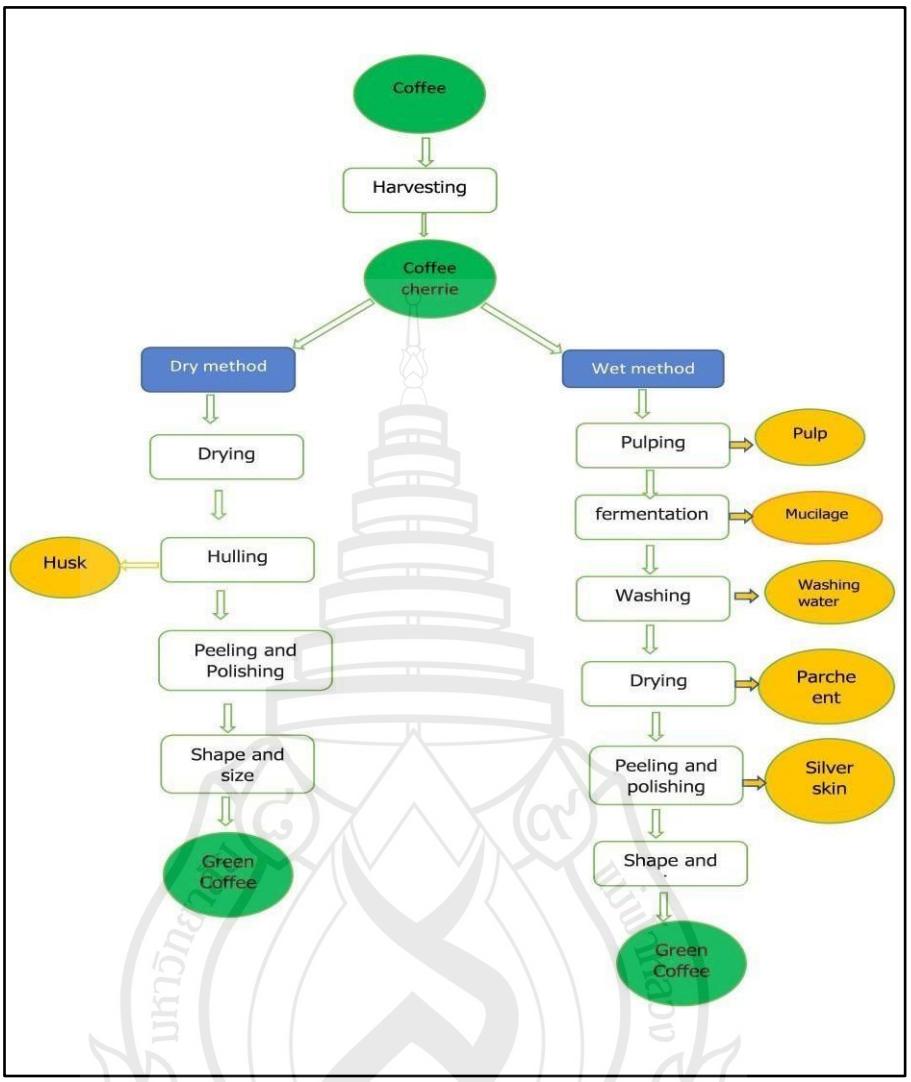
cherry, retaining their pulp and mucilage. This slows down the drying process, allowing chemical components to migrate from the pulp to the inner bean. However, ambient conditions greatly influence this method, leading to significant chemical transformations that can significantly impact the final coffee bean quality and brewed cup flavor. Coffee processing involves the extraction and utilization of coffee pulp, a by-product of dry processing. (Suarez-Quiroz et al., 2005).

The pulping stage of the wet method (Figure 2.2), which also involves the removal of the mucilaginous mesocarp through a carefully planned fermentation process, separates coffee beans from fruit and skin. The fermentation environment is water-based, with temperature regulation being a critical factor. This precise control results in minimal unwanted tastes, making washing coffee a frequent companion for superior cup quality, enhances the aroma quality of green and roasted coffee, especially when compared to mechanical mucilage removal. However, the wet process requires abundant freshwater resources, especially in regions with abundant water. The extraction and responsible utilization of coffee pulp by-products presents an opportunity for sustainability (Gonzalez-Rios et al., 2007).

Coffee processing plays a crucial role in determining the quality and flavor profile of coffee beans. The two primary processing methods—dry processing and wet processing—are widely used globally, including in Thailand. Each method has distinct advantages and implications for coffee quality, sustainability, and by-product utilization. Dry processing, also known as the natural process, is a cost-effective and traditional method predominantly used in regions with arid climates, such as Brazil and parts of Africa (Paudel & Parajuli, 2020). The process involves sorting and cleaning coffee cherries to remove impurities such as dirt, leaves, and twigs. The cleaned cherries are then spread under direct sunlight and periodically agitated to prevent fermentation. Unlike wet processing, dry processing retains the entire cherry, including its mucilage and pulp, prolonging the drying period and allowing chemical compounds from the pulp to transfer to the inner bean. This method often results in a coffee profile characterized by fruity and complex flavors due to prolonged exposure to natural sugars during drying (Suarez-Quiroz et al., 2005). However, external factors such as humidity, temperature, and airflow can significantly impact the final quality of the coffee beans, leading to potential inconsistencies in flavor and aroma.

The wet processing method, or washed process, involves mechanically removing the outer fruit layer before fermenting the beans in water to dissolve the mucilage (Gonzalez-Rios et al., 2007). Fermentation time and temperature regulation are critical in ensuring optimal enzymatic breakdown of mucilage, which directly influences cup quality. This process enhances acidity and clarity in the coffee flavor profile, making it a preferred choice for highquality specialty coffees (Worku et al., 2018). However, wet processing has a highwater demand, which poses environmental concerns, particularly in waterscarce regions. Sustainable practices, such as water recycling and ecopulping, have been introduced to mitigate water consumption and pollution (Mokhtar et al., 2019). Additionally, the by-products of wet processing, such as coffee pulp, present opportunities for waste reduction through innovative reuse strategies, including composting, biofuel production, and animal feed supplementation (Pandey et al., 2000).

Coffee pulp, a major by-product of both processing methods, has been widely studied for its potential applications. Research indicates that coffee pulp is rich in bioactive compounds such as polyphenols, antioxidants, and dietary fiber, making it suitable for various value-added uses (Murthy & Naidu, 2012). In agricultural contexts, coffee pulp can be processed into organic fertilizers or animal feed, contributing to circular economy practices in coffee production (Fan et al., 2020). Moreover, advancements in biotechnology have enabled the extraction of bioactive compounds from coffee pulp for use in nutraceuticals and pharmaceuticals (Rojas et al., 2021). These developments highlight the importance of integrating sustainability into coffee processing by optimizing byproduct utilization.



Source Rattan et al. (2015)

Figure 2.2 Coffee processing and production of coffee pulp

2.4 Health Benefits of Coffee Pulp Extract

Coffee pulp has recently gained attention for its potential health benefits. This seemingly insignificant byproduct of the coffee industry contains a treasure trove of bioactive compounds that could revolutionize our approach to wellness. One such compound is chlorogenic acid, which has shown remarkable antioxidant properties (Naveed et al., 2018). These antioxidants play a crucial role in neutralizing harmful free radicals and reducing oxidative stress within the body. Furthermore, research suggests that chlorogenic acid may also possess anti- inflammatory effects (Yun et al., 2012). By mitigating chronic inflammation, this bioactive compound offers protection against various diseases, such as cardiovascular disorders and type 2 diabetes. Additionally, coffee pulp extract contains high levels of dietary fiber, which can aid digestion and promote gut health (Khochapong et al., 2021).

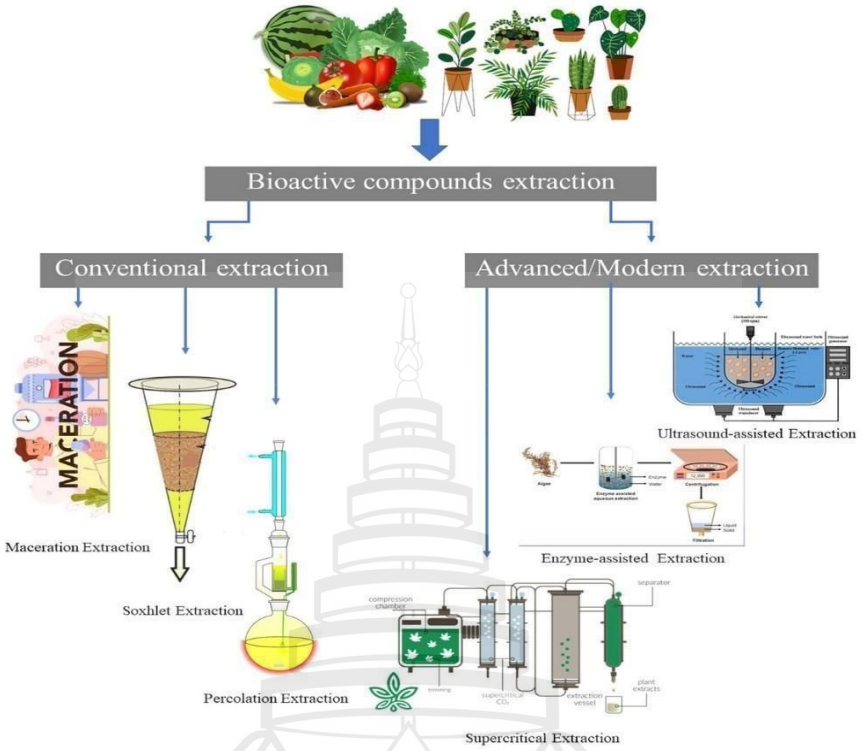
The soluble fibers found in coffee pulp are known to act as prebiotics, fueling beneficial gut bacteria and maintaining a healthy microbial balance (Machado et al., 2023). These findings highlight the untapped potential held within coffee pulp's bioactive compounds; utilizing them could lead us towards a healthier future. Bioactive compounds derived from coffee pulp have gained significant attention in recent years due to their potential health benefits.

The bioactive compounds found in coffee pulp include polyphenols, chlorogenic acids, caffeine, and diterpenes (Reznik, 2012). These compounds possess antioxidant properties and exhibit anti-inflammatory activity within the body. They have also been linked to various health-promoting effects, such as reducing the risk of chronic diseases like heart disease, type 2 diabetes, and certain types of cancer (Adefegha, 2017). Furthermore, bioactive compounds extracted from coffee pulp may contribute to weight management by enhancing metabolism and increasing fat oxidation (Konstantinidi & Koutelidakis, 2019). Notably, these beneficial attributes continue to grow rapidly as the specific mechanisms through which these compounds exert their positive impacts on human health

Coffee pulp extracts may reduce chronic diseases and improve liver function by modulating detoxification enzymes, Bioactive compound extracts from coffee pulp, rich in antioxidant properties like chlorogenic acids, caffeine, and melanoidins, have been shown to have potential health benefits. These extracts can combat oxidative stress, reduce chronic diseases, and alleviate inflammation. Consumption of these extracts has been linked to improved glucose metabolism and insulin sensitivity. Additionally, they have been found to enhance memory retention and reduce cognitive decline (Dominguez et al., 2021).

2.5 Extraction Techniques of Bioactive Compounds

The extraction of bioactive compounds from plant materials can be accomplished through various techniques, each with unique benefits and applications (Figure 2.3). Microwave-Assisted Extraction (MAE) is a technique that uses microwave energy to heat solvents in contact with plant materials, facilitating the rupture of cell walls and the release of bioactive compounds. MAE is recognized for its efficiency, rapid extraction process, and reduced solvent consumption. Ultrasound-Assisted Extraction (UAE), on the other hand, utilizes ultrasonic waves to generate microbubbles in the solvent. The collapse of these microbubbles near cell walls disrupts the structure, enhancing solvent penetration and the dissolution of bioactive compounds. UAE is particularly valued for its low operating temperature, energy efficiency, and the preservation of thermolabile compounds. Supercritical Fluid Extraction (SFE) employs supercritical fluids like CO_2 as the extracting solvent. These fluids possess unique properties that enable efficient penetration and extraction, making SFE an environmentally friendly option noted for producing high-purity extracts without toxic solvent residues. Lastly, conventional methods such as Soxhlet extraction, maceration, and infusion, while simple and cost-effective, often require longer extraction times and higher solvent usage, and may involve high temperatures that can degrade sensitive bioactive compounds.

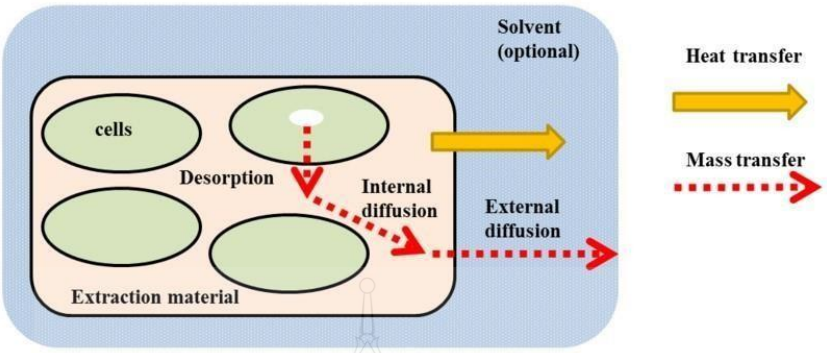


Source Hamed et al. (2019)

Figure 2.3 Summary of extraction techniques of bioactive compounds

2.5.1 Microwave-Assisted Extraction (MAE)

Microwave-assisted extraction (Figure 2.4) stands at the forefront of innovative separation techniques, poised to revolutionize flavonoid extraction and quantitative analysis (Coelho et al., 2021). This versatile method not only matches, but in many instances surpasses, modern extraction techniques in terms of yield, offering a straightforward and efficient approach for extracting flavonoids from plant materials with varying physicochemical properties (Stalikas, 2007). Many important things, like the type of solvent, the volume, the dielectric properties, the microwave power levels, the exposure time, the system specifications, and the temperature, have a big effect on how well MAE extracts (Mandal et al., 2008). In addition, the best solvent for MAE depends on the matrix-to-solvent ratio, how well the analyte dissolves in the solvent, and how well the solvent absorbs microwaves (Clodoveo et al., 2016). In MAE, the controlled application of microwaves plays a pivotal role in selectively segregating compounds of interest from the test sample into the solvent (Li et al., 2013).



Source Gomez et al. (2020)

Figure 2.4 Summary of microwave assisted extraction techniques

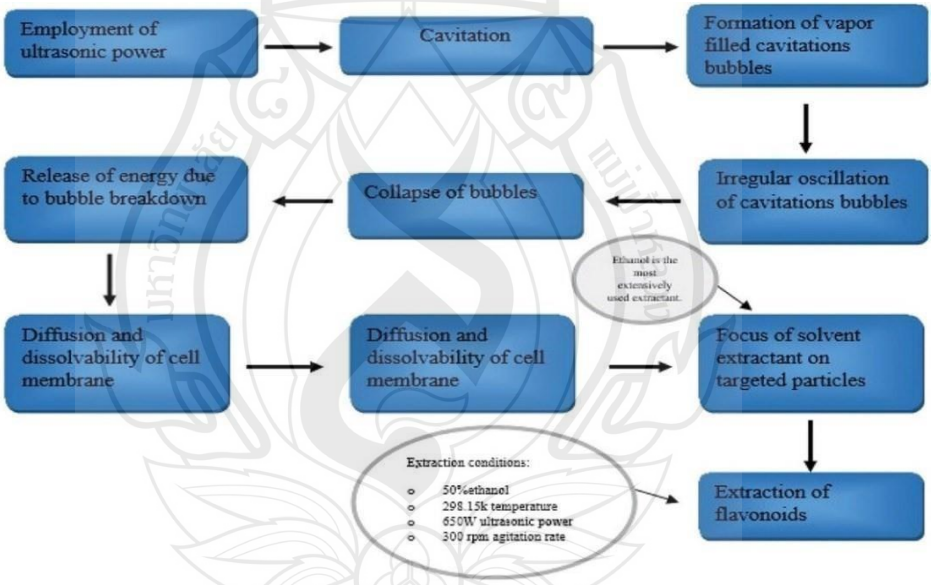
The cells are ruptured during the MAE extraction process in order to expose the analytes to the solvent (Mandal et al., 2008). It promotes the draining of target components from the sample to the solvent using microwave energy. Microwave flux in a microwave environment heats the natural water in plant cells, promoting cell wall breakdown and stimulating plant cell development. (Tariq et al., 2023) Important components (constituents and secondary metabolites) are released from the plant sample as a result of the opening of cell walls. In order to maximize the efficiency of pressurized liquid extraction (PLE) and microwave-assisted extraction (MAE), it was looked at how different amounts of ethanol, extraction time, and temperature affected the yield of the extract, the total phenolic content (TPC), the total flavonoid content (TF), the DPPH scavenging method, and the Trolox equivalent antioxidant capacity (TEAC) assay. The research by Rodríguez-Pérez et al (2016) shows that MAE extracts quercetin, quercetin-3Oglucoside, and kaempferol, both kaempferol acetyl glycoside isomers and kaempferol-3-O-glucoside isomers, better than Pressurized Liquid Extraction (PLE). This means that MAE is better than PLE because the first derivatives of quercetin and kaempferol are said to be stronger antioxidants than regular vitamins.

2.5.2 Ultrasound-Assisted Extraction (UAE)

Ultrasound-Assisted Extraction (UAE) is a modern extraction technique widely researched for its effectiveness in extracting bioactive compounds from various plant materials, including food and natural product by-products. This technique employs ultrasound waves to enhance the extraction process, leading to

higher efficiency, reduced energy consumption, and improved quality of the extracts (Figure 2.5). UAE has shown promising results in extracting valuable compounds such as phenolic compounds, antioxidants, and other bioactive substances from different plant sources.

UAE is particularly effective in extracting bioactive compounds from fruit and vegetable by-products, a process that not only utilizes waste materials but also contributes to environmental sustainability. This method has been applied in extracting prebiotic oligosaccharides, phenolic compounds, and other bioactive compounds from sources like Annona muricata by-products and pulp (Aguilar-Hernández et al., 2019) and from byproducts generated during the elaboration of isotonic beverages (Gonçalves et al., 2018). Furthermore, the application of UAE extends to marine macroalgae, highlighting its versatility in extracting bioactive compounds from a diverse range of plant materials (Cikoš et al., 2018).



Source Tariq et al. (2023)

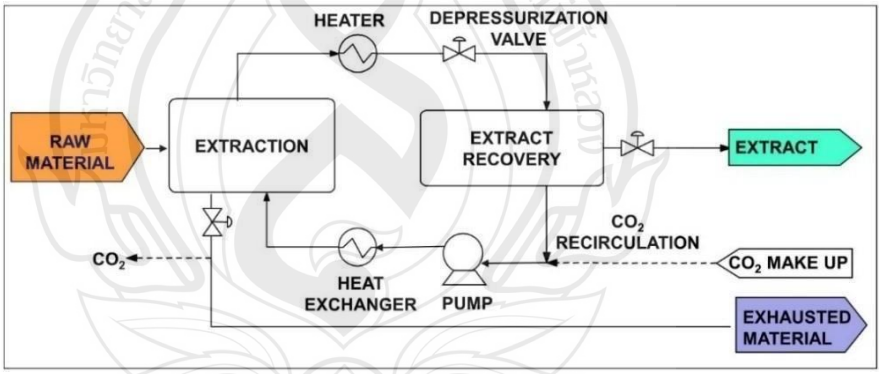
Figure 2.5 Summary of UAE

2.5.3 Supercritical Fluid Extraction (SFE)

Supercritical Fluid Extraction (Figure 2.6), particularly with CO_2, is a prominent method for extracting bioactive compounds from plant materials, including food waste. CO_2 is favored for its low cost, non-toxicity, nonflammability, and convenient critical point. SFE is efficient due to the unique properties of supercritical

fluids, like low viscosity and high diffusivity, which enhance the solubility and extraction rate of target compounds. Studies have shown SFE to be more efficient than traditional methods like Soxhlet extraction, requiring less solvent and time. For instance, phenolic compound extraction from orange pomace via SFE was significantly quicker and used much less solvent compared to conventional methods (Ballesteros-Vivas et al., 2019; Cavalcanti et al., 2011; Espinosa-Pardo et al., 2017; Varaee et al., 2019; Yu et al., 2007)

SFE using CO_2 is recognized for its effectiveness in extracting bioactive compounds from various plant materials, including food waste. This method is advantageous due to CO_2's low cost, non-toxicity, and eco-friendly properties. SFE stands out for its efficiency, often surpassing traditional extraction methods like Soxhlet extraction in terms of speed and solvent use. This technique has been successfully applied to extract compounds such as limonin and naringin from grapefruit seeds, demonstrating higher yields under specific conditions (Yu et al., 2007). Additionally, SFE has been utilized for extracting phenolic compounds from orange pomace, highlighting its potential in using less solvent.



Source Geeta et al. (2020)

Figure 2.6 Supercritical Fluid Extraction (SFE) summary

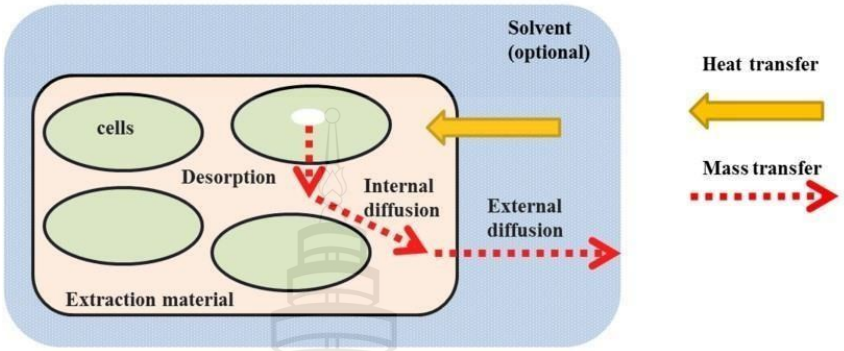
The extraction of bioactive compounds from waste materials using SFE is gaining prominence due to its efficiency and environmental benefits. SFE uses supercritical fluids that possess both liquid and gas properties to isolate complex compounds. This technique is advantageous over traditional methods involving conventional solvents, which may be unsustainable or cause thermal degradation of the compounds. CO_2 is commonly used in SFE due to its nontoxic, inexpensive, and non-flammable characteristics. Its near-ambient critical temperature allows for the extraction of thermolabile components without degradation. The solubility of nonpolar supercritical CO_2 can also be enhanced by adding cosolvents, making it versatile for different compounds. A study highlighted the use of SFE for extracting bioactive compounds from citrus peels using supercritical CO_2. This research demonstrated the potential of SFE in obtaining enriched extracts containing significant concentrations of compounds like limonene, linalool, and coumarin derivatives from agricultural by-products (Geeta et al., 2020; Wrona et al., 2017).

2.5.4 Conventional Methods

Various extraction procedures, such as percolation, maceration, boiling, reflux, soaking, hydro-distillation, and Soxhlet, have been proposed. (Hamed et al., 2019). Several solvents, including ethanol, chloroform, methanol, benzene, acetate and ethyl and others, (Figure 2.7) have been tested to see how they affected extraction yields (Chávez-González et al., 2020). Soxhle and Reflux extraction are both continuous and high temperature methods. Soxhlet extraction is performed with the Soxhlet apparatus: A porous ―thimble‖ is used to hold the fine grounded sample within a chamber. In the bottom flask, extraction solvents are heated, vaporized into the sample thimble, a condenser used to condense it, and then it dripped back. These procedures, in comparison to maceration, use a lesser amount of solvent found that the Soxhlet extraction of moringa leaves resulted in a decreased yield of phenolic compounds, including flavonoids (Routray & Orsat, 2011; Vergara- Jimenez et al., 2017).

Maceration is a wine making technique frequently used in medicinal plant research where plant materials steeped in a solvent at room temperature with frequent agitation result in softening and cracking of the plant’s cell wall, releasing soluble phytochemicals (Owona et al., 2020). Decoction- and infusion- like maceration

involve steeping in cold or boiling water. For infusion, however, the time of maceration is shortened, and the sample is cooked in a predetermined amount of water 1:4 or 1:16 for a set duration for decoction (Owona et al., 2020).



Source Gomez et al. (2020)

Figure 2.7 Summary of convectional extraction techniques

2.6 Response Surface Methodology (RSM)

The integration of Response Surface Methodology and MAE has been utilized to enhance the precision and efficiency of extracting bioactive components from coffee pulp. RSM is a well-established mathematical and statistical technique that has proven highly effective in optimizing complex processes (Basri et al., 2007). It offers structured experimental designs and data analysis, making it a powerful tool with versatile application that extends to diverse fields (Das & Agrawal, 2011).

RSM enables researchers to explore multifactorial relationships, precisely control variables, and reach optimal conditions. In the context of coffee pulp, RSM provides a data-driven approach to fine-tuning MAE parameters, balancing factors such as microwave power, exposure time, solvent characteristics, and temperature for maximal bioactive compound extraction efficiency (Yolmeh & Jafari, 2017). According to Sosa-Martínez et al. (2023), RSM has been very useful in getting bioactive compounds out of different plants. It has helped researchers figure out how to best use the key extraction variables. To improve the quality and yield of the extracts, RSM can be used to systematically look into how things like ethanol percentage, extraction time, temperature, particle size affects each other. Researchers

have shown that RSM can be used to get more of the useful chemicals in plants, like quercetin, kaempferol, and other strong antioxidants (Rodríguez-Pérez et al., 2016). This makes it an interesting tool for optimizing for coffee pulp extraction. The application of RSM in the field of bioactive compound extraction is not without its challenges. These include experimental design complexities, the need for a sufficient number of runs, and potential nonlinearity in responses (Maran & Priya, 2015) However, recent advances incorporate innovative techniques such as artificial intelligence and machine learning, enhancing predictive accuracy and enabling the modeling of intricate, high-dimensional systems (Wang et al., 2023).

CHAPTER 3

RESEARCH METHODOLOGY

3.1 Overall Research Methodology

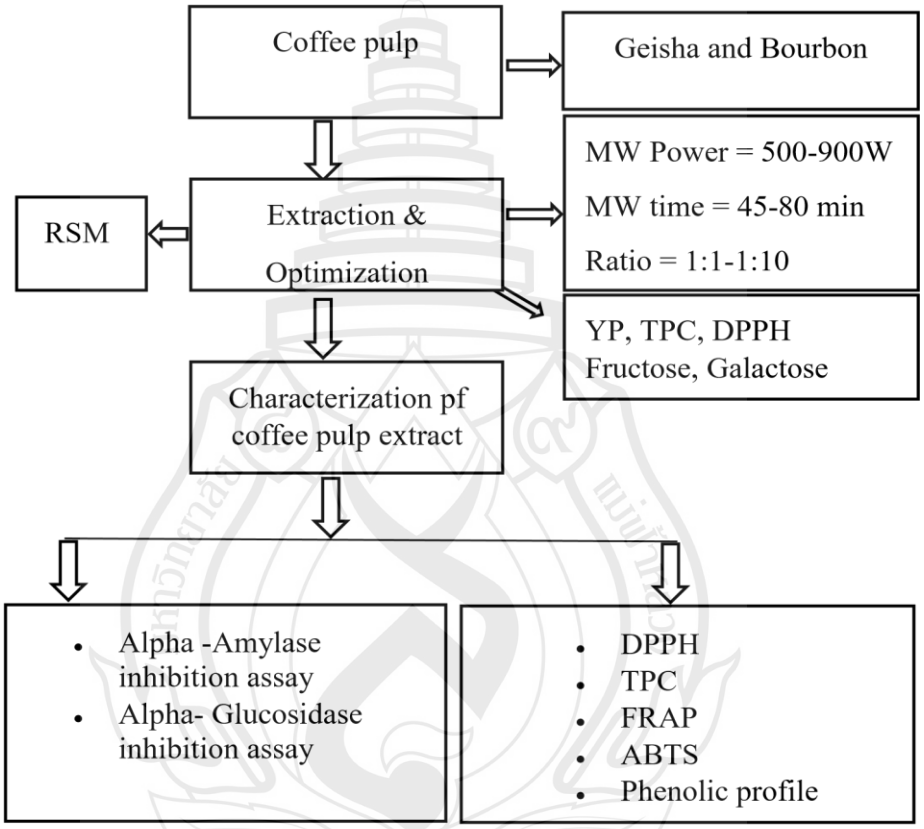


Figure 3.1 Overall research methodology

3.2 Raw Material

The coffee cherries of Bourbon and Geisha varieties were collected from various farms in Chiang Rai province, Northern Thailand, to obtain a representative sample. The cherries were immediately pulped on-site to separate the seeds from the fruit pulp. The fresh pulp was then hygienically sun-dried for several hours until its moisture content reduced. To preserve quality, the pulp was sealed in containers and transported within 24 hours under controlled conditions to the Laboratory of Food Science at Mae Fah Luang University, minimizing exposure to heat, moisture, or microbial contamination. Then the pulp was dried further at 60 °C until its moisture content of 10%. Finally, the dried pulp was ground into a fine powder and vacuum-sealed for ambient storage until analysis (Jiamjariyatam et al., 2020).

3.3 Chemical and Equipment

The study employed a range of laboratory equipment and reagents to optimize and analyze the extraction of bioactive compounds from Geisha and Bourbon coffee pulp. For the extraction process, an ETHOS X microwave extraction oven was utilized under varied conditions of microwave power, time, and solvent ratios. Additional steps included sonication, vortexing, and centrifugation at 5,000 rpm at 4°C using a centrifuge. Conventional extraction was carried out using a water bath and a rotary evaporator for solvent removal. Extracts were further dried using a freeze dryer and weighed using an analytical balance. For bioactivity analyses, a microplate reader (for DPPH, TPC, and enzyme inhibition assays) and a Multiskan FC microplate photometer (for alpha-glucosidase assays) were used. Sugar profiling employed High-Performance Liquid Chromatography (HPLC) with an amino column and a refractive index detector, while phenolic profiling was performed using an Agilent 1290 UPLC system coupled with a Quadrupole Time-of-Flight Mass Spectrometer (QTOF-MS) and operated via Agilent MassHunter Software.

Chemicals used included distilled water, methanol (for DPPH), Folin-Ciocalteu reagent, 7.5% sodium carbonate, gallic acid (standard for TPC), acetonitrile and formic acid (for HPLC mobile phases), deionized water, and 0.45 µm membrane filters. For enzyme inhibition assays, porcine pancreatic alpha-amylase, soluble starch, DNS reagent, sodium phosphate buffer, DMSO, alpha-glucosidase enzyme, and p-nitrophenyl-α-D-glucopyranoside (pNPG) were used, with acarbose serving as a positive control.

3.4 Optimization Study of Bioactive Compounds Extraction

3.4.1 Microwave-Assisted Extraction

ETHOS X microwave extraction ETHOS X Microwave Extraction System (Model: ETHOS X, Milestone Srl, Sorisole, Italy) oven was used for microwaveassisted extraction. The retrieval process was performed mixing coffee pulp powder with distilled water in various weight-to-volume ratios (w/v) at room temperature. The solution is subsequently sonicated and vortexed for 2 and 1 min at room temperature, respectively before undergoing heat treatment under different microwave power (Table 3.1) under various controlled conditions, based on parameters i.e. microwave power, extraction time, and solvent ratios. Following extraction with microwave assistance, the mixture was centrifuged twice using a centrifuge machine set to 5000 rpm and 4 °C for 10 min. For additional analysis, the liquid extract was kept and stored at 4°C before being freeze-dried (Li et al., 2010).

3.4.2 Conventional Extraction of Coffee Pulp

Distilled water and coffee pulp powder was mixed in a 1:2 ratio. Until the mixture was homogeneous, it was continuously stirred. A water bath was then used to heat the sample to 85°C, and it was kept there for 15 min. After that, cold water was used to cool the sample to room temperature. After centrifuging each sample for seven min at 3,000×g, the supernatant was collected. Coffee pulp extract powder samples was obtained by freeze-drying the supernatant in Falcon tubes after it had been concentrated using a rotary evaporator to remove excessive water (Thaiphanit et al., 2020; Wong & Nillian, 2023).

3.4.3 Experimental Design

The extraction and optimization method performed with various parameter levels using RSM. Microwave power, microwave time and sample-to-water ratios (Table 3.2) were maintained constant in the extraction condition. The selected independent variables were intended to study the effects of extraction conditions on the quantity and quality of extracts. BoxBehnken design (BBD) was used to study the linear and quadratic terms of input variables on the response and provide information about the response surface to get optimum conditions for the best possible responses. Impact of three microwave parameters including microwave power (X_1), microwave time (X_2) and sample-to-solvent ratio (X_3) on response variables namely extraction yield (Y_1), TPC (Y_2), DPPH (Y_3) and fructose and galactose (Y_4) were investigated. Each of the selected parameters was tested at three levels high (+1), medium (0) and low (-1), corresponding to the minimum and maximum readings respectively according to literature data and preliminary experimental findings. Water was used as a solvent due to its safety and high solubility of bioactive compounds. Experimental design and analysis were performed using Design-Expert® software (ver. 13.0, Stat-Ease, Inc).

Table 3.1 Factors and Levels of Independent Variables with Corresponding Coded Values of Microwave Assisted Extraction

Independent variables	**Code**	**Coded variable**		
		-1	**0**	**+1**
Microwave power (Watt)	X_1	500	700	900
Microwave time (min)	X_2	45	62.5	80
Sample-to-water ratios	X_3	1:1	1:5.5	1:10

This design consisted of a total of 15 runs, which was suitable for fitting a second-order polynomial equation with three factors. The design included three center points, which were used to estimate the pure error and assess the adequacy of the model. Additionally, the design incorporated eight axial points, which were utilized to estimate the quadratic terms and check for curvature in the response surface.

Table 3.2 RSM Output with Box-Behnken Design

Run	X_1 Microwave power (Watt)	X_2 Microwave time (Watt)	X_3 Water to sample (Watt)
1	900 (+1)	62.5 (0)	1:10 (+1)
2	900 (+1)	45 (-1)	1:5.5 (0)
3	700 (0)	80 (+1)	1:1 (-1)
4	700 (0)	45(-1)	1:1(-1)
5	700 (0)	62.5 (0)	1:5.5 (0)
6	700 (0)	62.5 (0)	1:5.5 (0)
7	700 (0)	45 (-1)	1:10 (+1)
8	500 (-1)	62.5 (0)	1:10 (+1)
9	900 (+1)	80 (+1)	1:5.5 (0)
10	700 (0)	80 (+1)	1:10 (+1)
11	500 (-1)	45 (-1)	1:5.5 (0)
12	900 (+1)	62.5 (0)	1:1 (-1)
13	500 (-1)	80 (+1)	1:5.5 (0)
14	500 (-1)	62.5 (0)	1:1 (-1)
15	700 (0)	62.5 (0)	1:5.5 (0)

3.4.4 Fitted Model Validation

The fitted models were evaluated using key statistical parameters, including R^2, R^2_adj, R^2_pred, coefficient of variation and lack of fit. High R^2 values (0.9054–0.9837) indicate a strong fit, while a small difference between R^2_adj and R^2_pred (<0.2) ensures good predictive accuracy. Adequate precision (>4) confirms a reliable signal-to-noise ratio, and a non-significant lack of fit ($p > 0.05$) supports model validity. P-values determined the significance of factors, with lower values indicating a stronger impact. While the models fit well, low R^2_pred for some responses suggests a need for refinement. These metrics affirm the models' reliability (Montgomery, 2020).

3.5 Method of Analysis

3.5.1 Extraction Yield (%)

A gravimetric technique was used to estimate the total harvest of bioactive compounds from coffee pulp. The extract obtained from MAE was concentrated with a rotary evaporator to remove the solvent (water). The residue was dried to a constant weight in an oven at 50°C. The dried extract was then re- weighed, and the weight of the dried and the initial weight were used to calculated a percentage yield (Zhang & Wang, 2021).

3.5.2 DPPH Radical-Scavenging Activity

DPPH of coffee pulp extract was carried out in microplates. Blois (1958) reported that DPPH was dissolved in methanol to get final concentration of 0.1 mM in the first step, Thus, 180 µL DPPH solution and 20 µL extract were added to each well. Following the addition of DPPH and incubation in the dark for 30 min in the dark at room temperature then absorbance was measured through microplate, DPPH scavenging activity was calculated. The percentage inhibition is determined and expressed in mg TE/g DW (Molyneux, 2004).

3.5.3 Total Phenolic Content

The Folin-Ciocalteu reagent with a microplate format analysis was used to analyze TPC in coffee pulp extracts. Briefly, each test was performed by adding 25 µL of the phenolic/biological extract or standard solution of gallic acid to individual wells on a 96-well plate. This was followed by addition of 125 µL diluted Folin-Ciocalteu reagent into each well left to stand for five min at room temperature. This was followed by adding 100 µL 7.5% w/v sodium carbonate for neutralization. The plate was then incubated for approximately 30 min at room temperature and in the dark, and further on the absorbance was determined at 765 nm using a microplate reader. TPC was reported as mg GAE/g DW (Magalhães et al., 2010).

3.5.4 Fructose and Galactose Analysis by HPLC

A High-Performance Liquid Chromatography was utilized to analyze fructose and galactose concentrations in coffee pulp extracts. 1 g of powdered coffee pulp extract was combined with 40 mL of deionized water to facilitate the efficient

extraction of sugar. The mixture was stirred for 30 min to ensure complete dissolution. Afterward, the solution was filtered through a 0.45 µm membrane filter to remove any solid particles that could interfere with the analysis, as suggested by Zhang et al. (2022). To obtain suitable concentration for HPLC analysis, the filtered extract was then diluted ten times with deionized water. An amino column was used to separate the diluted extract for HPLC analysis, and the monosaccharides were quantified using refractive index detection. The monosaccharides were separated using the mobile phase, which was a 75:25 (v/v) mixture of water and acetonitrile following the methodology outlined by Zhu et al. (2019). Using the calibration process described by Xie et al. (2021) the concentrations of fructose and galactose were measured by comparing retention periods and peak regions with those of recognized sugar standards.

3.5.5 Phenolic Profile Analysis

The Phenolic profile Analysis was performed using an Agilent 1290 UPLC system equipped with a Poroshell EC-C18 column (2.1 × 150 mm, 2.7 µm; Agilent Technologies, Santa Clara, USA). Solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile) made up the mobile phase. 0–10 min (95% A, 5% B), 10–20 min (83% A, 17% B), 20–27 min (100% B), and 27–30 min (re-equilibration to 95% A, 5% B) were the specified times for the gradient elution. The column was maintained at 37 °C with a flow rate of 0.2 mL/min, and the sample injection volume was 1 µL. Detection was carried out using a Quadrupole Time-of-Flight Mass Spectrometer (QTOF/MS) operated via Agilent MassHunter Software Version B.08.00. Fragmentation was achieved using collision energies of 10, 20, and 40 eV, and the MS/MS acquisition range spanned from 50 to 1100 m/z. Sheath gas temperature of 250 °C was set, with a flow rate of 10 L/min and an atomizing gas pressure of 35 psi. Analyses were conducted in both positive and negative ionization modes to ensure comprehensive mass peak identification (Zhang et al., 2017).

3.5.6 Alpha-Glucosidase Inhibition Assay

The Schmidt et al. (2012) method was used to perform the alphaglucosidase inhibition experiment. 90 µL of buffer (SPB; pH 7.5, 0.1 M) containing 0.02% sodium azide was put into a 96-well microplate. After that, each well received 10 µL of extract in DMSO. The α-glucosidase solution (2.0 U/mL) in SPB (80 µL) was

added. An 80 µL solution of α-glucosidase (2.0 U/mL) in SPB was added to each well microplate, and it was pre-incubated for 10 min at 28 °C. Acarbose served as the positive control. A 10% DMSO and sodium phosphate buffer mixture was used to treat the extracts, while sodium phosphate buffer was used to treat the negative controls, which were water extracts. Following incubation, a reaction was initiated by adding 20 µL of pNPG dissolved in SPB to the solution. To monitor the rate of pNPG conversion to p- nitrophenol, the absorbance of p-nitrophenol at 405 nm was measured using a Multiskan FC microplate photometer (every 30 seconds for 35 min).

3.5.7 Alpha-Amylase Inhibition Assay

Coffee pulp's α-amylase inhibitory activity was assessed using the 3,5dinitrosalicylic acid (DNS) colorimetric method since it is a simple and accurate way to measure the quantity of reducing sugars produced during enzymatic reactions. In this process, the coffee pulp extract was first incubated for 10 min at 25°C with porcine pancreatic α-amylase in a phosphate buffer (pH 6.9). The mixture was then incubated for ten more minutes at the same temperature after a soluble starch solution was added as the substrate. After adding DNS reagent and heating the mixture in boiling water for five min to halt the reaction, the mixture's color changed (Thongra-ar et al., 2021). After cooling, a spectrophotometer was used to detect absorbance at 540 nm. By contrasting the absorbance of the test sample with that of a control sample devoid of the extract, the degree of α-amylase inhibition was ascertained (Kazeem, Adamson, and Ogunwande, 2013).

3.6 Statistical Analysis

The measurements and analyses were analyzed in triplicate to ensure reliability of data. The trial designs for chemical properties followed a completely randomized design (CRD) to minimize bias. The obtained data underwent analysis of variance (ANOVA) to determine statistical significance.

To compare means and Duncan's multiple range test was applied. Statistical analysis was performed using SPSS version 16.0 (SPSS Inc., Chicago, IL, USA). Response Surface Methodology (RSM) and the Box-Behnken design was utilized to

evaluate the relationship between variables. The analysis was conducted using Design-Expert® software version 13 (Stat-Ease Inc., USA)**,** employing Box- Behnken Design for optimization of extraction parameters using Response Surface Methodology.

CHAPTER 4

RESULT AND DISCUSSION

4.1 Optimization Study of Bioactive Compounds Extraction

The extraction yield and bioactive properties of Geisha coffee variety extracts obtained under various Microwave Assisted Extraction (MAE) conditions, including different microwave power levels, extraction times, and sample-to-solvent ratios, were analyzed. Table 4.1 summarizes the experimental design, conditions, and corresponding responses namely, yield, TPC, DPPH values, and sugar content (fructose and galactose). The highest extraction yield (13.15%) was achieved in Run 15 (700Watt, 62.5 min, 1:5.5 ratio), while the lowest (7.16%) occurred in Run 13 (500Watt, 80 min, 1:5.5 ratio). TPC of 32.7 mgGAE/mL was observed in Run 10 (700Watt, 80 min, 1:10 ratio), whereas the lowest (20.35 mgGAE/mL) was recorded in Run 8 (500Watt, 62.5 min, 1:10 ratio). Antioxidant activity (DPPH value) peaked at 7.12 mgTE/g in Run 9 (700Watt, 80 min, 1:5.5 ratio) and was lowest at 1.31 mgTE/g in Run 14 (500Watt, 62.5 min, 1:1 ratio). Fructose content ranged from 2.84% (Run 14) to 7.96% (Run 10), while galactose content varied from 0.322% (Run 14) to 1.523% (Run 10).

Table 4.2 presents the extraction yield, TPC, antioxidant activity, and sugar composition of Bourbon coffee pulp variety, the findings suggest that microwave power, extraction time, and the sample-to-solvent ratio play a crucial role in determining extraction Among all runs, the highest extraction yield (11.41%) was observed in Run 10 (700Watt, 80 min, 1:10 ratio), while the lowest (6.2%) occurred in Run 3 (700Watt, 80 min, 1:1 ratio). TPC values showed considerable variation, ranging from 14.62 mg GAE/mL in Run 3 to 26.29 mg GAE/mL in Run 6 (700Watt, 45 min, 1:7 ratio). DPPH value showed a similar trend, with the highest value recorded at 6.43 mg TE/g in Run 10, while the lowest value (1.89 mg TE/g) was observed in Run 9 (500Watt, 80 min, 1:10 ratio). Sugar content varied as well, with fructose levels ranging from 2.63% in Run 14 (500Watt, 80 min, 1:5.5 ratio) to

7.85% in Run 3. Meanwhile, galactose content fluctuated between 0.54% in Run 14 and 1.71% in Run 10. Overall, the most effective runs particularly Runs 6, 10, and 15 suggest that the optimal conditions for maximizing bioactive compound recovery include using 700W microwave power, an extraction time of around 62.5 minutes, and a 1:5.5 sample-to-solvent ratio. These conditions provide a well-balanced approach, ensuring high extraction yields, efficient bioactive compound recovery, and optimal sugar content, making them ideal for an efficient extraction process.

Table 4.1 Independent Variables with Response Variables (RSM, BBD) of Geisha variety coffee pulp

Run	X_1	X_2	X_3	Yield (%)	TPC (mg GAE/mL)	DPPH (mg TE/g)	Fructose (%)	Galactose (%)
1	900 (+1)	62.5 (0)	1:10 (+1)	10.51	29.50	5.13	4.52	0.87
2	900 (+1)	45 (-1)	1:5.5 (0)	8.61	24.25	4.12	4.60	0.81
3	700 (0)	80 (+1)	1:1 (-1)	8.21	29.21	5.67	7.80	1.47
4	700 (0)	45(-1)	1:1(-1)	10.42	21.85	3.54	4.67	0.79
5	700 (0)	62.5 (0)	1:5.5 (0)	12.54	27.65	5.94	5.78	1.34
6	700 (0)	62.5 (0)	1:5.5 (0)	12.89	28.94	5.94	6.23	1.37
7	700 (0)	45 (-1)	1:10 (+1)	9.69	22.98	4.75	5.32	1.20
8	500 (-1)	62.5 (0)	1:10 (+1)	8.25	20.35	4.45	4.52	0.98
9	900 (+1)	80 (+1)	1:5.5 (0)	10.22	31.12	7.12	7.82	1.47
10	700 (0)	80 (+1)	1:10 (+1)	10.31	32.70	6.23	7.96	1.52
11	500 (-1)	45 (-1)	1:5.5 (0)	7.72	22.79	3.51	3.47	0.45
12	900 (+1)	62.5 (0)	1:1 (-1)	7.63	22.62	5.74	5.65	1.23
13	500 (-1)	80 (+1)	1:5.5 (0)	7.16	27.46	4.11	4.59	0.79
14	500 (-1)	62.5 (0)	1:1 (-1)	8.52	23.55	1.31	2.84	0.32
15	700 (0)	62.5 (0)	1:5.5 (0)	13.15	28.94	5.33	6.23	1.37

Table 4.2 Independent Variables with Response Variables (RSM, BBD) of Bourbon coffee variety

Run	X_1	X_2	X_3	Yield (%)	TPC (mg GAE/mL)	DPPH (mg TE/g)	Fructose (%)	Galactose (%)
1	900 (+1)	62.5 (0)	1:10 (+1)	9.52	22.72	3.87	4.12	1.13
2	900 (+1)	45 (-1)	1:5.5 (0)	8.40	22.31	3.31	3.86	0.92
3	700 (0)	80 (+1)	1:1 (-1)	6.20	14.62	2.31	7.85	0.79
4	700 (0)	45(-1)	1:1(-1)	7.41	19.13	3.51	5.12	1.41
5	700 (0)	62.5 (0)	1:5.5 (0)	9.12	25.52	4.92	6.23	1.57
6	700 (0)	62.5 (0)	1:5.5 (0)	8.98	26.29	4.51	6.54	1.61
7	700 (0)	45 (-1)	1:10 (+1)	8.21	20.41	3.33	5.62	1.02
8	500 (-1)	62.5 (0)	1:10 (+1)	8.34	21.22	5.32	5.21	0.99
9	900 (+1)	80 (+1)	1:5.5 (0)	7.76	15.87	1.89	7.74	0.75
10	700 (0)	80 (+1)	1:10 (+1)	11.4	21.74	6.43	7.51	1.71
11	500 (-1)	45 (-1)	1:5.5 (0)	6.24	20.56	2.15	3.98	0.72
12	900 (+1)	62.5 (0)	1:1 (-1)	7.51	20.74	3.27	4.76	1.08
13	500 (-1)	80 (+1)	1:5.5 (0)	7.32	17.11	2.88	4.10	0.89
14	500 (-1)	62.5 (0)	1:1 (-1)	7.76	21.61	3.04	2.63	0.54
15	700 (0)	62.5 (0)	1:5.5 (0)	9.61	24.41	4.86	7.52	1.49

The regression analysis (Tables 4.3 and 4.4) highlights the significant effects of microwave power (X_1), extraction time (X_2), and sample-to-solvent ratio (X_3) on extraction yield, total phenolic content (TPC), antioxidant activity (DPPH), and sugar composition in Geisha and Bourbon coffee pulp extracts. Extraction yield showed a positive correlation with microwave power ($p < 0.05$) and sample-to-solvent ratio ($p < 0.05$) in both coffee varieties. However, excessive microwave exposure resulted in lower yields, as indicated by the significant negative quadratic terms X_1^2 (-0.9754) and X_2^2 (1.45). The strong R^2 values above (0.92) confirm the model's reliability in predicting extraction efficiency. TPC was significantly influenced by extraction time (X_2 = -2.13, $p < 0.001$) for Bourbon, suggesting that prolonged exposure leads to phenolic degradation, consistent with prior studies on oxidative instability (Akanbi et al., 2022). Conversely, a higher sample-tosolvent ratio ($p < 0.01$) improved polyphenol solubilization, supporting findings that solvent availability enhances

extraction efficiency (Wong & Nillian, 2023).

DPPH antioxidant activity followed a similar pattern, with X_3 ($p < 0.01$) positively influencing radical scavenging activity, while X_1^2 (-1.17, $p < 0.01$) indicated degradation due to excessive microwave power. The interaction between extraction time and sample-to-solvent ratio ($X_2 X_3$ = 1.05, $p < 0.05$) in Bourbon suggests that moderate extraction times combined with sufficient solvent volumes help preserve antioxidant activity (Tran et al., 2020). Sugar composition varied between the two coffee varieties, with Bourbon coffee pulp containing higher fructose levels (7.02%) and Geisha exhibiting higher galactose content (1.692%) (Table 4.7). The positive impact of extraction time ($p < 0.001$) on fructose content confirms that extended exposure improves sugar diffusion. However, the negative quadratic effect of microwave power (-1.58, $p < 0.01$) suggests that excessive energy input leads to sugar degradation (Tran et al., 2020). The high adequate precision values above (7) and acceptable coefficient of variation (C.V.%) further validate the reliability of the models.

Table 4.3 Estimated Regression Coefficients for Geisha Variety Coffee Pulp Extracts for Yield%, TPC, DPPH, Fructose, and Galactose

Term	Yield	TPC	DPPH	Fructose	Galactose
Intercept	10.52	27.7	5.2	5.78	1.35
Linear					
X_1	1.05*	-0.0225	-0.0338	0.6762*	0.1159**
X_2	0.5175	-2.13***	0.0837	1.26***	0.0252
X_3	1.17*	1.25**	0.8450**	0.2	0.1379**
Quadratic					
X_1^2	-0.9754	-2.43*	-1.17**	-1.58**	-0.4094***
Interaction					
X_1X_2	-0.2975	-0.835	-0.6500*	0.525	0.0248
X_1X_3	0.9125*	0.525	-0.4375	-0.6425**	-0.2175
X_2X_3	1.18**	1.52	1.05*	-0.1225	0.2607**
R^2	0.9207	0.9183	0.9054	0.9419	0.9837
Adj. R^2	0.7779	0.7711	0.7351	0.8374	0.9542
Pred. R^2	0.0675	-0.0833	-0.4566	0.2303	0.7492
A. Precision	9.7451	8.1065	7.2638	10.2926	20.1925
C.V %	9.1	7.54	16.46	12.05	7.08
Model p-value	0.027	0.0289	0.0402	0.0131	0.0006
Lack of Fit p-value	0.4253	0.2842	0.0652	0.2838	0.0706

Note X_1: Microwave Power X_2: Extraction Time X_3: Sample to Solvent Ratio R^2: Coefficient of Determination Adj. R^2: Adjusted R^2 Pred. R^2: Predicted R^2 A. Precision: Adequate Precision C.V %: Coefficient of Variation % Significance levels: * significant at $p < 0.05$

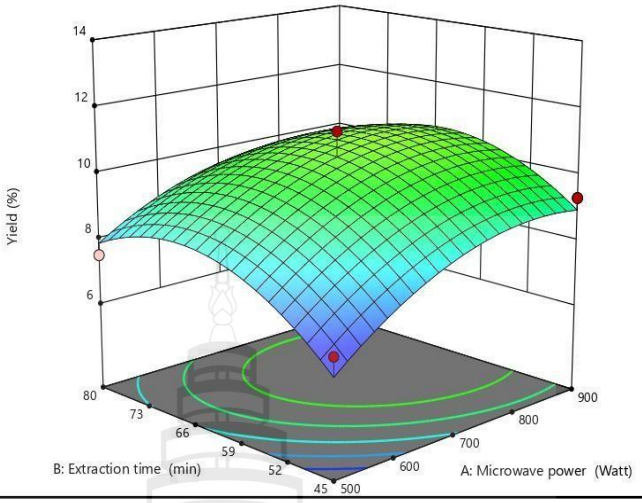
Table 4.4 shows estimated regression coefficients for bourbon variety coffee pulp extracts, yield is positively impacted by microwave power ($p < 0.05$) and sample-to-solvent ratio ($p < 0.05$), with negative impacts from their quadratic terms ($p < 0.05$). TPC is significantly reduced by extraction time ($p < 0.001$) but increased by sample-to-solvent ratio ($p < 0.01$). DPPH activity increases with sample-to-solvent ratio ($p < 0.01$) but decreases with microwave power and extraction time squared ($p < 0.01$). Fructose is significantly increased by microwave power ($p < 0.05$) and extraction time ($p < 0.001$), while galactose increases with microwave power and sample-to-solvent ratio ($p < 0.01$) but decreases with their quadratic terms ($p < 0.001$). The models show high explanatory power and fit, indicating reliable predictions.

Table 4.4 Estimated Regression Coefficients for Bourbon Variety Coffee Pulp Extracts for Yield, TPC, DPPH, Fructose, and Galactose

Term	Yield	TPC	DPPH	Fructose	Galactose
Intercept	10.52	27.7	5.2	5.78	1.35
Linear					
X_1 (Microwave power)	1.05*	-0.0225	-0.0338	0.6762*	0.1159**
X_2 (Extraction time)	0.5175	-2.13***	0.0837	1.26***	0.0252
X_3 (Sample-to-solvent ratio)	1.17*	1.25**	0.8450**	0.2	0.1379**
Quadratic					
X_1^2	-0.9754	-2.43*	-1.17**	-1.58**	-0.4094***
X_2^2	-1.45*	-4.76**	-1.06**	0.9175*	-0.1647**
X_3^2	0.3946	-1.76	0.1292	-0.2600	-0.1239*
Interaction					
X_1X_2	-0.2975	-0.835	-0.6500*	0.525	0.0248
X_1X_3	0.9125*	0.525	-0.4375	-0.6425**	-0.2175
X_2X_3	1.18**	1.52	1.05*	-0.1225	0.2607**
R^2	0.9207	0.9183	0.9054	0.9419	0.9837
Adj. R^2	0.7779	0.7711	0.7351	0.8374	0.9542
Pred. R^2	0.0675	-0.0833	-0.4566	0.2303	0.7492
A. Precision	9.7451	8.1065	7.2638	10.2926	20.1925
C.V %	9.1	7.54	16.46	12.05	7.08
Model p-value	0.027	0.0289	0.0402	0.0131	0.0006
Lack of Fit p-value	0.4253	0.2842	0.0652	0.2838	0.0706

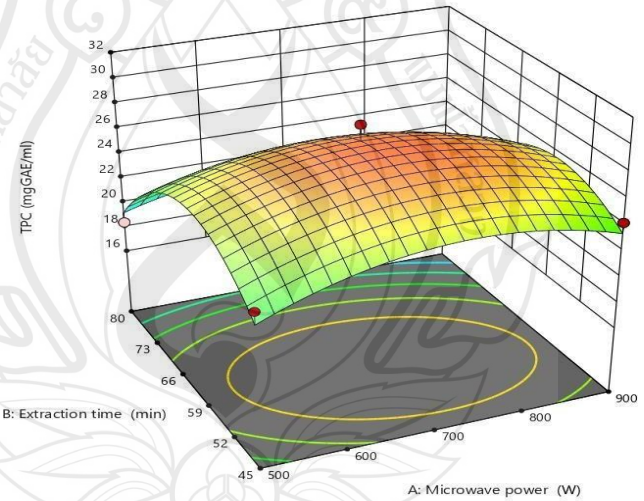
Note X_1: Microwave Power, X_2: Extraction Time, X_3: Sample to Solvent Ratio, R^2: Coefficient of Determination, Adj. R^2: Adjusted R^2 Pred. R^2: Predicted R^2, Adeq. Precision: Adequate Precision, C.V %: Coefficient of Variation %, Significance levels: * significant at $p < 0.05$

(a)



$$\text{Yield \%} = 9.23667 + 0.44125\ X_1 + 0.30375\ X_2 + 1.075\ X_3 - 0.43\ X_1X_2 + 0.3575\ X_1X_3 + 1.1025\ X_2X_3 + -0.915833\ X_1^2 - 0.890833\ X_2^2 - 0.0383333\ X_2^2$$

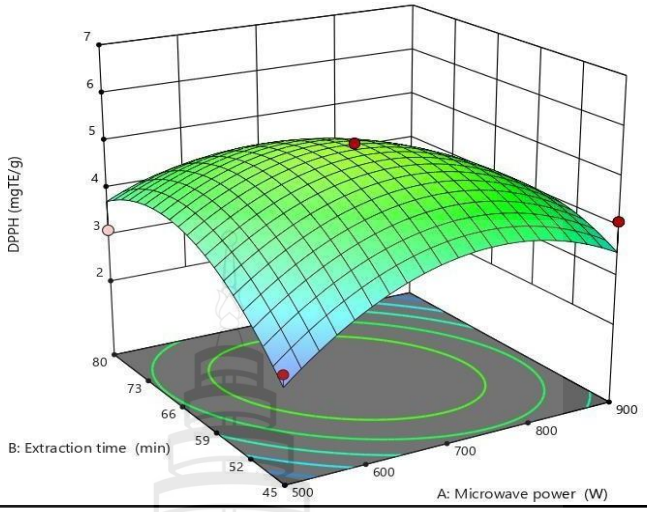
(b)



$$\text{TPC} = 27.6967 - 0.0225\ X_1 - 2.13125\ X_2 + 1.25375\ X_3 - 0.835\ X_1X_2 + 0.525\ X_1X_3 + 1.5225\ X_2X_3 - 2.43208\ X_1^2 - 4.76458\ X_2^2 - 1.75958\ X_3^2$$

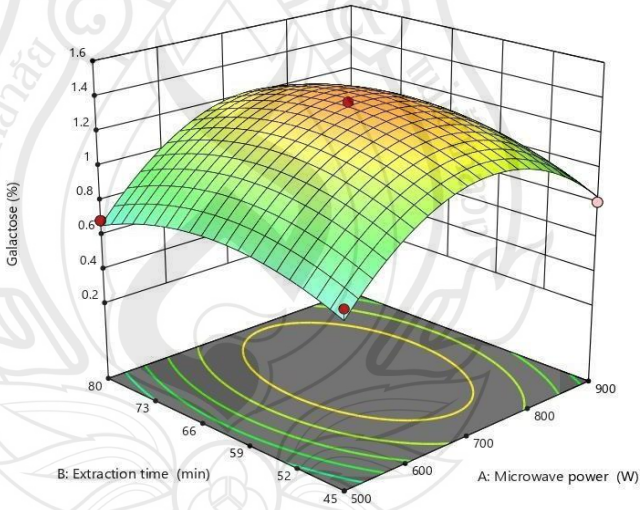
Figure 4.2 3D surface plot showing the effects of microwave power (A) and extraction time (B) on the (a), (b), (c), (d), and (e) content of Geisha and (f), (g), (h), (i), and (j) content of Bourbon variety coffee pulp extract respectably, with the sample-to- solvent ratio held constant at 1:10 g/mL

(c)



$$DPPH = 5.19667 - 0.03375\ X_1 + 0.08375\ X_2 + 0.845\ X_3 + -0.65\ X_1X_2 - 0.4375\ X_1X_3 + 1.0525\ X_2X_3 - 1.16833\ X_1^2 - 1.06333\ X_2^2 + 0.129167\ X_3^2$$

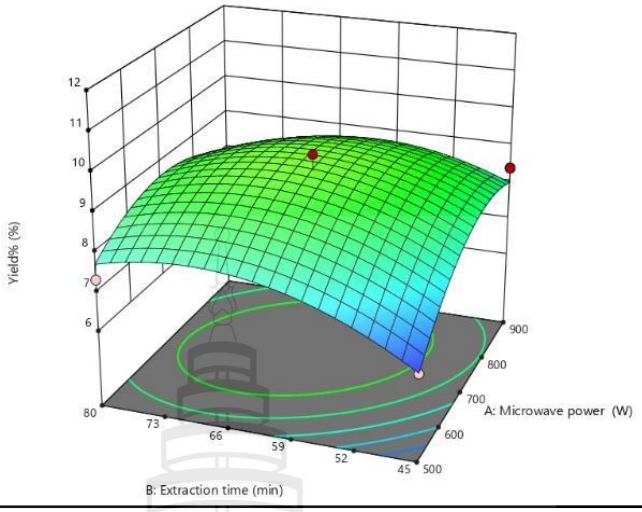
(d)



$$Galactose = 1.34933 + 0.115875\ X_1 + 0.02525\ X_2 + 0.137875\ X_3 + 0.02475\ X_1X_2 - 0.2175\ X_1X_3 + 0.26075\ X_2X_3 - 0.409417\ X_1^2 + -0.164667\ X_2^2 + -0.123917\ X_3^2$$

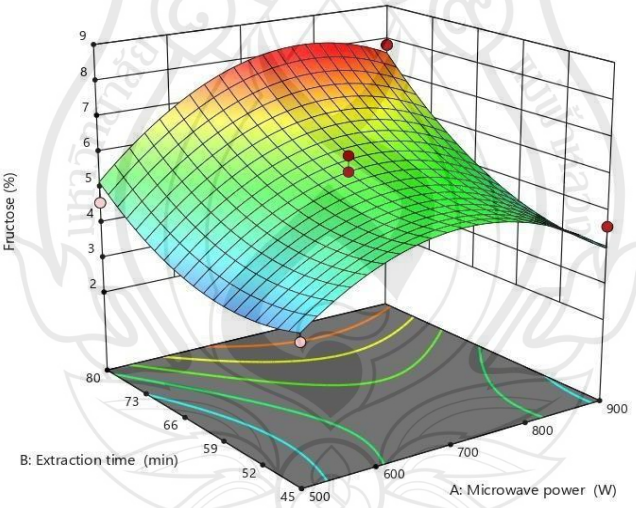
Figure 4.2 (continued)

(e)



$$\text{Fructose} = 5.78 + 0.67625\ X_1 + 1.26375\ X_2 + 0.2X_3 + 0.525\ X_1X_2 - 0.6425\ X_1X_3 - 0.1225\ X_2X_3 - 1.5775\ X_1^{\,2} + 0.9175\ X_2^{\,2} - 0.26X_3^{\,2}$$

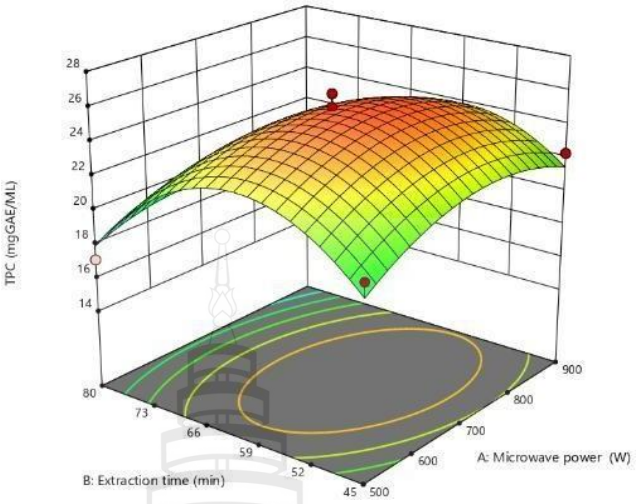
(f)



$$\text{Yield \%} = 9.23667 + 0.44125 * X_1 + 0.30375 * X_2 + 1.075\ X_3 - 0.43\ X_1X_2 + 0.3575\ X_1X_3 + 1.1025\ X_2X_3 - 0.915833\ X^2 - 0.890833\ X^2 - 0.0383333\ X^2$$

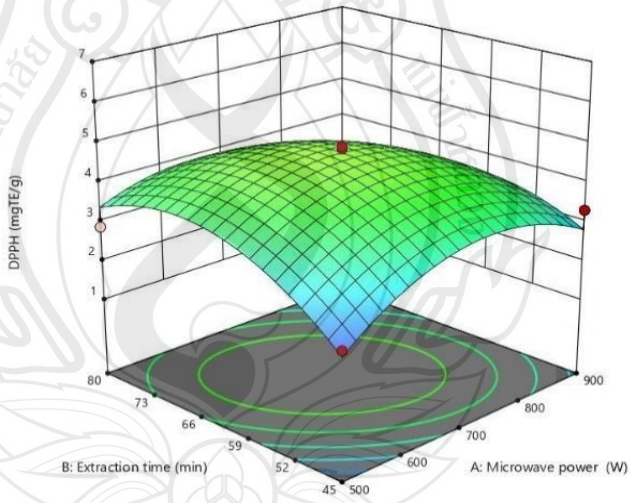
Figure 4.2 (continued)

(g)



$$TPC = 25.4067 + 0.1425\ X_1 + -1.63375\ X_2 + 1.24875\ X_3 - 0.7475\ X_1X_2 + 0.5925\ X_1X_3 + 1.46\ X_2X_3 + -1.92333\ X_1 + -4.52083\ X_2 + -1.91083\ X^2$$

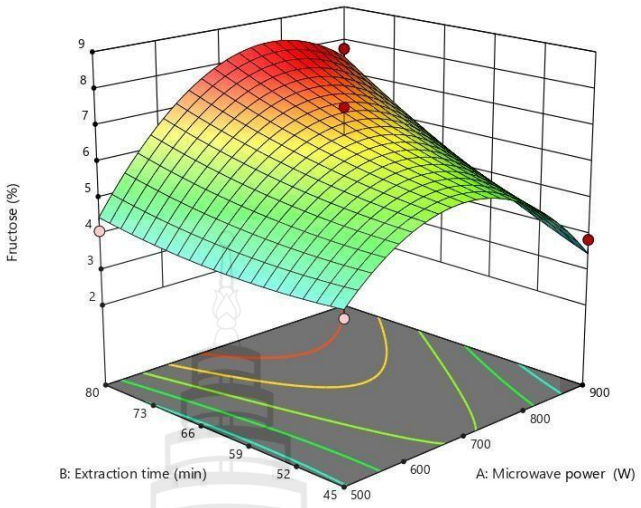
(h)



$$DPPH = 4.76333 + -0.13125\ X_1 + 0.15125\ X_2 + 0.8525\ X_3 - 0.5375\ X_1X_2 - 0.42\ X_1X_3 + 1.075\ X_2X_3 - 1.11292\ X_1^2 - 1.09292\ X_2^2 + 0.224583\ X_3^2$$

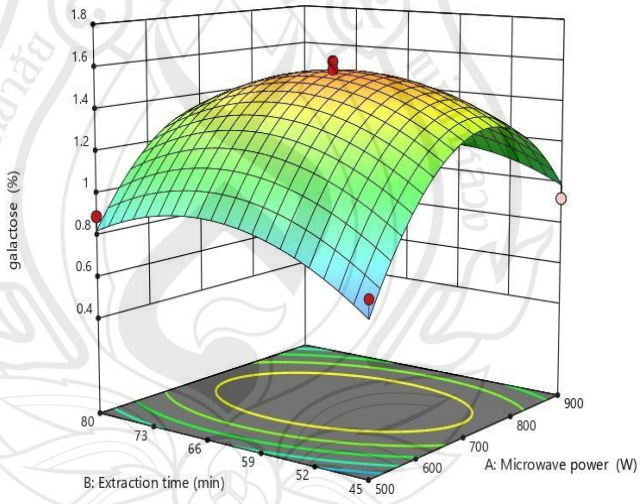
Figure 4.2 (continued)

(i)



$$\text{Fructose} = 6.76333 + 0.57\,X_1 + 1.0775X_2 + 0.2625\,X_3 + 0.94\,X_1X_2 - 0.805\,X_1X_3 - 0.21\,X_2X_3 - 2.09417X_1^{\,2} + 0.250833\,X_2^{\,2} - 0.489167\,X_3^{\,2}$$

(j)



$$\text{Galactose} = 1.55667 + 0.092875\,X_1 + 0.008375\,X_2 + 0.12875\,X_3 - 0.08575\,X_1X_2 + -0.1\,X_1X_3 + 0.3275\,X_2X_3 + -0.516708\,X_1^{\,2} - 0.219208\,X_2^{\,2} - 0.104958\,X_3^{\,3}$$

Figure 4.2 (continued)

Table 4.5 presents the optimal MAE conditions and corresponding desirability scores for the Geisha coffee pulp variety. The experiments were conducted using a Box-Behnken Design within the RSM framework, focusing on microwave power (X_1), extraction time (X_2), and sample-to-water ratio (X_3). All runs yielded consistent values for bioactive compounds, including total phenolic content (TPC), antioxidant activity (DPPH), fructose, and galactose, with a maximum desirability score of 0.84. The most favorable condition was identified at 708.70-Watt, 72.20 min, and a 1:10 ratio, which was selected based on its optimal yield and highest desirability.

Table 4.5 Optimal Microwave-Assisted Extraction Conditions and Desirability Scores for Geisha Variety Coffee Pulp Bioactive Compounds

No	X_1	X_2	X_3	Yield%	TPC	DPPH	Fructose	Galactose	Desirability	Score
1	708.70	72.20	10	12.60	25.30	6.40	6.60	1.40	0.84	Selected
2	709.80	72.30	10	12.60	25.30	6.40	6.70	1.40	0.84	
3	708.00	72.10	10	12.60	25.40	6.40	6.60	1.40	0.84	
4	707.00	72.30	10	12.60	25.30	6.40	6.70	1.40	0.84	
5	710.00	72.00	10	12.60	25.40	6.40	6.60	1.40	0.84	
6	708.50	72.50	10	12.60	25.20	6.40	6.70	1.40	0.84	
7	708.30	71.90	10	12.60	25.40	6.40	6.60	1.40	0.84	
8	704.90	72.20	10	12.60	25.30	6.40	6.60	1.50	0.84	
9	712.60	72.20	10	12.60	25.30	6.40	6.60	1.50	0.84	
10	712.50	71.90	10	12.60	25.40	6.40	6.60	1.40	0.84	

Table 4.6 displays the optimal microwave-assisted extraction (MAE) conditions and corresponding desirability scores for the Bourbon coffee pulp variety. Using a Box-Behnken Design under the Response Surface Methodology (RSM), experiments were carried out by varying microwave power (X_1), extraction time (X_2), and a fixed sample-to-water ratio (X_3 = 1:10). The runs consistently resulted in desirable yields of bioactive compounds, including TPC, DPPH, fructose, and galactose, with the highest desirability score of 0.87. The selected optimal condition was 699.20 W microwave power, 71.00 minutes extraction time, and a 1:10 ratio.

Table 4.6 Optimal Microwave-Assisted Extraction Conditions and Desirability Scores for Geisha Variety Coffee Pulp Bioactive Compounds

No	X_1	X_2	X_3	Yield%	TPC	DPPH	Fructose	Galactose	Desirability	Score
1	699.20	71.00	10	10.70	23.50	6.10	7.00	1.60	0.87	Selected
2	700.10	70.90	10	10.70	23.60	6.10	7.00	1.60	0.87	
3	698.40	70.80	10	10.70	23.60	6.10	7.00	1.60	0.87	
4	700.20	71.20	10	10.70	23.50	6.10	7.00	1.60	0.87	
5	697.70	71.20	10	10.70	23.50	6.10	7.00	1.60	0.87	
6	696.10	70.90	10	10.70	23.50	6.10	7.00	1.60	0.87	
7	696.90	70.70	10	10.70	23.60	6.10	6.90	1.60	0.87	
8	696.50	70.50	10	10.70	23.70	6.10	6.90	1.60	0.87	
9	703.80	71.60	10	10.70	23.40	6.10	7.00	1.60	0.87	
10	689.30	71.40	10	10.70	23.40	6.20	7.00	1.60	0.87	

Table 4.7 outlines the ideal conditions for MAE of Geisha coffee pulp, highlighting the key parameter ranges that most effectively enhance the recovery of bioactive compounds. The best extraction conditions 708.70-Watt microwave power, 72.30 min, and a 1:10 sample-to-solvent ratio led to a predicted yield of 12.66%. TPC reached 25.36mg GAE/g, while DPPH measured 6.43mg TE/g, emphasizing the notable presence of polyphenols.

Table 4.7 Optimal Condition for Microwave-Assisted Extraction Conditions for Bioactive Compounds from Geisha Coffee Pulp

Variables	Lower Limit	Upper Limit	Optimum
Microwavepower (Watt)	500	900	708.7
Extraction time (min)	45	80	72.3
Sample-to-solvent ratio (g/mL)	1	10	1:10
Yield (%)	6.8	13.11	12.66
TPC (mg GAE/g)	16.32	28.94	25.36
DPPH (mgTE/g)	2.12	6.89	6.43
Fructose (%)	2.84	7.96	6.65
Galactose (%)	0.322	1.52	1.46

Moreover, the extraction process preserved 6.65% fructose and 1.46% galactose, showcasing its effectiveness in retaining essential sugars. These results highlight how optimizing MAE parameters improves both extraction yield and the functional qualities of the final product.

Table 4.8 presents the optimization study for Bourbon coffee pulp, examining various microwave power levels, extraction times, and sample- towater ratios. The most effective conditions 699.30-Watt, 71.05 min., and a 1:10 ratio achieved a desirability score of 0.87, resulting in a 10.74% extraction efficiency. TPC reached 23.57mg GAE/g, DPPH measured 6.18mg TE/g, while fructose and galactose levels were 7.02 g/L and 1.69 g/L, respectively. Similar tested conditions yielded nearly identical desirability scores, ranging from 0.87 to 0.87, indicating that the optimized parameters reliably enhance extraction efficiency with minimal variation. This consistency highlights the strength of the MAE process in efficiently extracting bioactive compounds from coffee pulp.

Table 4.8 Optimal Condition for Microwave-Assisted Extraction Conditions for Bioactive Compounds from Bourbon Coffee Pulp

Variables	Lower Limit	Upper Limit	Optimum
Microwavepower (Watt)	500	900	699.26
Extraction time (min)	45	80	71.05
Sample-to-solvent ratio (g/mL)	1	10	1:10
Yield (%)	6.2	11.41	10.74
TPC (mg GAE/g)	14.62	26.29	23.57
DPPH (mgTE/g)	1.89	6.43	6.18
Fructose (%)	2.63	7.85	7.02
Galactose (%)	0.54	1.71	1.69

4.1.1 The Effects of Extraction Conditions on the Yield

The highest extraction yield for Geisha coffee pulp (12.6%) was achieved at 708.7-Watt microwave power, 72.3 min, and a 1:10 g/mL sampleto-solvent ratio (Table 4.7). Similarly, Bourbon coffee pulp yielded 10.74% under 699.3-Watt, 71.1 min, and a 1:10 ratio (Table 4.8). These findings align with previous research, indicating that moderate microwave power (650–750 Watt) with controlled exposure

time enhances extraction efficiency while preventing excessive degradation (González-González et al., 2022).

High-energy exposure leads to oxidation, polymerization, and molecular breakdown, reducing the functional properties of bioactive compounds (Tran et al., 2020). These trends are consistent with previous studies showing that exceeding optimal microwave conditions may enhance extraction efficiency but also accelerate the degradation of thermolabile compounds (Chen et al., 2023; Kim et al., 2021). A statistical model confirmed that microwave power (X_1), extraction time (X_2), and sample-tosolvent ratio (X_3) interact to optimize extraction yield. Maintaining a balance among these parameters is crucial to maximizing efficiency while preserving compound stability (Wong & Nillian, 2023) Notably, a higher sample-tosolvent ratio (1:10) improved extraction yield, likely due to better solute diffusion and solvent penetration into the coffee pulp matrix (Salamatullah et al., 2021).

4.1.2 The Effects of Extraction Conditions on Total Phenolic Content and Antioxidant Activity

The extraction of total phenolic content and antioxidant activity is a multi-stage process influenced by microwave settings, solvent availability, and the structural characteristics of coffee pulp. Initially, microwave irradiation disrupts the cell walls, allowing polyphenols to diffuse into the solvent. Under optimized conditions, Geisha coffee pulp demonstrated a higher polyphenol concentration (25.36 mg GAE/g) than Bourbon (23.57 mg GAE/g) (Tables 4.7 and 4.8), suggesting that Geisha's cellular matrices break down more efficiently. This effect is further enhanced by microwave-induced pressure fluctuations, which promote solvent penetration into plant tissues, accelerating the release of bioactive compounds (Doulabi & Golmakani, 2020). As the extraction progresses, antioxidant activity increases due to the improved solubilization of free and bound polyphenols. The DPPH radical scavenging assay showed that Geisha pulp exhibited 6.43 mg TE/g DW, while Bourbon pulp yielded 6.18 mg TE/g DW, likely due to differences in polyphenol composition and structural integrity. Microwave radiation plays a crucial role in this stage, stimulating molecular movement to enhance the extraction rate while minimizing oxidative degradation (Kim et al., 2021; Roselló-Soto et al., 2015). In the final phase, the stabilization of phenolic compounds occurs through solvent diffusion. At this stage, the sample-

tosolvent ratio (X_3) significantly influences the retention of bioactive compounds. A higher solvent volume improves mass transfer efficiency, ensuring polyphenols and antioxidants remain soluble and bioavailable rather than undergoing precipitation or thermal degradation (Dai & Mumper, 2010; Wong, Tan, & Ho, 2013). Controlled microwave exposure at this stage prevents overheating, reducing oxidative stress that could otherwise compromise the antioxidant potential of the final extract (Wong & Nillian, 2023). By maintaining moderate microwave power and precise exposure times, the process optimizes both phenolic yield and antioxidant stability, further highlighting the effectiveness of MAE for recovering bioactive compounds from coffee pulp (Tran et al., 2020).

4.1.3 The Effects of Extraction Conditions on Sugar Content: Fructose and Galactose

Fructose and galactose were analyzed due to their nutritional and functional roles in food products. These sugars contribute to sweetness, possess prebiotic benefits, and help assess the potential of coffee pulp for use in functional foods and natural ingredients. The sugar composition of Bourbon and Geisha coffee pulps showed distinct differences, reflecting variations in solubility, extraction efficiency, and functional potential. Bourbon coffee pulp had a higher fructose concentration (7.02%) than Geisha (6.65%), suggesting better solubility within Bourbon's cellular matrix. In contrast, Geisha pulp had a higher galactose content (1.69%) compared to Bourbon (1.46%), studies have characterized the free-sugar profile of coffee pulp, highlighting its potential as a source of functional monosaccharides. Teixeira and Mussatto (2011) isolated the sugar fraction from Arabica coffee pulp (6.64 g/100 g sample) and reported fructose and glucose concentrations of 7.34 and 4.52 mg/g extract, respectively (Urbaneja et al., 1996). Due to structural differences affecting sugar retention and release (Yan et al., 2023). These differences suggest potential applications, with Bourbon pulp being more suitable for sweetener-based formulations, while Geisha may be better suited for polysaccharide-based functional foods and prebiotic development (Bhandarkar et al., 2021; Cangussu et al., 2021). Beyond varietal differences, extraction parameters significantly influenced sugar yield. The statistical model indicated that microwave exposure time (X_2) and the sample-to-solvent ratio (X_3) positively affected fructose recovery, whereas excessive

microwave power (X_1) had a negative impact (Tables 4.3 and 4.4). This aligns with previous research showing that moderate microwave settings enhance sugar solubilization, while prolonged high-energy exposure leads to degradation (Fideles et al., 2022; Zhang et al., 2021). Maintaining microwave power between 650–700Watt was found to optimize sugar preservation, preventing degradation caused by caramelization and thermal decomposition (Tran et al., 2020). While moderate power maximizes sugar recovery, excessive heating can alter sugar structures, reducing bioavailability and functionality (Zhou et al., 2020). The study further highlights microwave-assisted extraction MAE as a sustainable and efficient alternative to traditional solvent-based methods. Although water as a solvent yielded slightly lower sugar extraction compared to organic solvents, it remains a cost-effective, environmentally friendly, and scalable option (Chemat et al., 2017; Roselló-Soto et al., 2015). Additionally, MAE significantly reduces extraction time and energy consumption, making it a viable choice for industrial-scale applications (Kim et al., 2021). Comparing these findings with conventional methods confirms that MAE offers a balance between efficiency and sustainability, reinforcing its effectiveness in bioactive compound recovery from coffee by-products Finally, the study identified the ideal microwave power settings as 708.7 Watt for Geisha and 699.3 Watt for Bourbon, which resulted in the highest concentrations of phenolic compounds and antioxidant activity. Microwave power levels around 700 Watt proved optimal for breaking down cellular structures through intracellular boiling, a process that enhances the release of bioactive compounds by effectively disrupting plant cell walls (González-González et al., 2022; Wong & Nillian, 2023). Research suggests this method significantly improves polyphenol recovery compared to traditional extraction techniques (López-Salazar et al., 2023). Higher power levels also facilitate faster solvent penetration and diffusion, optimizing extraction efficiency (Lovrić et al., 2017). However, excessive exposure to high microwave energy can lead to thermal degradation of heat-sensitive compounds, causing structural breakdown and reduced bioactivity (Khristian, 2021; Tran et al., 2022). Previous studies highlight the importance of balancing microwave intensity to maximize efficiency while minimizing oxidative damage (Salamatullah et al., 2021; Sangta et al., 2021). Preserving antioxidants is particularly crucial for maintaining the functional properties

of coffee pulp extracts, emphasizing the need for carefully controlled processing conditions (Tran et al., 2020).

Extraction duration is a critical variable in Microwave-Assisted Extraction, affecting both extraction yield and the stability of bioactive compounds. Optimized extraction times of 72.1 min for Bourbon coffee pulp and 72.3 min for Geisha coffee pulp ensure effective cell wall rupture while minimizing thermal degradation of sensitive bioactive compounds. Studies suggest that extraction times of around 70 min maximize antioxidant activity and polyphenol yield in coffee pulp (Bollamma, 2023; Braojos et al., 2020). Controlled microwave irradiation enhances mass transfer rates and improves polyphenol recovery while reducing oxidative losses (Wang et al., 2022) Carefully controlling extraction intervals in microwave-assisted extraction is essential to prevent the degradation of bioactive compounds and preserve their properties. Research highlights that prolonged microwave exposure can break down sensitive compounds, emphasizing the need for precise timing (Quintero-Quiroz et al., 2019). Prolonged extraction times in microwaveassisted extraction can degrade polyphenols and flavonoids through oxidation, depolymerization, and structural breakdown, leading to by-product formation and reduced functionality of coffee pulp extracts. This highlights the need for precise time management to retain bioactive compounds while minimizing degradation risks (Pyrzynska, 2024). The sample-to-solvent ratio plays a crucial role in Microwave-Assisted Extraction, impacting solubility, diffusion, and mass transfer of bioactive compounds. An optimal ratio enhances extraction efficiency while avoiding solvent saturation or excessive dilution, both of which can reduce yield and compound stability. A 1:10 g/mL ratio provided the highest bioactive compound recovery by ensuring sufficient solvent for solubilization and maintaining an ideal concentration gradient (Buck et al., 2021). Increasing the ratio to 1:8 g/mL further improved polyphenol recovery, but excessive solvent use may dilute extracts, requiring additional processing (Predescu et al. 2016). Conversely, higher sample- tosolvent ratios risk saturation, limiting solute solubility and reducing extraction efficiency (Ince et al., 2022; Zhou et al., 2020). Maintaining an optimal solvent-to-sample ratio is essential for maximizing bioactive yield and ensuring efficient processing.

4.2 Characterization of Coffee Pulp Extract from Optimum Extraction Conditions

4.2.1 Scope of Extraction and Bioactivity Evaluation

This section of the study compares optimized MAE and Conventional extraction methods applied to Bourbon and Geisha coffee pulp, to recover phenolic compounds and antioxidants. Antioxidant activity was assessed via ABTS, DPPH, FRAP, and TPC assays, as well as enzyme inhibition assays for α-amylase and α-glucosidase. The study further incorporates a mass spectrometry based phenolic profiling to reveal the specific bioactive constituents and their relative abundance. It aims to show by analyzing extraction yields and chemical diversity of those obtained, to establish the effectiveness of MAE in increasing coffee pulp's nutritional and functional potential.

TPC was significantly higher in the MAE samples (Figure 4.3), Geisha MAE yielded 25.36 ± 0.5 mg GAE/g, and Bourbon MAE had 24.50 ± 0.3 mg GAE/g. These were notably higher than Geisha Conv. Ext. (21.3 ± 0.4 mg GAE/g) and Bourbon Conv. Ext. (22.50 ± 0.4 mg GAE/g). This reflects a 21% increase in phenolic recovery for Geisha and 10% increase for Bourbon due to MAE. In comparison, Nillian et al. (2023) found TPC values of 24.9 mg GAE/g from MAE-extracted Liberica coffee pulp, while Tran et al. (2022). Figure 4.3 Total Phenolic Content (TPC) of Geisha and Bourbon coffee pulp extracts as determined by MAE and Conv. Ext., expressed in mg GAE/g reported 22.0 mg GAE/g using conventional methods. These figures align well with our data and support the claim that MAE improves phenolic yield by breaking down cell wall structures and releasing phenolics bound to polysaccharides or proteins.

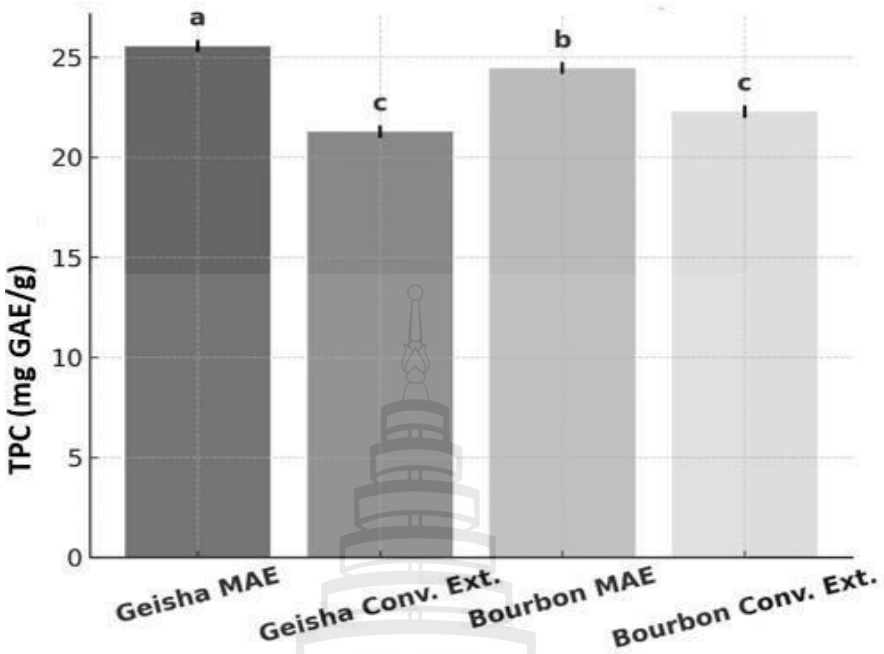


Figure 4.3 Total Phenolic Content (TPC) of Geisha and Bourbon coffee pulp extracts by MAE and conventional extraction, expressed in mg GAE/g DW. Values are mean ± SD (n = 3). Different letters indicate significant differences ($p < 0.05$).

The DPPH assay showed a similar enhancement pattern. Bourbon MAE and Geisha MAE recorded DPPH scavenging values of 7.56 ± 0.8 mg TE/g and 7.51 ± 0.6 mg TE/g, respectively, while the conventional counterparts were significantly lower (3.61 ± 0.7 mg TE/g for Bourbon and 3.16 ± 0.9 mg TE/g for Geisha). This indicates that MAE led to over a twofold increase in DPPH scavenging activity. Comparable studies support this outcome. Nillian et al. (2023) reported DPPH values of 6.1 mg TE/g from Liberica coffee pulp extracted via MAE, and Tran et al. (2022) reported 4.3 mg TE/g using conventional extraction of coffee pulp. These values suggest that our DPPH results are within the expected range for MAE and higher than traditional methods, reinforcing the improved radical scavenging efficiency due to MAE.

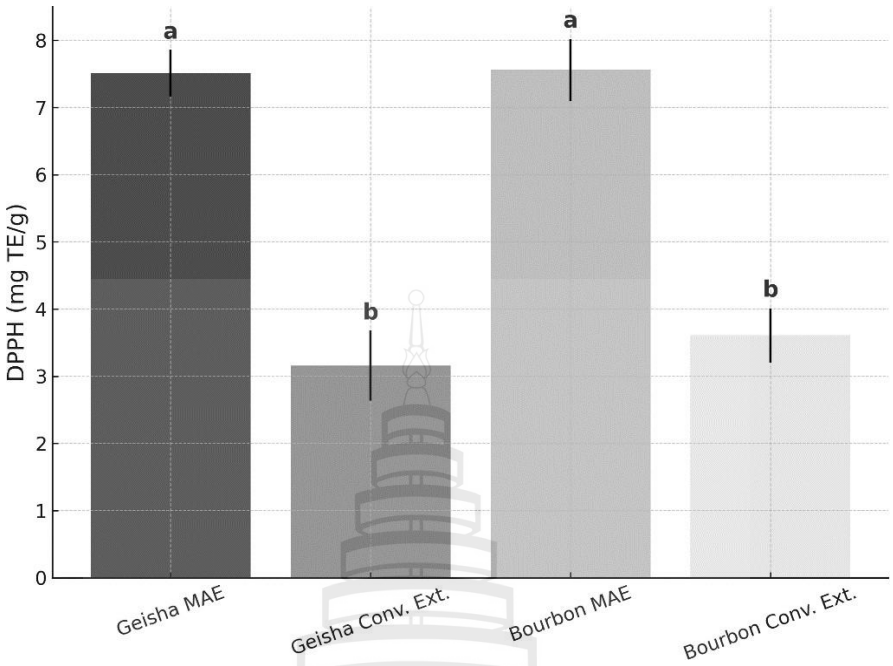


Figure 4.4 DPPH radical scavenging activity of Geisha and Bourbon coffee pulp extracts using MAE and conventional extraction, expressed in mg TE/g. Values are mean ± SD (n = 3). Different letters indicate significant differences (ANOVA, DMRT, $p < 0.05$).

In this study, Geisha MAE exhibited the highest ABTS radical scavenging activity at 33.78 ± 0.6 mg TE/g, followed by Bourbon MAE (32.43 ± 0.5 mg TE/g). The conventional extracts were significantly lower: Geisha Conv. Ext. recorded 21.25 ± 0.5 mg TE/g, and Bourbon Conv. Ext. showed 20.89 ± 0.4 mg TE/g. These results are consistent with Thaiphanit et al. (2022), who reported an ABTS value of 32.0 mg TE/g in MAE-extracted coffee pulp, and Oliveira et al. (2021), who found 21.1 mg TE/g using conventional methods. The similarity between our MAE results and those reported in literature reinforces the role of microwave energy in enhancing the release of antioxidant compounds from the coffee pulp matrix, primarily by disrupting cell walls and facilitating mass transfer of bound phenolics

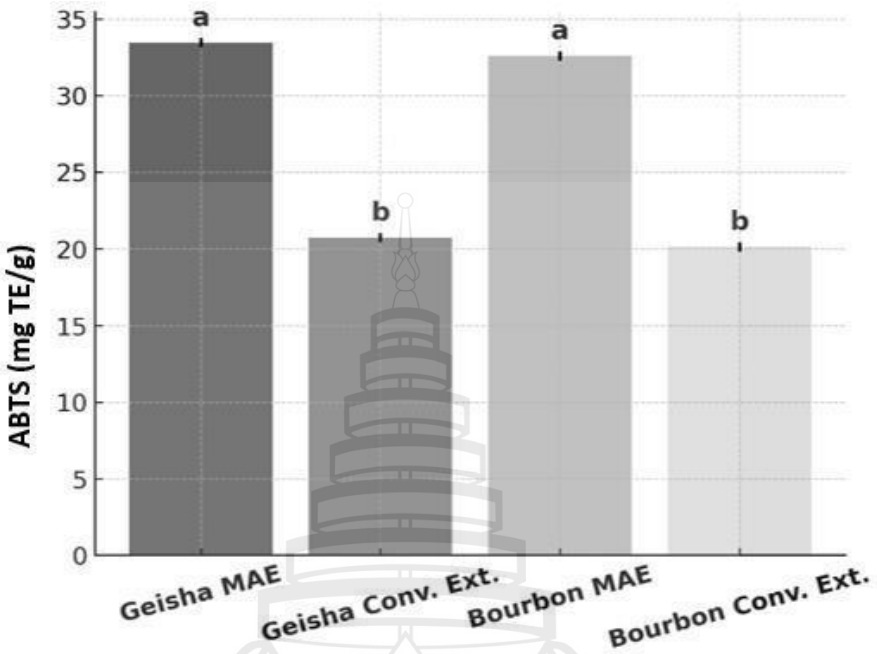


Figure 4.5 ABTS radical scavenging activity of Geisha and Bourbon coffee pulp extracts obtained by MAE and conventional extraction, expressed in mg TE/g. Values are mean ± SD (n = 3). Different letters indicate significant differences (ANOVA, DMRT, $p < 0.05$).

The FRAP values were highest in Geisha MAE (4.56 ± 0.3 mg TE/g), followed by Bourbon MAE (4.12 ± 0.2 mg TE/g). In contrast, Geisha Conv. Ext. and Bourbon Conv. Ext. exhibited values of 2.44 ± 0.2 mg TE/g and 2.32± 0.1 mg TE/g, respectively. This nearly doubled the ferric reducing power in both varieties when extracted with MAE. Our findings are strongly aligned with those of Thaiphanit et al. (2022), who recorded a FRAP value of 4.2 mg TE/g using MAE for coffee pulp, and Oliveira et al. (2021), who found 2.4 mg TE/g with conventional methods. The results support the hypothesis that MAE recovers more redox-active compounds particularly phenolics capable of reducing Fe^{3+} to Fe^{2+} than traditional solvent extraction.

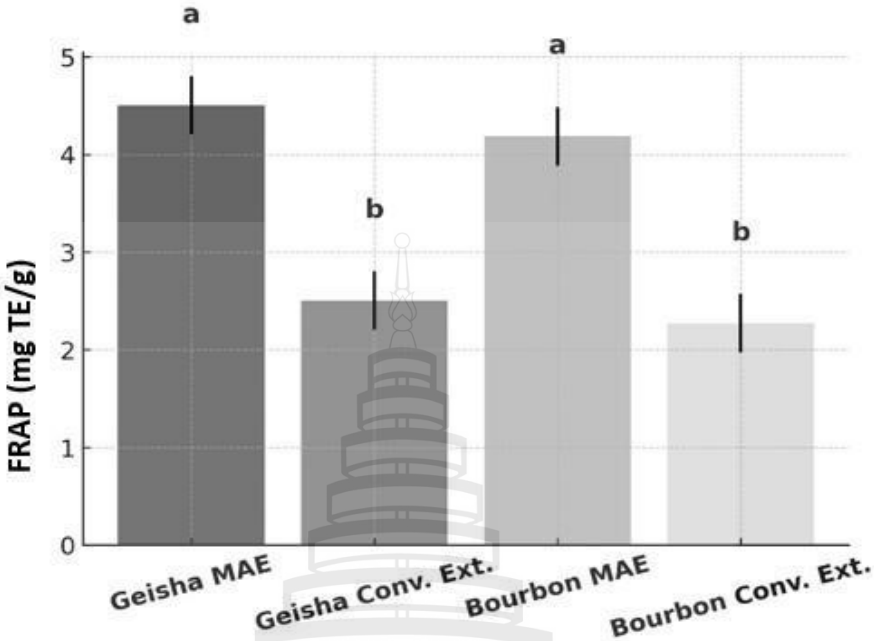


Figure 4.6 Ferric reducing antioxidant power (FRAP) of Geisha and Bourbon coffee pulp extracts obtained by MAE and conventional extraction, expressed in mg TE/g. Values are mean ± SD (n = 3). Different letters indicate significant differences (ANOVA, DMRT, $p < 0.05$).

MAE significantly improved the recovery of antioxidant compounds from coffee pulp compared to conventional solvent extraction. In both Bourbon and Geisha varieties, MAE extracts exhibited higher antioxidant activity, as indicated by increases in ABTS radical scavenging capacity (from 20.89 to 32– 34 g Trolox equivalents per 100 g), DPPH scavenging activity (from 4.5–4.6 to 6.3–6.4 mg Trolox equivalents per gram), and ferric reducing antioxidant power (from 2.32 to 4.12 mg/mL). TPC also increased slightly, from 21.3–22.5 to 24.5–25.8 mg gallic acid equivalents per gram.

The enhanced antioxidant activity corresponds with the increased TPC, consistent with the role of phenolic compounds in contributing to antioxidant capacity through their redox behavior. The greater relative increase in ferric reducing power suggests that MAE may favor the extraction of phenolics with strong reducing potential. Although DPPH and ABTS values were relatively high in control samples, all antioxidant measures showed a positive correlation with TPC, affirming the

central role of phenolic compounds. These findings align with previous research (Hu et al., 2023; Lestari et al., 2022).

4.2.2 Phenolic Compound Profile and Extraction Method Comparison

Phenolic profiling of coffee pulp from Bourbon and Geisha varieties, extracted via microwave-assisted and conventional methods, revealed a broad spectrum of bioactive compounds. Most were identified in the positive ionization mode $[M+H]^+$, with fewer in the negative mode $[M–H]^–$. Consistently detected compounds included caffeine, trigonelline, and daidzein- 4'-sulfate, with caffeine exhibiting a stable m/z of 194. Flavonoids and alkaloids, such as 3,3',4',5,6,7,8-heptahydroxyflavone and hibiscetin, were observed in the negative mode, suggesting deprotonation due to functional group composition. Fenofibric acid appeared in both modes, underscoring the chemical diversity. The alignment of m/z values with molecular weights confirmed compound identities and demonstrated the efficiency of MAE in extracting a wider array of bioactives with higher identification.

Table 4.9 Phenolic Compound Profile of Bourbon Using Microwave-Assisted Extraction

Compound Name	Formula	RT (min)	Ionization	Score (%)	Observed m/z	RA (%)
Caffeine	$C_8H_{10}N_4O_2$	9.078	[M+H] +	94.96	194.0780	21.20
Trigonelline	$C_7H_7NO_2$	1.735	[M+H] +	94.22	137.0466	21.11
Daidzein-4'sulfate	$C_{15}H_{10}O_7S$	2.748	[M+H] +	92.62	334.0151	20.93
Caffeine sodium salicylate	$C_{15}H_{15}N_4NaO_5$	8.948	[M+H] +	98.58	354.0940	21.63
3,9-Dimethyluric acid	$C_7H_8N_4O_3$	1.801	[M-H] -	94.68	196.0601	21.17
Pteridine	$C_6H_4N_4$	1.852	[M-H] -	94.43	132.0437	21.14
3,3',4',5,6,7,8-Heptahydroxy flavone	$C_{15}H_{10}O_9$	2.700	[M-H] -	94.63	334.0321	21.16
2-Iodo-5methylbenzoic acid	$C_8H_7IO_2$	1.102	[M-H] -	97.59	261.9486	21.51
5-((2hydroxy-2methylpropoxy) carbonyl)-2- (hydroxymeth yl)-6-methyl-4-(2- nitrophenyl)-1,4dihydropyridine-3carboxylic acid	$C_{19}H_{22}N_2O_8$	3.694	[M+H] +	85.64	406.1368	20.10

Table 4.10 Phenolic Compound Profile of Bourbon Using a Conventional Method

Compound Name	Formula	RT (min)	Ionization	Score (%)	Observed m/z	RA (%)
Trigonelline	$C_7H_8N_2O_2$	1.727	$[M+H]^+$	94.2	137.046	17.69
Theophylline	$C_8H_{11}N_5O_6$	1.685	$[M+H]^+$	82.19	273.071	15.43
Caffeine	$C_8H_{10}N_4O_2$	9.085	$[M+H]^+$	96.26	194.077	18.07
Fenofibric acid	$C_{17}H_{15}ClO_4$	2.718	[M-H]-	90.34	318.065	16.96
N-Pyrazinylthioura	$C_5H_6N_4S$	5.927	$[M-H]^-$	88.01	154.03	16.52
Dimethyl disulfide	$C_2H_6S_2$	2.266	$[M+H]^-$	81.43	93.9907	15.29

Table 4.11 Phenolic Compound Profile of Geisha Coffee Pulp Extracted Using a Conventional Method

Compound Name	Formula	RT (min)	Ionization	Score (%)	Observed m/z	RA (%)
Trigonelline	$C_8H_{11}N_5O_6$	1.736	$[M+H]^+$	93.56	137.0465	14.62
Betaine	$C_8H_{10}N_4O_2$	1.770	$[M+H]^+$	95.33	117.0783	15.08
Caffeine	$C_{17}H_{15}ClO_4$	9.067	$[M+H]^+$	96.11	194.0778	15.29
Kojic acid	$C_5H_6N_4S$	2.779	$[M+H]^+$	94.99	142.0253	14.99

Table 4.12 Phenolic Compound Profile of Geisha Using Microwave- Assisted Extraction

Compound Name	Formula	RT (min)	Ionization	Score (%)	Observed m/z	RA (%)
Theophylline, 7hydroxymethyl-	$C_8H_{11}N_5O_6$	1.669	[M+H] +	81.5	273.071	19.19
Betaine	$C_5H_{11}NO_2$	1.737	[M+H] +	96.5	117.078	20.88
Trigonelline	$C_7H_8NO_2$	1.822	[M+H] +	94.3	137.044	20.63
Daidzein- 4'sulfate	$C_{15}H_{10}O_7S$	2.722	[M+H] +	88	334.015	19.92
Caffeine	$C_8H_{10}N_4O_2$	9.054	[M+H] +	96.5	194.078	20.88
3,3',4',5,6,7,8-Heptahydroxyflavone	$C_{15}H_{10}O_9$	2.718	[M-H]-	94.6	334.032	20.66
Quercetin	$C_{15}H_{10}O_9$	2.7	[M-H]-	94.6	334.032	20.66
8Hydroxyquercet a getin	$C_{15}H_{10}O_9$	2.7	[M-H]-	94.6	334.032	20.66

Table 4.12 (continued)

Compound Name	Formula	RT (min)	Ionization	Score (%)	Observed m/z	RA (%)
Caffeine sodium salicylate	$C_{19}H_{19}N_7O_6$	8.948	[M+H] +	98.5	354.094	21.1
Fenofibric acid	$C_{17}H_{15}ClO_4$	2.72	[M-H]-	47.6	318.065	15.36

Phenolic profiling of coffee pulp from Bourbon and Geisha varieties, extracted via microwave-assisted and conventional methods, revealed a broad spectrum of bioactive compounds. Most were identified in the positive ionization mode $[M+H]^+$, with fewer in the negative mode $[M–H]^–$. Consistently detected compounds included caffeine, trigonelline, and daidzein-4'-sulfate, with caffeine exhibiting a stable m/z of 194. Flavonoids and alkaloids, such as 3,3',4',5,6,7,8-heptahydroxyflavone and hibiscetin, were observed in the negative mode, suggesting deprotonation due to functional group composition. Fenofibric acid appeared in both modes, underscoring the chemical diversity. The alignment of m/z values with molecular weights confirmed compound identities and demonstrated the efficiency of MAE in extracting a wider array of bioactives with higher identification confidence.

MAE recovered a wider range of bioactive compounds, while the conventional method mainly extracts out a limited set, largely composed of alkaloids. Among all samples, core alkaloids are caffeine (m/z 194) and trigonelline (m/z 138) were consistently found, but they showed up in greater relative abundance in the MAE extracts, caffeine accounts about 21.2% of the total relative abundance in Bourbon MAE samples and 20.88% in Geisha MAE, compared to just 18.07% and 15.29%, respectively, in the conventionally extracted versions. Trigonelline followed the same pattern, with higher levels detected in the MAE samples. Interestingly, several compounds appeared only in the MAE extracts. Flavonoids like 3,3',4',5,6,7,8-heptahydroxyflavone, hibiscetin, and 8-hydroxyquercetagetin were exclusive to these samples and absent from those extracted conventionally. These phenolics, identified in negative ion mode, are wellregarded for their strong antioxidant and anti-inflammatory effects (Romualdo et al., 2018). Their absence in the conventional extracts highlights

MAE's superior ability to release complex or tightly bound phenolic compounds (Santos et al., 2021).

Common bioactive compounds like caffeine and trigonelline were found in samples from both extraction methods, though they were more abundant in the MAE samples. This aligns with earlier studies that highlight MAE's effectiveness in extracting alkaloids and phenolics, thanks to its rapid energy transfer and lower risk of thermal degradation (Mizuno et al., 2014). Other compounds daidzein-4'-sulfate and caffeine sodium salicylate were also detected in both methods but showed higher relative abundance in the MAE extracts. On the other hand, the conventional method mainly recovered alkaloids and small, water-soluble molecules, including theophylline, kojic acid, and dimethyl disulfide. While these are easier to extract with hot water, they don't offer the same antioxidant benefits as the larger flavonoids (Alboofetileh et al., 2017).

The type of coffee variety also played a key role in shaping both the phenolic profile and the effectiveness of the extraction method. Geisha pulp, in particular, stood out especially when extracted using MAE. It not only delivered a higher yield of phenolic compounds but also showcased greater chemical diversity compared to Bourbon pulp. MAE extracts from Geisha contained a wider range of unique compounds, including a richer array of flavonoids. Compounds including daidzein-4'-sulfate and flavonoids hibiscetin and quercetagetin derivatives appeared more prominently in Geisha samples. This points to genetic differences between the two varieties influencing their secondary metabolite profile, with Geisha pulp offering a deeper or more extractable pool of phenolics under optimized conditions (Hidalgo et al., 2020). Geisha MAE not only captured a broader spectrum of compounds but also edged out Bourbon MAE in total phenolic yield. This may be due to structural or compositional traits in Geisha pulp that make it more responsive to microwave-assisted breakdown, helping to release tightly bound phenolics more effectively. Previous studies have shown that coffee genotype can influence the concentration of phenolic subclasses of chlorogenic acids and flavonoids, which aligns well with the observed abundance and diversity in Geisha's profile.

The improved chemical profile observed with MAE also translated into significantly higher total phenolic content in the extracts. For both coffee varieties,

TPC was notably greater in the MAE samples, reinforcing the method's qualitative edge. In Bourbon pulp, MAE produced 24.5 ± 0.3 mg GAE/g, compared to 22.5 ± 0.4 mg GAE/g using the conventional method. Geisha pulp showed an even more pronounced boost, 25.8 ± 0.5 mg GAE/g with MAE, with 21.3 ± 0.4 mg GAE/g in the control. These numbers reflect a TPC increase of 9% for Bourbon and a striking 21% for Geisha under MAE, which aligns with earlier findings suggesting Geisha's phenolic compounds are especially responsive to microwave extraction. The differences between MAE and the conventional method were statistically significant ($p < 0.05$). These gains in TPC were also mirrored by higher antioxidant activity, as shown by both DPPH and ABTS assay results. The flavonoids identified in the extracts are well-known for their potent antioxidant and anti-inflammatory effects (Feng et al., 2020), while alkaloids both caffeine and trigonelline contribute cognitive and metabolic benefits (Zhang & Speakman, 2024). Its ability to preserve heat-sensitive bioactives and minim to nonuse of solvent makes it a sustainable and cost-efficient option for large-scale extraction.

4.2.3 Enzyme Inhibition Efficacy

The results clearly indicate that MAE produced coffee pulp extracts with significantly higher enzyme inhibitory activities than the conventional extraction for both Geisha and Bourbon varieties. In the Geisha pulp, MAE yielded 90% α-amylase inhibition, compared to 70% for the conventional extraction. A similar trend was observed in Bourbon (89% vs 68% inhibition). Figure 4.7 compares the αamylase inhibitory activity of Geisha and Bourbon coffee pulp extracts obtained by MAE and conventional extraction**.** The results show a dose-dependent increase in inhibition, with MAE extracts exhibiting greater potency than their control counterparts and reflected in lower IC_{50} values. Geisha MAE showed the strongest inhibition, suggesting its higher potential as a natural α-amylase inhibitor.

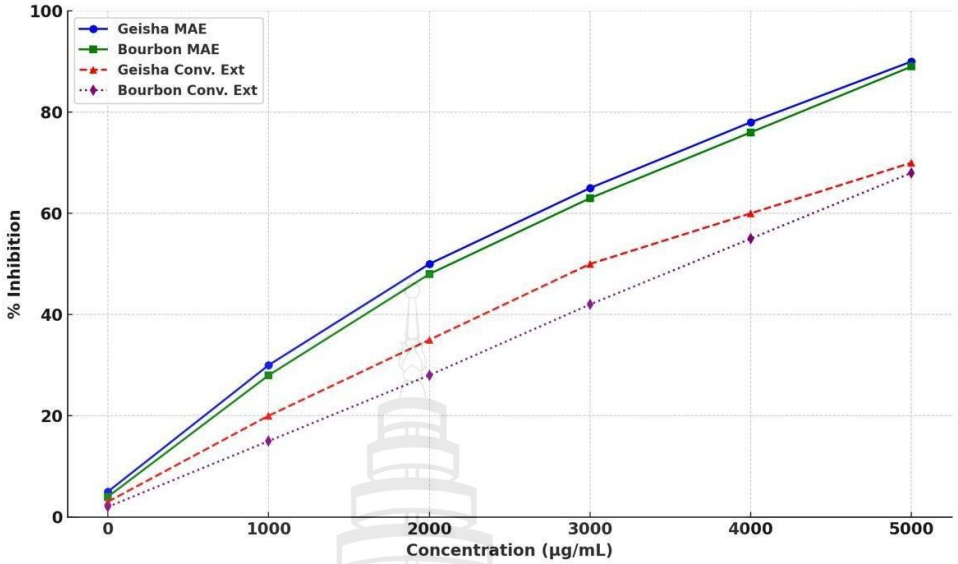


Figure 4.7 Dose–Response Curve for α-Amylase Inhibition by Geisha and Bourbon Coffee Pulp Extracts

Current study results indicated that MAE significantly enhanced the inhibition of α-amylase, achieving 90% inhibition for Geisha and 89% for Bourbon, compared to conventional hot water extracts, which showed comparatively lower activities (Geisha: 70%; Bourbon: 68%). These findings are supported by Nemzer et al. (2021), who reported a substantial inhibitory effect on α-amylase (IC_{50} = 1.74 mg/mL) from ethanol-water extracts of whole coffee cherry. Although their IC_{50} values indicate greater potency than our extracts (Geisha MAE IC_{50} 2.8 mg/mL; Bourbon MAE 3.1 mg/mL), differences may arise due to varietal differences, extraction solvents, or concentration methods. In contrast, Patil et al. (2022) reported relatively lower α-amylase inhibitory activity from aqueous coffee pulp extracts (IC_{50} = 13.2 mg/mL), suggesting the extraction efficiency and inhibitory potency of MAE observed in our study.

Table 4.13 IC_{50} Values of Coffee Pulp Extracts for Enzyme Inhibition

Sample	**Extraction Method**	**IC_{50} (mg/mL)**
Geisha Coffee Pulp	MAE	2.8
Bourbon Coffee Pulp	MAE	3.1
Geisha Coffee Pulp	Conventional Extraction	4.7
Bourbon Coffee Pulp	Conventional Extraction	5.1

Coffee pulp extracts, including those from Geisha and Bourbon varieties, inhibit α-amylase activity primarily due to bioactive compounds such as chlorogenic acids, caffeine, and other polyphenols. These compounds may inhibit α-amylase through competitive inhibition, where they compete with starch for the enzyme's active site, or non-competitive inhibition, where they alter the enzyme's structure without competing for the active site. In some cases, irreversible inhibition may occur if compounds bind covalently to the enzyme. Chlorogenic acids, in particular, are the main inhibitors responsible for these effects (Pan et al., 2013). The effectiveness of inhibition is often measured by IC_{50} values, with lower values indicating stronger inhibition, typically linked to higher concentrations of phenolic compounds. This inhibition could have therapeutic implications, especially for conditions of diabetes, where regulating starch breakdown is crucial (Aruoma et al., 2017).

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

This study demonstrated that Microwave-Assisted Extraction (MAE) is an effective, rapid, and eco-friendly technique for recovering bioactive compounds from coffee pulp, particularly when optimized for each coffee variety. For Geisha, the optimal conditions—708.7 W microwave power, 72.3 minutes extraction time, and a 1:10 sample-to-solvent ratio—resulted in enhanced recovery of phenolic compounds, antioxidant activity, and sugars. These findings reinforce the potential of MAE as a superior alternative to conventional extraction methods. Geisha pulp exhibited slightly higher Total Phenolic Content (TPC) and galactose, while Bourbon pulp contained more fructose. Across all tested bioactivities—DPPH, ABTS, FRAP assays, and αamylase/α-glucosidase enzyme inhibition—MAE extracts outperformed those obtained via conventional hot water extraction. Furthermore, phenolic profiling through mass spectrometry confirmed a wider spectrum and higher abundance of bioactive compounds such as flavonoids (e.g., hibiscetin) and alkaloids (e.g., caffeine and trigonelline) in MAE-treated samples. Statistical modeling and regression analysis validated the strong influence of microwave power, extraction time, and solvent ratio on extraction efficiency. Importantly, excessive exposure to microwave energy showed degrading effects on phenolics and sugars, emphasizing the need for precisely controlled extraction parameters.

The results strongly support the use of MAE-extracted coffee pulp compounds as natural ingredients for health-oriented applications, particularly in the nutraceutical, functional food, and natural health product industries. Based on the demonstrated antioxidant capacity and enzyme inhibition (notably α-amylase and α-glucosidase), these extracts exhibit promising health benefits. Specifically, they offer antioxidant

support by neutralizing free radicals, which are known to contribute to aging, inflammation, and the development of chronic diseases. Additionally, the extracts show potential in blood sugar regulation by inhibiting key enzymes involved in carbohydrate metabolism. This suggests their usefulness in managing postprandial hyperglycemia and providing supportive care for individuals with diabetes. Collectively, these bioactivities highlight the therapeutic relevance of the extracts and align well with the increasing global demand for clean-label, plant-based supplements derived from sustainable food waste valorization

5.2 Recommendations and Suggestions

5.2.1 Future studies should explore the feasibility of scaling up MAE for industrial applications, including pilot-scale extraction systems and economic assessments for commercial use.

5.2.2 Although water was used in this study for its environmental safety, future research could explore food-grade organic solvents or natural deep eutectic solvents (NADES) to improve extraction efficiency and compound selectivity.

5.2.3 Further analysis on the stability of the extracted compounds under various storage conditions will be important to understand the shelf life and formulation strategies for functional food applications.

5.2.4 Further research is needed to assess the bioavailability and in vivo effects of the extracted phenolics and sugars, particularly for their antidiabetic and antioxidant potentials.

5.2.5 Expanding this work to include other coffee varieties and agroindustrial by- products will help generalize the findings and support sustainable waste valorization in the food and nutraceutical industries.

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