OPTIMIZATION OF MICROWAVE-ASSISTED EXTRACTION AND ENCAPSULATION OF BLACK MULBERRY RICH ANTHOCYANIN EXTRACT AS FUNCTIONAL INGREDIENT

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ABSTRACT

Black mulberry fruit contains high level of anthocyanins and this compound is known for its beneficial health properties. This fruit is easily perishable and thus, it must be transformed into powder form to preserve its health benefits. The project consists of three objectives. First, to optimise the processes of microwave-assisted extraction, secondly to optimize microwave-assisted encapsulation of black mulberry extract and thirdly to evaluate the storage stability of the encapsulated black mulberry extract and analyse its anti-diabetic property. To obtain the optimised conditions, a response surface methodology was applied. The factors studied for extraction were microwave power (200 – 800 W), extraction time (5 - 15 min), and solid to liquid ratio (0.1 - 1.0 g/ml). The optimised extraction condition was at power of 475.16 W, time of 10.73 min and solid to liquid ratio of 0.59 g/ml. The total anthocyanin content (TAC), 2,2 diphenyl, 1-picrylhydrazyl (DPPH) inhibition and ferric reducing antioxidant properties (FRAP) obtained at this optimized condition were 21.75 mg/g dry basis, 92.4 % inhibition and 0.49 mg Trolox/ml, respectively. For microwave-assisted encapsulation optimisation, the factors studied were the core to wall ratio (0.1 - 0.3 ml/ml), encapsulation time (2 - 4 min) and microwave power (450 - 640 W). Based on the results from the analysis, the optimized encapsulation condition found to be at core to wall ratio of 0.19 ml/ml, power at 450 W and time at 3 min. Under this optimized encapsulation condition, the encapsulated black mulberry extract contained TAC of 23.02 mg/g, with encapsulation efficiency (EE) of 90.08 %, DPPH inhibition of 85.58 %, FRAP of 0.4 mg Trolox/ml and moisture content of 4.31 %. From storage stability study, the best storage condition was at 4 ^{0}C where TAC, DPPH and FRAP experienced the lowest degradation (k = 0.0033, 0.0011, 0.0059 mg/day) and highest half-life ($t_{1/2}$ = 201, 230, 117..5 days). In addition, this study demonstrated that the encapsulated black mulberry extract was capable of inhibiting alpha-glucosidase enzyme and thus, it can be used for preventing or treating diabetes. In conclusion, the results of this study show the potential of black mulberry fruit extract as a functional ingredient.

ABSTRAK

Buah mulberi hitam mempunyai sumber antosianin yang tinggi dan sebatian ini terkenal dengan khasiat kesihatannya yang bermanfaat. Namun buah ini mudah rosak dan oleh itu ia perlulah dijadikan serbuk untuk mengekalkan faedah kesihatannya. Projek ini mengandungi tiga objektif. Pertama, untuk mengoptimumkan proses pengekstrakan berbantukan gelombang mikro, kedua untuk mengoptimumkan enkapsulasi ekstrak mulberi hitam berbantukan gelombang mikro dan ketiga untuk menilai kestabilan penyimpanan ekstrak mulberi hitam dan menganalisis sifat anti-diabetesnya. Untuk mendapatkan keadaan yang optimum, kaedah sambutan permukaan telah digunakan. Faktor-faktor yang dikaji untuk pengekstrakan adalah daya gelombang mikro (200 - 800 W), masa pengekstrakan (5 - 15 min), dan nisbah pepejal terhadap cecair (0.1 - 1.0 g/ml). Keadaan pengekstrakan yang dioptimumkan adalah pada kuasa 475.16 W, masa 10.73 min dan nisbah pepejal terhadap cecair 0.59 g/ml. Jumlah keseluruhan antosianin (TAC), 2,2-difenil-1-pikrilhidrazil (DPPH) dan kuasa antioksidan penurunan ion ferik (FRAP) yang tertinggi masing-masing adalah 21.75 mg/g , 92.4 % dan 0.49 mg Trolox/ml. Bagi enkapsulasi berbantukan gelombang mikro yang optimum, faktor yang dikaji ialah nisbah teras terhadap dinding (0.1 - 0.3 ml/ml), masa enkapsulasi (2 - 4 min) dan kuasa gelombang mikro (450 - 640 W). Berdasarkan keputusan daripada analisis ini, keadaan enkapsulasi dioptimumkan didapati berada pada 0.19 ml/ml nisbah teras terhadap dinding, pada kuasa 450 W kuasa dan pada masa 3 min. Di bawah keadaan enkapsulasi yang dioptimumkan ini , ekstrak mulberi hitam yang dienkapsulasi mengandungi TAC 23.02 mg/g , kecekapan enkapsulasi (EE) 90.08 % , perencatan DPPH 85.58 %, FRAP 0.4 mg Trolox / ml dan kandungan lembapan sebanyak 4.31%. Daripada kajian kestabilan penyimpanan, keadaan penyimpanan yang terbaik ialah pada 4 ^{o}C yang di mana TAC, DPPH dan FRAP mengalami kemerosotan terendah (k = 0.0033, 0.0011, 0.0059 mg/hari) dan separuh hayat tertinggi ($t_{1/2}$ = 201, 230, 117.5) hari. Tambahan pula, kajian ini memperlihatkan bahawa ekstrak mulberi hitam mampu menghalang enzim alpha-glucosidase dan oleh itu ia boleh digunakan untuk mencegah ataupun merawat diabetes. Kesimpulannya, hasil kajian ini menunjukkan potensi ekstrak buah mulberi hitam sebagai bahan berfungsi.

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LIST OF ABBREVIATIONS

NHMS	-	National Health and Morbidity Survey
MOH	-	Ministry of Health
DPPH	-	2,2-diphenyl-1 picrylhydrazyl
T2DM	-	Type 2 Diabetes Mellitus
FBG	-	Fasting Blood Glucose
HbA1C	-	Haemoglobin A1C
ESR	-	End Stage Renal Failure
ACE	-	Angiotensin Converting Enzyme
EE	-	Encapsulation Efficiency
EPC	-	Endothelial Progenitor Cells
ROS	-	Reactive Oxygen Species
APP	-	Amyloid Precursor Protein
T1DM	-	Type 1 Diabetes Mellitus
PPG	-	Post-prandial sugar
ESRP	-	End Stage Renal Failure
DHD	-	Diabetic Heart Disease
VSM	-	Vascular Smooth Muscle
RAS	-	Renin-Angiotensin System
ANG	-	Angiotensin
ANG II	-	AngiotensinII
MAE	-	Microwave-Assisted Extraction
TAC	-	Total Anthocyanin Content
TPC	-	Total Phenolic Content
FRAP	-	Ferric Reducing Antioxidant Power
STZ	-	Streptozotocin
SEM	-	Scanning Electron Micrograph
GA	-	Gum Arabic
MD	-	Maltodextrin

LIST OF SYMBOLS

α	-	Alpha
Aβ	-	beta-amyloid
μL	-	Microliter

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CHAPTER 1

INTRODUCTION

1.1 Research Background

Black mulberry (*Morus nigra*) is native to Middle Eastern countries such as Iran and Turkey. It is also planted for commercial purposes in Mediterranean countries. Black mulberry can be eaten raw or processed further into jam, natural dye, whiskey, or juice. Black mulberry has become a phenomenon in fruit markets and the food industry due to its high phytochemical level and distinctive flavor (Dincer, Tontul, and Topuz, 2016; Fazaeli et al., 2012). Today, black mulberry is being cultivated in Malaysia for commercial purposes. They are marketed as raw fruit and are ready to be eaten fresh. This is because black mulberry is easy to plant and does not require low temperatures to survive. Various types of mulberry fruits can be found on hypermarket shelves or in nursery farms.

Besides, plant-based functional food has been proven to reduce the risk of diabetes or cardiovascular diseases (CVD). Zhang et al. (2012) found that the consumption of mulberry could promote insulin secretion, reduce the glycaemic index and blood pressure, as well as promote a healthy diet. The consumption of plant-based functional foods can decrease Fasting Blood Glucose (FBG) level and enhance the blood lipid in patients with Type 2 diabetes and hypertensive problems. This is due to the content of the functional food itself that is rich in polyphenols, vitamins, minerals, and antioxidants (Rudkowska et al., 2007; Hunyadi et al., 2012).

Recently, many studies have investigated the properties of black mulberry. This current study is a continuous process and is still ongoing up to the time of writing. Of the many studies in this area, some have investigated the concentration and storage of black mulberry (Dincer et al., 2016), the heating method of black mulberry (Fazaeli et al., 2013), as well as compared the phytochemical content of

different mulberries (Mena et al., 2016). Others have optimized various types of extraction methods (Espada-Bellido et al., 2017), investigated the anti-oxidant content in the juice of black mulberry (Tomas et al., 2015), studied the different content of anthocyanin in fruit and juice matrices (Tomas et al., 2015), the effect of drying on the physical properties of blackberry powder (Fazaeli et al., 2012), and many more. The wealth of studies on the black mulberry shows that the fruit has very high potential as a functional food and has an even greater prospect in the nutraceutical field. All key findings in this research can be used to develop mulberry derived-products rich in phenolic content.

Generally, this study aims to determine the optimum condition for the microwave-assisted extraction and encapsulation of anthocyanin in black mulberry fruit. The effectiveness of the antioxidant and anti-diabetic properties of the anthocyanin-rich black mulberry extract was tested. This study also evaluated the effectiveness of the anthocyanins after extraction and encapsulation. The extract of black mulberry was chosen, as it has a higher anthocyanin level compared to the plant's other parts. Also, this study provides a comprehensive understanding of the phenolic compounds of the blackberry fruit for future improvement and expansion of mulberry products rich in phenolic compounds.

1.2 Problem Statement

Black mulberry fruit contains a high amount of anthocyanin (Minhas et al., 2016). Anthocyanin has many beneficial functions that improve human health (Deylami et al., 2016). If anthocyanin is converted into a functional ingredient, its potency could be enhanced but unfortunately, the compound is unstable and very sensitive to heat (Han et al., 2016). Since the anthocyanin in black mulberry is easily perishable, it needs to be extracted and encapsulated to maintain its antioxidant properties in order to extend its shelf life and achieve stability (Fazaeli et al., 2012).

In Malaysia, black mulberry is still underutilized so many people are not aware of its importance. There is also a lack of studies on the optimization of black mulberry extraction, encapsulation, and stability. Many extraction methods used in the past include harmful solvents such as methanol; thus making it harmful to the environment due to the accumulation of toxic waste. Longer extraction time and a high cost are also factors inherent in previous extraction methods. Microwave-assisted extraction saves time and cost. Moreover, this method can be done using only 10% solvent, making it more eco-friendly. Besides, it can also produce a higher extraction yield. The method involves mixing organic solvent with water to help extract all compounds that dissolve in both water and organic solvent. Water is also a good microwave-absorbing solvent, so water can serve as a good solvent for microwave extraction (Do et al., 2014). At the same time, the temperature of the microwave can be controlled at the desired degree. Water is also a good microwave-absorbing solvent, so it could be a good solvent for microwave extraction.

Previously, no research has studied black mulberry encapsulation using a combination of gum Arabic and maltodextrin. The encapsulation method, which is the microwave-encapsulated technique, can also save lots of time, thus making the whole process more economical and cost-effective.

This study is important, as it can achieve the highest recovery rate of anthocyanin from the black mulberry extract, besides ensuring its effectiveness after being encapsulated and its rate of kinetic degradation. Moreover, it provides the latest information about black mulberry extracts and meets the demand for black mulberry in today's booming nutraceutical industry.

1.3 Objectives of Study

The ultimate aim of this study was to produce an encapsulated black mulberry extract powder that is stable, with a longer shelf life. To achieve this aim, the specific objectives below were derived:

(a) To optimize the microwave extraction process of black mulberry rich anthocyanin extract.

(b) To optimize the microwave encapsulation process of black mulberry rich anthocyanin extract.

(c) To evaluate the stability of encapsulated black mulberry rich anthocyanin extract and analyze its anti-diabetic properties.

1.4 Scope of Study

The scope were limited to:

a) Optimization of black mulberry extraction process using Central Composite Design (CCD) of Response Surface Methodology (RSM) by varying the microwave power, solid to liquid ratio and time. While the temperature was fixed at 50 ^{o}C

b) Optimization of microwave encapsulation process using Central Composite Design (CCD) of Response Surface Methodology (RSM) by differing the core to wall ratio, time and power. Whereas the coating material comprise of combination of gum Arabic and maltodextrin.

c) Storage stability study.
The storage stability study towards the degradation kinetics of the microwave encapsulated black mulberry extract was performed with two months of shelf-life. The temperature selected were 4 ^{o}C and 30 ^{o}C. The responses studied were encapsulation efficiency, TAC, DPPH, FRAP and half-life (days).

d) Study of anti-diabetic properties.

The anti-diabetic properties of black mulberry rich anthocyanin extract was evaluated using α-glucosidase assay with different concentration. The percentage inhibition of α-glucosidase was set as the response.

1.5 Significance of Study

Microwave-assisted extraction (MAE), using water as a solvent, is one of the most effective phytochemical extraction methods. The equipment is easy to operate and produces non-harmful waste. The MAE method is capable of reducing extraction time and energy consumption, besides producing high yields, as well as improving precision and accuracy. Nowadays researchers are looking for a safer and more environmentally-friendly method to extract phytochemicals from plants. Hence, MAE can be used as a potential alternative extraction method. The MAE optimization process is also crucial in process engineering to achieve a better extraction yield and encapsulation efficiency based on precise factors (solid-to-liquid ratio, core-to-wall ratio, microwave power, and process time).

There is also a lack of optimization studies on the extraction and encapsulation of black mulberry. The stability of the black mulberry extract has also been under-explored. This study, therefore, provides a systematic method to achieve the best yield of anthocyanin extracted from black mulberry fruit. It also provides valuable information on the stability of the anthocyanin after encapsulation. Besides, the findings also serve as a reference for future efforts to produce more functional foods based on mulberry-based anthocyanin, as well as information regarding the quality control and shelf life of this extract.

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