OPTIMIZATION OF POLYPHENOLS RECOVERY FROM DEFATTED ROSELLE SEED USING MICROWAVE ASSISTED EXTRACTION

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by

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LIST OF ABBREVIATIONS

Abbreviation	Description
ANOVA	Analysis of variance
CCD	Central composite design
DW	Dry weight
DOE	Design of experiment
GAE	Gallic acid equivalent
GC-MS	Gas chromatography-mass spectrometry
MAATPE	Microwave assisted aqueous two-phase extraction
MAE	Microwave assisted extraction
PHWE	Pressurized hot water extraction
PI	Prediction interval
PLE	Pressurized liquid extraction
QE	Quercetin equivalent
RSM	Response surface method
SA-MSPD	Soxhlet-assisted matrix solid phase dispersion
SFE	Supercritical fluid extraction
TFC	Total flavonoid content
TPC	Total phenolic content
UAE	Ultrasonic assisted extraction

LIST OF SYMBOLS

Symbol	Description	Unit
t	Time	min
T	Temperature	°C
ε''	Loss factor	-
ε'	Dielectric constant	-
$\tan\delta$	Dissipation factor or loss tangent	-
X_i, X_j	Actual independent variables	-
$\alpha_\circ$	Constant coefficient at the central point	-
α_i	Regression coefficients of the linear terms	-
α_{ii}	Regression coefficients of the quadratic terms	-
α_{ij}	Regression coefficients of the interaction terms	-

PENGOPTIMUMAN PEMULIHAN POLIFENOL DARIPADA BIJI ROSELLE YANG DINYAH LEMAK MENGGUNAKAN PENGEKSTRAKAN TERBANTU GELOMBANG MIKRO

ABSTRAK

Roselle terkenal dengan kelopaknya yang digunakan untuk menyediakan minuman herba, jem dan jeli kerana ia telah lama diiktiraf sebagai sumber antioksidan. Walau bagaimanapun, biji roselle dalam kapsul baldu biasanya dibuang sebagai sisa walaupun pada hakikatnya ia mengandungi unsur pemakanan yang baik. Ini adalah kerana eksploitasi yang terhad terhadap kegunaan dan manfaat biji roselle sebagai alternatif yang berpotensi sebagai sumber makanan manusia. Oleh itu, kajian ini bertujuan untuk menentukan kandungan hasil ekstrak, jumlah kandungan fenolik dan jumlah kandungan flavonoid daripada ekstrak biji roselle yang dinyah lemak yang disediakan dengan menggunakan pengekstrakan terbantu gelombang mikro (MAE). MAE belum pernah digunakan dalam pengekstrakan biji roselle, oleh itu kajian ini adalah penting. Dalam kajian ini, polifenol telah diekstrak menggunakan campuran etanol-air (70 % (i/i)) di dalam bekas bertutup di bawah penyinaran gelombang mikro dengan dan tanpa kawalan suhu. Pengaruh parameter yang berbeza semasa proses pengekstrakan (masa pengekstrakan (min), kuasa gelombang mikro (W), nisbah pelarut kepada pepejal (mL/g), suhu pengekstrakan (°C)) telah dikaji. Kaedah gerak balas permukaan (RSM) telah digunakan untuk membangunkan model ramalan dan pengoptimuman proses pengekstrakan polifenol. Tanpa kawalan suhu, keadaan MAE yang diramalkan pada 10 min, 300 W dan 97.7178 mL/g memberikan hasil ekstrak (65.0367 ± 1.2450 %) dan kandungan fenolik (18.2244 ± 0.3310 mg GAE/g) yang tinggi kerana mencapai keadaan subgenting. Walau bagaimanapun, kandungan flavonoid yang rendah (6.4524 ± 0.3035 mg QE/g) tidak dijangkakan diperolehi

kerana ekstrak telah terdegradasi pada 163 °C. Sebaliknya, keadaan optimum MAE dengan kawalan suhu (12 min, 290 W, 125 mL/g dan 150 °C) juga dikenal pasti menghasilkan fenolik (21.0950 ± 0.4907 mg GAE/g) dan kandungan flavonoid (8.9452 ± 0.1873 mg QE/g) yang tertinggi. Ekstrak MAE optimum dengan kawalan suhu mengandungi jumlah fenolik yang sama dengan ekstrak lazim dan Soxhlet, tetapi hasil ekstrak dan kandungan flavonoid meningkat 173 % dan 443 %, masing-masing berbanding ekstrak Soxhlet. Analisis gas kromatografi-jisim spektrometri telah digunakan untuk menyiasat sebatian yang mungkin terdapat dalam ekstrak optimum. 1,2-epoxyhexadecane dan asid palmitik masing-masing adalah sebatian utama yang terdapat dalam ekstrak yang dioptimumkan untuk MAE dengan kawalan suhu dan MAE tanpa kawalan suhu. Oleh itu, MAE berpotensi menjadi satu kaedah pengekstrakan alternatif untuk pengekstrakan polifenol dari biji roselle kerana ia menawarkan tempoh yang pendek dan ekstrak yang berkualiti tinggi.

OPTIMIZATION OF POLYPHENOLS RECOVERY FROM DEFATTED ROSELLE SEED USING MICROWAVE ASSISTED EXTRACTION

ABSTRACT

Roselle is well known for its calyces that are used to prepare herbal beverages, jams and jellies because it has been long recognized as a source of antioxidants. However, roselle seeds in the velvety capsules are usually discarded as waste despite the fact that they contain good nutritional constituents. This is due to the limited exploitation of their usefulness and benefits as a potential alternative for human food source. Therefore, this study aims to determine the yield content, total phenolic content and total flavonoid content of defatted roselle seed extract prepared using microwave assisted extraction (MAE). MAE has not been applied in the extraction of roselle seed to the best knowledge, therefore this study is important. In this work, polyphenols were extracted using ethanol-water (70 % (v/v)) in a closed vessel under microwave irradiation with and without temperature control. The influence of different parameters on the extraction process (extraction time (min), microwave power (W), solvent to solid ratio (mL/g), extraction temperature (°C)) were studied. Response surface methodology (RSM) was used to develop predictive models and optimization of the polyphenols recovery process. Without temperature control, the predicted MAE conditions of 10 min, 300 W and 97.7178 mL/g resulted in high yield (65.0367 ± 1.2450 %) and phenolic content (18.2244 ± 0.3310 mg GAE/g) due to the reaching of subcritical state. However, low flavonoids content (6.4524 ± 0.3035 mg QE/g) was unexpectedly obtained due to extract degradation at 163 °C. On the other hand, the optimum conditions of MAE with temperature control (12 min, 290 W, 125 mL/g and 150 °C) were also identified to yield the highest phenolic (21.0950 ± 0.4907 mg

GAE/g) and flavonoid content (8.9452 ± 0.1873 mg QE/g). The optimum MAE extract with temperature control contains similar phenolic content to the extracts of maceration and Soxhlet, but the yield and flavonoid content increased 173 % and 443 %, respectively than the Soxhlet extracts. Gas chromatography-mass spectrometry analysis was further used to investigate the possible compounds available in the optimum extract. 1,2-epoxyhexadecane and palmitic acid were the major compounds found in the optimized extract for MAE with temperature control and MAE without temperature control, respectively. Thus, MAE appears to be a potential alternative for polyphenols recovery from roselle seed as it offers short extraction duration and high quality of extract.

CHAPTER ONE

INTRODUCTION

1.1 Introduction to roselle

Roselle (*Hibiscus sabdariffa* L.) is widely cultivated in tropical and subtropical regions as a traditional remedy. It is an annual erect and bushy plant with a height up to 2.5 m (Mohamed et al., 2007). The plant takes approximately 3 – 4 months to reach the maturity stage before the flowers are harvested. More importantly, it can tolerate Malaysia's climate which is warm and humid with night temperature at 21 °C and above (Ismail et al., 2008). The roselle flowers grow singly on different branches with a red or pale yellow edible calyx (Figure 1.1). Its velvety capsules contained 16 – 20 kidney-shaped light brown seeds.

Figure 1.1: The roselle (*Hibiscus sabdariffa* L.) plant.

This plant is a relatively new crop in Malaysia. It was introduced into the country in early 1990s. Its commercial planting was first promoted by the

Department of Agriculture (DOA) in Terengganu in 1993, and it has spread to many states ever since (Mohamad et al., 2009). To date, only a handful of small companies are active in processing, expanding and marketing of roselle products, mainly for the local market. Malaysia supplies abundant of roselle raw materials to the world, but Australia has been identified to be the major manufacturer of roselle tea (Bureau, 2016). The export market has not been fully explored by Malaysian even though roselle extract is a popular functional ingredient in beverages, food and nutritional supplements which are commonly exported to the US and Europe. The limited exploration of global market is mainly caused by our low competence in processing technology.

Interestingly, the plant has considerable industrial, pharmaceutical and economic values. Besides being used as the coloring agent in the food industry, the fleshy calyces (sepals of the flower) of roselle flowers are commercialized in the form of beverages, juices, jam, dried fruit and syrup products due to their bioactive content. Roselle was historically used by Egyptian pharaohs for its nutritional and health value (Bureau, 2016) and also has been used in traditional medicine for several diseases (Ali et al., 2005). Moreover, previous studies have reported the crucial role of roselle extracts in preventing chronic diseases especially on hypertension (Ali et al., 2005, McKay et al., 2010, Maganha et al., 2010) cardiovascular disease (Ali et al., 2003, Chen et al., 2004) and diabetes (Agoreyo et al., 2008) since it contained bioactive properties and are known to be rich in vitamins.

Most studies were focused on the benefits of roselle calyces because it exhibited a high content of anthocyanins and ascorbic acid (Wong et al., 2003). However, local researchers discovered that seed oil and extract also exhibited high antioxidant activities (Hainida et al., 2008, Susanti et al., 2012). Instead of discharging the seeds in velvety as waste, the roselle seeds can be further processed into valuable herbal products (Nyam et al., 2012). According to Nyam et al. (2009), roselle seed contains approximately 15 wt % of highly unsaturated triacylglycerols and small amounts of other lipid components. The roselle seed oil contains oleic acid, linoleic acid and α-tocopherol (Nyam et al., 2012). α-tocopherols are well-known antioxidants that can prevent or retard the oxidation of body lipids (Mohamed et al., 2014). Unlike roselle calyces, the processing roselle seeds require the advanced technology in order to achieve effective extraction. To date, the conventional maceration extraction and Soxhlet method are still being used for roselle seed extraction even though they are time-consuming. Thus, the integration of modern technology such as microwave heating with a short extraction time is preferable.

1.2 Introduction to microwave assisted extraction (MAE)

Extraction is the primary step to recover and purify the bioactive phytochemicals compound from plant materials. It is affected by different factors such as extraction yield, production cost and safety and environmentally friendly solvents (Desai et al., 2010). A number of conventional extraction methods have been used in the past few years, including maceration extraction, heat reflux extraction and Soxhlet extraction. These conventional methods involved complex operation techniques, a high amount of solvents and long processing time. Besides that, these methods can cause thermal degradation of the target compounds at high temperature (Gao et al., 2006). Driven by these effects, a number of advanced extraction methods such as microwave assisted extraction (MAE) (Letellier and Budzinski, 1999, Lopez-Avila, 1999, Camel, 2000), supercritical fluid extraction (SFE) (Bøwadt and Hawthorne, 1995) and pressurized liquid extraction (PLE) (Björklund et al., 2000) have been developed. MAE has many advantages over the conventional methods, including the shorter extraction time, the higher extraction efficiency and selectivity as well as the lower consumption of solvents (Veggi et al., 2013). However, the acceptance of MAE in food processing is slow due to a high capital cost (Lopez-Avila, 1999).

MAE has been extensively employed to extract highly valuable polyphenols from various parts of plant materials like Sea Buckthorn leaves (Asofiei et al., 2016), *Clinacanthus nutans* Lindau (Mustapa et al., 2015) *Quercus* bark (Bouras et al., 2015) and grape seed (Li et al., 2011). Using the electromagnetic radiations of 0.3 to 300 GHz, MAE is identified as a promising green extraction method (Chemat et al., 2012, Rombaut et al., 2014). In MAE, the samples were heated simultaneously in both directions, internally and externally without heating the vessel (Li et al., 2011). Therefore, the solution reaches its boiling point rapidly, leading to a very short extraction time. In returned, the functional compounds can be extracted efficiently and protectively.

One of the first applications of MAE was performed by Ganzler et al. (1986). They used a household microwave oven to extract organic compounds from soils, seeds and food. The efficiency and reproducibility of MAE were found to be comparable with the conventional extraction methods. The laboratory and industrial microwave systems were further developed in the last decade (Camel, 2000). These microwave systems were specifically designed for extraction purpose completed with the safety features to avoid any accident during extraction. These microwave systems can be used with two types of microwave heating vessels, either closed-vessels or open-vessels. The closed-vessel in microwave system offers the extraction at elevated temperatures and pressure which accelerate the mass transfer of target compounds from the sample matrix under subcritical conditions.

1.3 Problem statement

Roselle is an ideal crop for developing countries like Malaysia. It is drought tolerance, easy to grow and can be grown as part of the multi-cropping system. Demand for roselle has steadily increased over the past decades. It was reported by Bureau (2016) that roselle has a growing global market because of its known medical value. Statistics for the volume or global consumption of roselle however, were not available as for now, but the major clients for roselle importers are herbal tea manufacturers. According to Plotto (2004), roselle is used as base materials in herbal and fruit teas, along with apple peel, orange peel and lemon twist. Additionally, roselle has been labelled as versatile plant similar to the coconut tree by Quezon (2005) since nearly all parts of the plant are usable and consumable.

As mentioned, the most exploited part of a roselle plant is its calyces (sepal of the flower). Ismail and Yee (2006) have reported that approximately 50 wt % of the raw materials (roselle) are underutilised seeds which are abundantly produced as the by-products in the roselle products manufacturing. Roselle seed also possesses the highest protein (Hainida et al., 2008) compared to other seeds like *Simmondsia chinensis* (Cappillino et al., 2003), *Acacia colei* (Adewusi et al., 2003), *Pisum sativum* (Black et al., 1998) and *Nigella sativa* L. (Al-Jassir, 1992). In some countries, roselle seeds have been exploited as a substitute of coffee for human consumption (Morton, 1987) and extracted for their oil that resembles cotton seed oil (Mohamed et al., 2007).

At present, the vast majority of studies related to the bioactive extract of roselle seeds were conducted using conventional extraction methods such as maceration (Mohd-Esa et al., 2010, Ismail and Yee, 2006) and Soxhlet (Susanti et al., 2012). In order to address the issue of roselle seed extraction, the shortcomings of current most favourable extraction technique; conventional maceration and Soxhlet extraction have to be identified. Using this approach, Bimakr et al. (2011) and Khoddami et al. (2013) have found the drawbacks in their studies which consist of high solvent consumption, high operating temperature resulted in high energy costs, injuries to thermolabile (heat sensitive) substances due to high temperature and solvent residue in the solute causing lower quality extracts.

Another major problem faced by roselle seed extraction is the time required to finish the extracting cycle. Based on the previous studies, the average duration needed by maceration and Soxhlet extraction ranged from 2 – 24 hours. Such long time of extraction has stirred the attention among researchers to find an advanced method which allows drastic improvement in extraction duration such as MAE, SFE and ultrasonic-assisted extraction (UAE). MAE is a promising advanced method using electromagnetic radiations for extraction since it has been reported by Pellati et al. (2013) to have a shorter extraction time and can reduce the amount of solvent used. Moreover, a thorough literature search did not yield in any reference on the polyphenols recovery from roselle seed using MAE which means it has not been studied yet and shows the importance of this study.