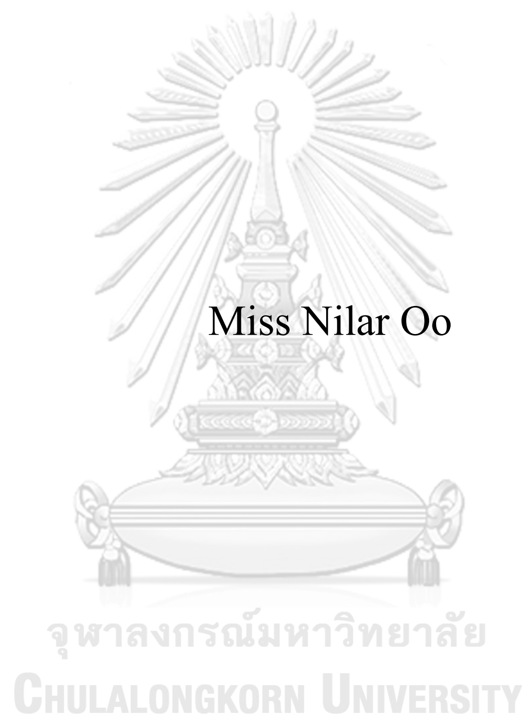
Biological Properties of Marigold Flower Extract and Its Microcapsule



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A Thesis Submitted in Partial Fulfillment of the Requirements
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สมบัติทางชีวภาพของสารสกัดดอกดาวเรืองและไมโครแคปซูล

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

น.ส.นิลาร์ อู

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยา
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นิลาร์ อู : สมบัติทางชีวภาพของสารสกัดดอกดาวเรืองและไมโครแคปซูล . (Biological Properties of Marigold Flower Extract and Its Microcapsule) อ.ที่ปรึกษาหลัก : กิติพงศ์ อัศตรกุล

งานวิจัยนี้มุ่งเน้นการศึกษาภาวะการสกัดด้วยวิธีอัลตราซาวด์ช่วยสกัดในการสกัดสารสกัดดอกดาวเรืองโดยใช้วิธีการพื้นผิวตอบสนง(response surface methodology, RSM) และผลิตผงไมโครแคปซูลด้วยวิธีการทำแห้งแบบพ่นฝอยโดยใช้สารห่อหุ้มและอัตราส่วนระหว่างสารห่อหุ้มและสารสกัดดอกดาวเรืองที่แตกต่างกันในงานวิจัยนี้ศึกษาปัจจัยที่ส่งผลต่อการสกัด คือ อุณหภูมิ (30-50 ℃) เวลา (5 -15 นาที)และความเข้มข้นของเอทานอล (60 - 100% v/v) โดยเลือกภาวะที่เหมาะสมจากปริมาณสารประกอบฟีนอลิกทั้งหมดปริมาณฟลาโวนอยด์ทั้งหมด ปริมาณแคโรทีนอยด์ทั้งหมดและฤทธิ์การต้านอนุมูลอิสระด้วยวิธี 2,2-diphenyl-1-picrylhydrazyl (DPPH) และ FRAPจากผลการทดลองพบว่า ภาวะที่เหมาะสมที่สุดในการสกัดสารสกัดดอกดาวเรืองด้วยวิธีอัลตราซาวด์ช่วยสกัด คือ อุณหภูมิ40 ℃ เวลา 15 นาที และความเข้มข้นของเอทานอล 68% v/v ซึ่งที่ภาวะนี้สารสกัดดอกดาวเรืองมีปริมาณสารประกอบฟีนอลทั้งหมด ปริมาณฟลาโวนอยด์ทั้งหมด ฤทธิ์การต้านอนุมูลอิสระทั้งวิธี DPPH และ FRAP และปริมาณแคโรทีนอยด์ทั้งหมด เท่ากับ 75.699 mg GAE/100 g db, 86.740 mg QCE/100g db, 630.369 mM Trolox/100g db, 3226.171 mM Trolox/100g db และ 234.741 mg carotenoid/100g db ตามลำดับ จากนั้นเลือกภาวะนี้เพื่อวิเคราะห์ฤทธิ์การต้านจุลชีพด้วยวิธีก disc diffusion method และความเข้มข้นต่ำสุดของสารที่สามารถยับยั้งการเจริญ (minimum inhibition concentration, MIC) ของสารสกัดดอกดาวเรือง และศึกษาการเอนแคปซูเลชันวิธีการทำแห้งแบบพ่นฝอย โดยใช้กัมอารบิก (gum Arabic, GA)และมอลโตเดกซ์ทริน (maltodextrin, MD)เป็นสารห่อหุ้ม จากผลการทดลองพบว่าสารสกัดดอกดาวเรืองที่สกัดด้วยภาวะที่เหมาะสมมีค่า IZ (inhibition zone) เท่ากับ9.66 mm และ 8.33 mm สำหรับ S. aureus และ E. coli ตามลำดับ และมีค่า MIC เท่ากับ 25 mg/mL สำหรับจุลินทรีย์ทั้งสองชนิด นอกจากนี้สารสกัดดอกดาวเรืองที่ห่อหุ้มด้วยมอลโตเดกซ์ทรินในอัตราส่วนของสารสกัดดอกดาวเรืองและมอลโตเดกซ์ทรินได้ผลผลิตและประสิทธิภาพการห่อหุ้มสูงที่สุด โดยความสามารถในการละลายน้ำ ปริมาณความชื้น และค่ากิจกรรมของน้ำไม่แตกต่างกันอย่างมีนัยสำคัญ (P>0.05) ในขณะที่ผลจากกล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราด (SEM) แสดงให้เห็นว่าโครงสร้างทางจุลภาคของผงไมโครแคปซูลที่เคลือบด้วยมอลโตเด็กซ์ตรินมีรูปร่างเป็นทรงกลม ผิวเรียบ และหดตัวน้อยกว่าผงไมโครแคปซูลที่ใช้กัมอารบิกเป็นสารห่อหุ้ม และตัวอย่างที่มีฤทธิ์การต้านอนุมูลอิสระที่ดีที่สุด คือผงไมโครแคปซูลที่ใช้กัมอารบิกในอัตราส่วนของสารสกัดดอกดาวเรืองและกัมอารบิก 1:2 (w/v) และสรุปได้ว่า ภาวะที่เหมาะสมจากการสกัดด้วยวิธีอัลตราซาวด์ช่วยสกัดและการห่อหุ้มด้วยวิธีการทำแห้งแบบพ่นฝอยสามารถใช้ในการผลิตอาหารเสริมเพื่อสุขภาพที่มีสารออกฤทธิ์ทางชีวภาพที่ดีขึ้น

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Nilar Oo : Biological Properties of Marigold Flower Extract and Its Microcapsule. Advisor: Assoc. Prof. Dr. KITIPONG ASSATARAKUL

This research focuses on the optimization of ultrasound-assisted extraction (UAE) conditions of marigold flower extract (MFE) using response surface methodology (RSM) and to produce microcapsule using spray drying with different wall materials and ratios. The extraction factors including temperature (30-50 °C), time (5 -15 min), and ethanol concentration (60 - 100% v/v) were investigated. The optimum condition was investigated according to the maximum concentration of total phenolic, total flavonoid, total carotenoid, and antioxidant activity determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical and ferric reducing antioxidant power (FRAP) assay. The optimized extraction conditions were temperature (40 °C), time (15 min), and ethanol concentration (68% v/v) which showed the highest total phenolic, total flavonoid, antioxidant activity by DPPH and FRAP assay, and total carotenoid content with the values of 75.699 mg GAE/100g db, 86.740 mg QCE/100g DB, 630.369 mM Trolox/100g db, 3226.171 mM Trolox/100g dB and 234.741 mg carotenoid/100g db, respectively. Depending on this optimal condition, the MFE determined antimicrobial activity by disc diffusion method and minimum inhibition concentration (MIC) and was further encapsulated by spray drying using gum Arabic (GA) and maltodextrin (MD) as the coating materials. The optimum extract had an inhibitory zone of 9.66 mm and 8.33 mm against *S. aureus* and *E. coli*, respectively. The MIC value of the optimized extract was 25 mg/mL. It was found that MFE encapsulated with maltodextrin at ratio of MFE and maltodextrin revealed the highest encapsulation yield (%) and encapsulation efficiency (%). Water solubility, moisture content and water activity showed no significant changes ($P>0.05$). The scanning electron microscope (SEM) showed that the microstructures of microcapsules coated by maltodextrin had a spherical shape, smooth surface and less shrinkage than the microcapsules using gum Arabic as the coating material. The best antioxidant activity was found from the microcapsule used GA at ratio of MFE and GA 1:2 (w/v). In conclusion, optimal condition from UAE and encapsulation by spray drying was suggested to be used for the production of functional food with improved bioactive compounds.

Field of Study:	Food Science and Technology	Student's Signature ....
Academic Year:	2021	Advisor's Signature ....

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Chapter 1

1.1 Introduction

Consumers have become increasingly concerned about their health, resulting in a significant desire for foodstuffs containing bioactive or beneficial ingredients that enhance the food products' nutrient content and health advantages. The use of bioactive chemicals in a variety of industries, including pharmaceutical, food, and chemical, has necessitated the development of a reliable and consistent process for extracting active compounds from plants. Different portions of various plants could be a natural source of bioactive chemicals. Total phenolic and carotenoid compounds are key bioactive molecules that have been linked to human health benefits in numerous studies. Furthermore, improved technologies are required to incorporate and safeguard sensitive bioactive ingredients as well as nutritional loss in new functional meals (Spinelli 2016).

Marigold (*Tagetes* spp.) is an *Asteraceae* family attractive plant with about 50 species of annual or perennial herbaceous plants with high levels of carotenoids (lutein, fatty acid esters and diesters). Carotenoid is abundant in marigold flowers, which are a common source of lutein pigment (Kaimainen, Järvenpää et al. 2015). Since the Middle Ages, marigolds have been utilized as a culinary and medicinal herb. Marigold flowers and leaves have been mentioned as a source of physiologically active chemicals in prior studies. Antioxidants are found in the essential oil extracted from the flowers. Antimicrobial agents are commonly found in medicinal plants. Microbiologists are becoming increasingly interested in screening medicinal plants for antimicrobial activity and phytochemicals that could be used to develop new treatments. Several marigold species have been widely employed in medicinal and industrial applications for a variety of illnesses including skin complaints, wounds, burns, and menstrual irregularities. The leaves of this plant have been said to help with kidney problems, muscular pain, ulcers, and wounds. Marigold flowers have petal colors ranging from white to yellow to orange, indicating the presence of flavonoid, essential oil, and pigments. Flowers can be used for fevers, epileptic fits (Ayurveda), astringent, carminative, stomachic, and liver ailments, among other things, and they have a variety of pharmacological properties, including antimicrobial action, insecticidal activity, wound healing activity, and antioxidant activity. Tagetes

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species have pigments that can be utilized as a culinary coloring additive (Priyanka, Shalini et al. 2013).

Secondary metabolites, or compounds other than primary metabolites, are bioactive compounds found in plants that serve to boost the ability to live and overcome problems by reacting with the environment (Azmir, Zaidul et al. 2013). The combination of secondary metabolites in plants, such as alkaloids, steroids, tannins and phenolic compounds, flavonoids, resins, and fatty acids, can have positive effects (Priyanka, Shalini et al. 2013). The term "bioactive compound" refers to a substance that has physiological and cellular effects. They are substances or dietary supplements that are responsible for alterations in the health of humans and/or animals who ingest them (Walia, Gupta et al. 2019). They are often found in fruits and vegetables, and they can give nutrients as well as a variety of bioactive components include phytochemicals (phenolic, flavonoids and carotenoids). Consumption of bioactive compounds in food products that have positive health effects could lead to the development of functional foods that have protective and preserving characteristics (Walia, Gupta et al. 2019).

According to epidemiological research, bioactive chemicals derived from natural sources can lower reactive oxygen in biological systems, allowing the endogenous antioxidant system to neutralize free radicals. Bioactive chemicals from edible flowers, according to molecular and mechanical research, have the potential to scavenge free radicals and control lipid peroxidation, which reduces the sequence of oxidative stress and affects cellular macromolecules. As edible flower consumption has grown in popularity, researchers have begun to focus on their nutritional worth and therapeutic capabilities, as well as the health-promoting substances that may be found in various anatomical regions of edible flowers (Janarny, Ranaweera et al. 2021).

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Extraction is a crucial process for separating useful chemicals from raw materials. Bioactive compounds can be extracted using a variety of techniques, including conventional (Soxhlet, maceration, and hydro distillation) and non-conventional (ultrasound assisted extraction or (UAE), enzyme assisted extraction,

microwave assisted extraction, and pulsed electric field assisted extraction, supercritical fluid extraction, and pressurized liquid extraction).Traditional extraction processes take time and need a high extraction temperature, which consumes energy due to prolonged heating, stirring in a boiling solvent, and the evaporation of a considerable amount of solvent. However, when compared to novel extraction techniques, the traditional extraction process Soxhlet is one of the reference procedures for extracting valuable bioactive compounds from various plant sources and is utilized as a model (Azmir, Zaidul et al. 2013). To improve production efficiency and contribute to the green environment by reducing solvent usage, energy consumption, and hazardous substance emissions, a new extraction technique called ultrasound assisted extraction method (UAE) has been introduced as an alternative to traditional extraction techniques. UAE is a successful extraction process for valuable bioactive compounds extracted from plant sources since it uses fewer synthetic and organic chemicals, takes less time to operate, produces better extract quality, and yields more in a shorter period of time (Azmir, Zaidul et al. 2013). In order to facilitate penetration of the matrix and increase mass transfer and extraction rates, the UAE disrupts the bulk substance's cellular structure. When compared to traditional extraction procedures, the UAE methodology has better efficiency and quicker extraction time.

Ultrasound is a type of sound wave that is beyond human hearing and travels through the medium by compressing and expanding. It has frequencies ranging from 20 kHz to 100 MHz and requires an elastic medium to travel through. In the UAE, the choice of solvent is crucial. Depending on the solvent employed, UAE can give a high extraction yield. Furthermore, critical physical parameters such as ultrasonic power, temperature, and extraction duration have an impact not only on the extraction yield but also on the extract composition (Otles 2016).

Microencapsulation is a useful method for protecting sensitive compounds like active compounds, flavor compounds, and vitamins from external environmental factors like heat, light, oxygen, and interactions with other compounds, as well as improving their oxidative stability, bioavailability, and functional properties. Microencapsulation is becoming increasingly popular in the food and beverage

sectors throughout the world to improve bioactive ingredient dispersion. The most common method of encapsulation is spray drying because it would be affordable, flexible, and produces high-quality particles (Šeregelj, Ćetković et al. 2017).

Response Surface Methodology (RSM) is a collection of statistical and mathematical methodologies that can be used to build and optimize a functional relationship between a response of interest and a set of control variables (Morshedi and Akbarian 2014). With univariate or multivariate approaches, RSM is a series of processes that evaluates the relationship between one or more output responses and various individual parameters using a second-degree polynomial model (Insang, Kijpatanasilp et al. 2021). RSM is commonly employed in three methods to identify the values of process variables (Myers and Montgomery, 20020: (1) statistical experimental design, which is two level factorial or fractional factorial design, (2) regression modeling techniques, and (3) optimization method.

Most researchers have examined the microencapsulation of bioactive chemicals from various plant sources utilizing spray drying in several studies. However, there is a lack of information on how to optimize the extraction yield of bioactive compounds with the highest antioxidant activity from Marigold flower extract (MFE) using RSM and the effect of microencapsulation conditions (types of coating material and MFE/coating material ratio) on the physiochemical properties of MFE microcapsules.

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Chapter 2

2.1 Marigold flowers

2.1.1 Characteristics of Marigold Flowers

Marigold flower, scientifically named *Tagetes erecta*, is classified in the Asteraceae family (Table 1). The marigold (*Tagetes*) flower is highly well-liked in South and Southeast Asia. Marigold is frequently utilized in a variety of lucky ceremonies. Marigolds come in three main varieties: (1) The short variation of American marigolds extends from 10 to 14 inches, the medium variety from 14 to 16 inches, and the long variety from 16 to 36 inches (tall variety). (2) The single flower variant of French marigolds ranges in size from 1.5 to 2 inches to 1.5 to 3 inches (double flower variety). (3) Marigold hybrids, a cross between the American and French varieties.

Table 1 Marigold Flower Classification

Sequence	Call Name
Kingdom (kingdom)	Plantae
Clade	Angiosperms
Rank (order)	Asterales
Wong (family)	Asteraceae – aster family
Division	Magnoliophyta
Genus (genus)	*Tagetes*
Type (species)	*Erecta*

Source: (Priyanka, Shalini et al. 2013)

Marigold flowers have overlapping petals. Moreover, marigold plants seem like flowering shrubs (25-60 cm high) and they can grow in almost every part of Thailand (Ruangram & Pimchan, 2016). Marigold flowers have different colors and different fragrances. Among them, yellow is the common color, as shown in Figure 1.

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Figure 1 Composition of Marigold flower
Source: (Shetty, Sakr et al. 2015)

Phenolics and carotenoids are the most abundant phytochemical antioxidants in marigold flower extracts. Lutein is one of the principal carotenoids present in marigold petals, whereas phenolics, which include gallic acid and quercetin, are detected in marigold flower extract (Rivas 1991). The principal pigment groups present in *Tagetes* spp. marigold blooms are flavonoids and carotenoids. Flavonoids are secondary plant metabolites with antioxidant and chelating characteristics that have various health benefits for humans (Číž, Čížová et al. 2010).

The composition of marigold flower consists of protein, fat, total carbohydrates, and fiber (Table 2). Tagetes is a genus of 50 annual and perennial herbaceous plants in the Compositae/Asteraceae family. Depending on the regional context, it has a variety of applications in food, perfumes, pharmaceuticals, and ornamentals. Fevers, epileptic fits, astringent, carminative, stomachic, scabies, liver problems, and other eye ailments are all treated with different components of this plant, particularly the blooms.

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Table 2 Composition of Marigold flower

Parameter	*Tagetes erecta*
Moisture (%)	83.39
Total carbohydrates (%)	14.15
Total Dietary Fiber (TDF) (%)	9.20
Protein (%)	1.32
Fat (%)	0.32
Ash (%)	0.80
Energy (kcal/100g)	28.02

Flowers are edible and can be used to color and flavor foods as well as serve as a coloring agent and a yellow dye. This plant's leaves are used as an antiseptic in

renal illnesses, muscular soreness, piles, and carbuncles. Moreover, it also contain many minerals and vitamins such as calcium and phosphorus as shown in Table 3 (Singh, Gupta et al. 2020).

Table 3 Mineral Composition of Marigold Flower

Mineral	*Tagetes erecta*
Calcium (mg/100g)	0.110
Copper (mg/100g)	0.104
Iron (mg/100g)	1.026
Potassium (mg/100g)	0.215
Magnesium (mg/100g)	0.060
Manganese (mg/100g)	0.303
Sodium (mg/100g)	0.015
Phosphorus (mg/100g)	0.065
Sulfide (mg/100g)	0.045
Strontium (mg/100g)	1.017
Zinc (mg/100g)	0.568

2.1.2 Chemical Constituents

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Phytochemical studies of this plant have shown the presence of thiophenes, flavonoids, carotenoids, and triterpenoids, among other chemical elements. *T. erecta* contains quercetagetin, a glucoside of quercetagetin, phenolics, syringic acid, methyl-3, 5- dihydroxy-4-methoxy benzoate, quercetin, vinyl, and ethyl gallate, according to previous research. Lutein is an oxycarotenoid, or xanthophyll, with two cyclic end groups (one beta and one alpha-ionone ring) and the fundamental C-40 isoprenoid structure common to all carotenoids. It is a significant ingredient and principal pigment of *Tagetes erecta*. The flowers contain carotenoids including lutein, zeaxanthin, neoxanthin plus violaxanthin, β-carotene, lycopene, α-Cryptoxanthin, phytoene and phytofluene. According to the results from (Xu, Wang et al. 2011), *T. erecta* compoments were β-sitosterol, daucosterol, 7β-hydroxysitosterol, erythrodiol-3-palmitate, lupeol, erythrodiol, 1-[5-(1-propyn-1-yl)-[2, 2-bithiophen]-5-yl]-ethanone,

α-terthienyl, quercetagetin, quercetagetin-7-methylether, quercetagetin-7-O-glucoside, kaemferol, syringic acid, gallic acid, 3-α-galactosyl disyringic acid, 3-β-galactosyl disyringic acid, 6-ethoxy-2, 4- dimethylquinoline, oplodiol, (3S, 6R, 7E)-hydroxy-4, 7- megastigmadien-9-one, palmitin, ethylene glycollinoleate, and n-hexadecane. The chemical structures of lutein, quercetagetin and syringic acid are described in Figure 2.

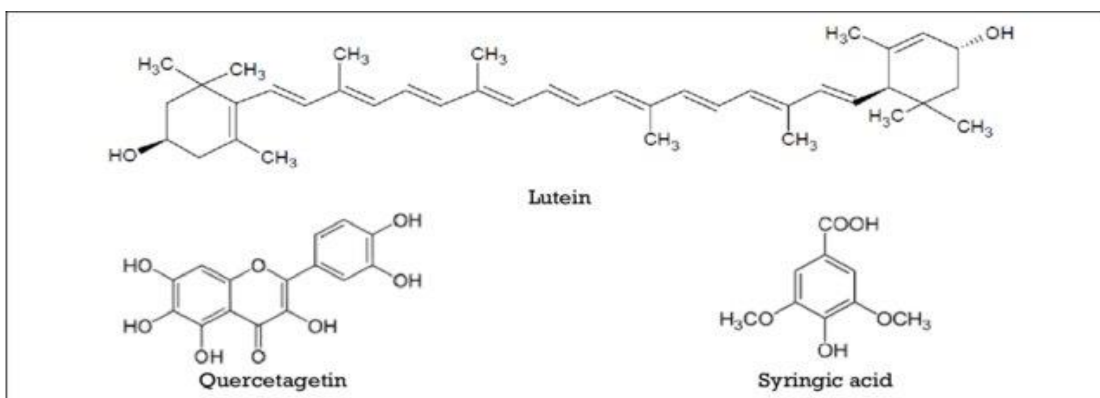

Figure 2 Major chemical constituents of *Tagetes erecta*
Source: (Singh, Gupta et al. 2020)

2.1.3 Utilization of marigold flowers in food products

Marigold flower (*Tagetes erecta*) petals are common sources of lutein (yellow orange) pigments and food additives as a coloring agent. Flowers and fruits high in carotenoids are important for human nutrition and health. Lutein accounts for over 90% of total carotenoids, while zeaxanthin makes up roughly 5% of total carotenoids. The presence of lutein and zeaxanthin in humans has been linked to good health. The daily consumption of macular carotenoid-rich meals and lutein supplements is significantly linked to the accumulation of lutein and zeaxanthin in the human body. This is owing to the fact that humans are unable to biosynthesize carotenoids. When compared to other sources of lutein and zeaxanthin, such as corn, vegetables, and so on, the marigold flower is a natural common source (Kurniawan, Yusuf et al. 2019). Lutein and its isomer zeaxanthin are found in the flower in the ester form, acetyl ester as shown in Figure 3 and 4, and can help protect the eyes from oxidative damage by filtering out harmful UV rays. Antioxidants can stop free radicals from causing damage by quenching singlet oxygen. Lutein and zeaxanthin, which can be found in

marigold flowers, have been employed as coloring agents and supplements in a variety of foods, including baked goods, drinks, cereals, chewing gums, dairy products, frozen dairy products, processed fruits, and juices. One of the carotenoid pigments, xanthophylls, can also be employed in commercial items as natural pigments. Carotenoids are pigments that are good for skin malignancies, dermatological illnesses, and cancer prevention in general (Gupta 2014).

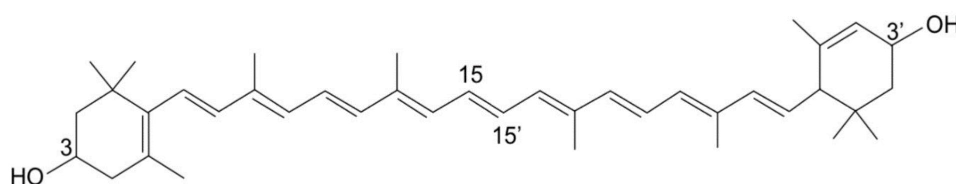

Figure 3 Lutein

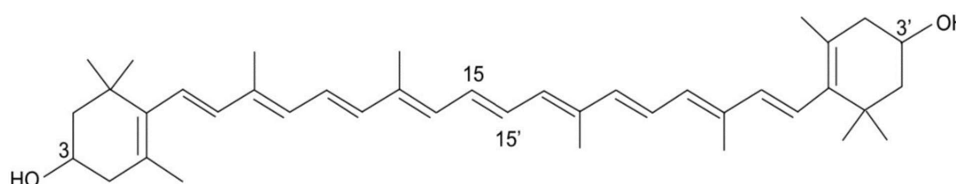

Figure 4 Zeaxanthin

2.2 Bioactive Compounds

Consumer preferences for food production have shifted dramatically in recent years. Consumer demand for meals that aid in human metabolism as well as food supplements containing various chemical compounds with beneficial effects on our health is increasing. Bioactive compounds are substances that are classified as non-nutritive ingredients (Koçak and Pazır 2018). Furthermore, the food processing business has been confronted with problems in terms of both technical and economic changes, as well as attempting to pique consumer interest in food items. In the food business, new technology and inventions were launched as a result. Functional meals have a key role in obtaining health advantages, increasing life expectancy, and improving the quality of life for older people, according to consumer demand (Spinelli 2016).

Bioactive substances are extra-nutritional secondary metabolites that are generally found in meals and give health benefits beyond the fundamental nutritional

value. Secondary metabolites, which comprise phenolic chemicals and carotenoids, have health-promoting properties (Biesalski, Dragsted et al. 2009). Reduced incidence of degenerative diseases such as cancer and diabetes, reduced risk of cardiovascular diseases, antioxidant, anti-mutagenic, anti-allergenic, anti-inflammatory, and anti-microbial actions are the key benefits (Ignat, Volf et al. 2011). Many studies have found that bioactive compounds act as health promoters when they act as a cofactor or inhibitor in enzymatic reactions, as a substrate in biochemical reactions, as an absorbent to remove unwanted compounds from the gastrointestinal tract, as a fermentation substrate for microorganisms, as inhibitors to prevent harmful microorganisms from growing, and as scavenging agents for reactive and toxic chemicals (Koçak and Pazır 2018).

2.2.1 PHENOLIC COMPOUNDS

Flowering plants produce phenolic chemicals, which are secondary plant metabolites. The quality and quantity of polyphenols found in plant materials is affected by plant genetics, soil composition, growing conditions, and post-harvest factors. The radical-scavenging activity of polyphenols is mostly found in antioxidant activities. Phenolic compounds can function as hydrogen donors by inhibiting the oxidation of low-density lipoproteins (LDL). Antimicrobial, anticarcinogenic, antiallergic, antimutagenic, anti-inflammatory, anti-allergenic, anti-atherogenic, and anti-thrombotic activities of phenolic compounds have been linked to improved human health. Furthermore, phenolic compounds have been linked to protect from cancer, coronary artery disease, and cardiovascular diseases. Secondary metabolites such as phenolic compounds can protect plants from oxidants and UV light. Secondary metabolites are divided into three categories: Depending on the biosynthetic structure, there are (1) flavonoids, related phenolic, and polyphenolic substances; (2) terpenoids; and (3) nitrogen-containing alkaloids and sulfur-containing compounds. Building blocks and biosynthetic enzymes link the majority of these compounds to main metabolites (Vuolo, Lima et al. 2019). Secondary metabolites of the pentose phosphate pathway produce phenolic chemicals, which are antioxidants. C_6H_5OH is the infrastructure of phenylpropanoid routes. The aromatic ring of benzene can be joined to one or more groups of (-OH) as seen in Figure 5. By interacting with hydroxyl groups, phenolic substances can be integrated with one or more sugar

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molecules; monosaccharides, disaccharides or oligosaccharides (Randhir, Lin et al. 2004).

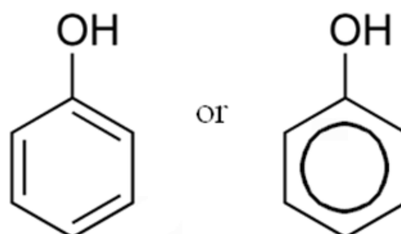

Figure 5 Structure of phenolic compounds

Source: (Vermerris and Nicholson 2007)

Phenolic compounds have an aromatic ring with one or more hydroxyl substituents in their structure and can range from basic phenolic molecules to highly polymerized compounds. Most phenolic compounds relate to one or more phenolic groups after being conjugated with mono- and polysaccharides. They can be connected to esters and methyl esters as well. Even though phenolic compounds appear in a wide variety of configurations in nature, more than 8000 phenolic compound structures have been discovered to date. They can be categorized into several classes as shown in Figure 6:

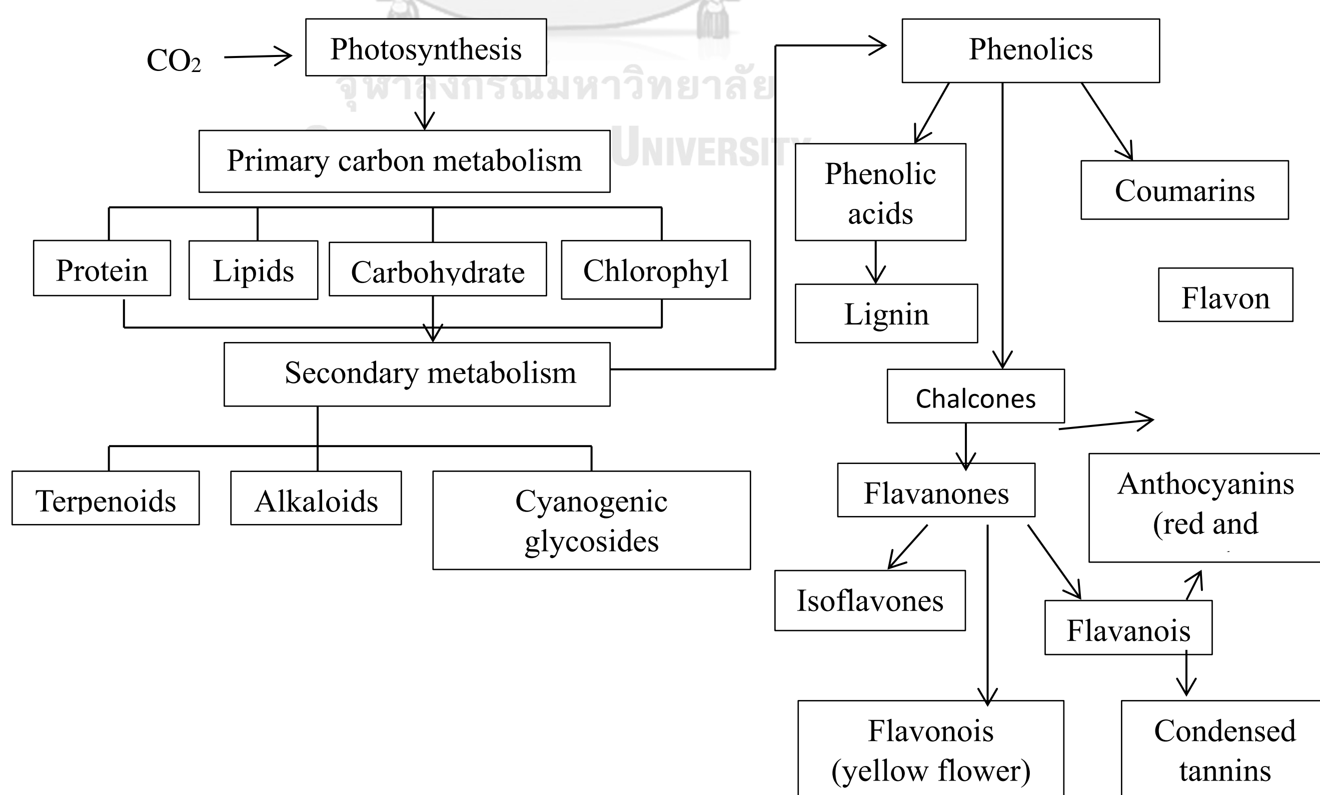

Figure 6 Primary and secondary metabolism linkage in plants
Source: (Das and Gezici 2018)

Free radicals are inhibited by phenolic substances that transfer hydrogen atoms from the hydroxyl group. According to the theory of a phenolic compound with a peroxyl radical (ROO*) is that the hydrogen cation from the phenol is transferred to the other radical, forming a transition state of an H-O bond with electron. he antioxidant activity of phenolic compounds is decreased because the reaction uses a solvent that can easily create a hydrogen bond with the phenolic molecules. Alcohol, for instance, has a two-fold effect on the interaction between phenol and the peroxyl radical. In addition to acting as a hydrogen acceptor, alcohol also ionizes phenols, producing anion phenoxides that can start a reaction with peroxyl radicals by transferring electrons. The degree of ionization of the final components determines the influence of the solvent on the antioxidant activity of phenolic compounds (Santos-Sánchez, Salas-Coronado et al. 2019). Polyphenol substances can be categorized in several ways such as depending on the amount of carbon in molecules as shown in Table 4 (Vermerris and Nicholson 2007).

Table 4 Classification of phenolic compounds

Structure	Phenolic compounds
C6	simple phenolics
C6 – C1	phenolic acids and related compounds
C6 – C2	acetophenones and pehnylacetic acids
C6 – C3	cinnamic acids, cinnamyl aldehydes, cinnamyl alcohols
C6 – C3	coumarins, isocoumarins, and chromones
C15	chalcones, aurones, dihydrochalcones
C15	Flavans
C15	flavones
C15	flavanones
C15	Flavanonols
C15	Anthocyanidins

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C15	Anthocyanins
C30	Biflavonyls
C6-C1-C6-C2-C6	benzophenones, xanthones, sitlbenes
C6, C10, C14	Quinones
C18	Betacyanins
Lignans, neolignans	dimers or oligomers
Lignin	Polymers
Tannins	oligomers or polymers
Phlobaphenes	Polymers

Source : (Vermerris and Nicholson 2008)

Polyphenols are secondary metabolites found in nature, and they are made up of the amino acids' phenylalanine and tyrosine. At least one hydroxyl group are present in the phenyl ring. Plant phenols include cinnamic acids (C6-C3) and benzoic acids (C6-C1) as seen in Figure 7. Plant tissues contain phenolics, which play a significant role in biotic and abiotic stress. Although polyphenols are abundant in plants, their quantities fluctuate depending on the quality and quantity of meals consumed. Flavanol is the most prevalent phenolic component in fruits.

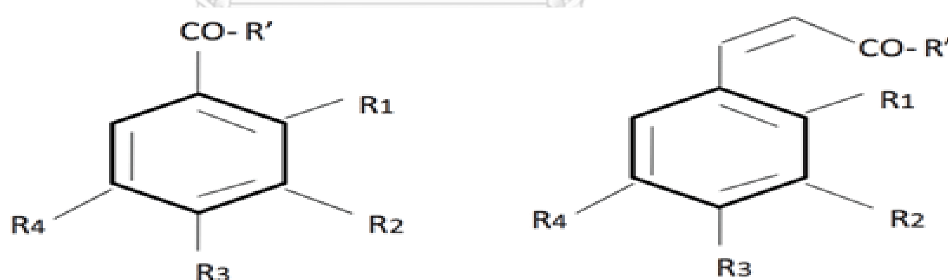

Figure 7 Molecular structure of benzoic and cinnamic acid

Source : (Castellano, Tena et al. 2012)

An aromatic ring with a hydroxyl substituent and a functional residue makes up the structure of phenolic compounds. Flavonoids, phenolic acids, tannins, stilbenes, and lignans are the most common types of polyphenols. Polyphenols can be found in a wide range of industrial applications, including food production and cosmetics, where they contain natural colorants and preservatives. In addition, employing microencapsulated polyphenols can improve the bioavailability of the

substance in vivo and in vitro by reducing the unpleasant taste and odors (Spinelli 2016).

The Folin-Ciocalteau reagent and the DPPH (2,2-diphenyl-1-picrylhydrazyl scavenging capacity) method can be used to quantify the amount of total phenolic compounds. The capacity of phenolic to react with oxidizing substances is described in these ways. Any form of phenol reacts with Folin-Ciocalteau reagent, which contains sodium molybdate and tungstate. According to the oxidation, electron transfer converts sodium molybdate and tungstate to tungsten (W_8O_{23}) and molybdenum (Mo_8O_{23}), resulting in a vivid blue color solution. The quantity of hydroxyl groups in the molecule determines the color intensity of the solution (Santos-Sánchez, Salas-Coronado et al. 2019).

2.2.1.1 Flavonoids

Plants synthesize polyphenolic chemicals called flavonoids, which have chemical and biological effects. They are also the most common type of phenolic compound: low molecular weight molecules with fifteen carbon atoms arranged in a C6-C3-C6 structure (Figure 8).

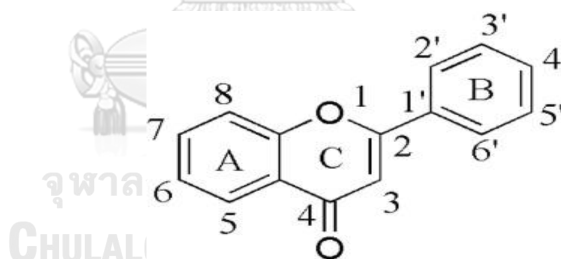

Figure 8 Structure of flavonoids

Source: (Wang, Li et al. 2018)

Flavonoids are divided into two groups based on variations in the heterocyclic ring substitution pattern: (a) anthocyanins (glycosylated derivatives of anthocyanins found in colorful flowers and fruits), and (b) anthoaxthins (colorless compounds including flavones, flavans, flavonoids, isoflavones, and their glycosides). Blackberries, black currants, blueberries, grapes, strawberries, cherries, plums, pomegranate, and raspberries are all good sources of flavonoids. Because of its antiradical (OH), anti-lipoperoxidation (R, ROO, RO), and metal cheating capabilities, it is linked to significant antioxidant activity (Spinelli 2016).

Flavonoids are polyphenolic-structured natural products and secondary metabolites of plants that are often found in fruits, vegetables, and some beverages. It's a type of phenolic chemical with a low molecular weight that's found all over the plant kingdom. Because of their antioxidative, anti-inflammatory, anti-mutagenic, and anti-carcinogenic qualities, as well as their ability to influence important cellular enzyme processes, it has health-promoting properties. Antioxidant properties have been linked to the development of diseases like cancer, cardiovascular disease, and heart disease. Xanthine oxidase (XO), cyclo-oxygenase (COX), lipoxygenase, and phosphoinositide 3-kinase are among the enzymes that they can block. Chalcones, flavones, flavanols, and isoflavones are all subgroups of flavonoids shown in Figure 9. Onions and tea, for example, are key sources of flavanols and flavones in the diet (Panche, Diwan et al. 2016).

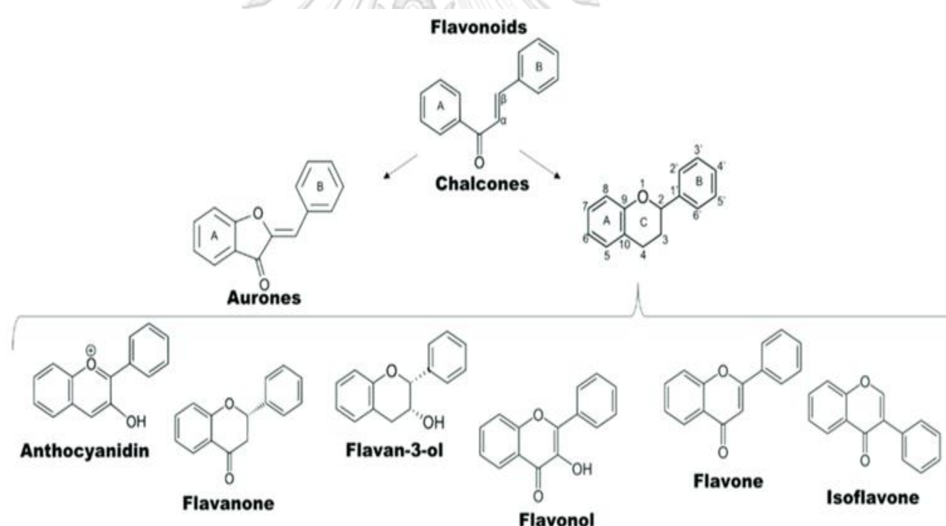

Figure 9 Sub-group of flavonoids

Source : (Carvalho Lemos, Reimer et al. 2019)

Quercetin is a flavonoid component that can be found in a variety of foods, including fruits, vegetables, nuts, seeds, flowers, and bark. In vitro, it can prevent platelet aggregation and reduce thromboxane production in vivo. The majority of *in vitro* and *in vivo* studies demonstrated that flavonoids, which have antioxidant and anticarcinogenic properties, may suppress numerous cancer processes.

Flavonoids have antioxidant properties and can be used in a variety of ways. They can scavenge radicals by breaking the chain of antioxidants, for example. They can also act as lipid peroxidation by recycling antioxidants, such as -tocopherol, and contributing hydrogen to the tocopherol. As a result, flavonoids' antioxidant qualities help to prevent carcinogenesis. Flavonoids can also protect DNA from oxidative damage, as well as suppress the expression of mutant genes and the action of enzymes that promote cancer. Previous research has demonstrated that the flavonoid quercetin can prevent tumor initiation, progression, and hyperproliferation in animal models (Kris-Etherton, Hecker et al. 2002).

Plants, animals, and microbes all use flavonoids in their biological processes. Flavonoids have been generated in sites for plants, and they are responsible for the color and perfume of flowers and fruits. Flavonoids have been linked to improved human and animal health, such as in treatment and chemoprevention. Approximately 6000 flavonoids are currently found in the vivid pigments of fruits, herbs, vegetables, and medicinal plants. They reported on the preventive benefits of flavonoids against human diseases as well as their actions in plants (Kumar and Pandey 2013).

2.2.1.2 Antioxidants

Antioxidants are any compounds or nutrients that can help to slow or stop molecules from oxidizing. Free radicals are produced via oxidation, a chemical reaction in which electrons are transferred from a substance to an oxidizing agent. Free radicals are extremely reactive and toxic chemicals that can damage DNA, lipids, and carbohydrates, resulting in diseases like cancer and atherosclerosis. Furthermore, antioxidants have the capacity to prevent alternative oxidation events and interrupt chain reactions. As a result, antioxidants relate to reducing agents like ascorbic acid and polyphenols (JP 2019). Antioxidants are chemicals that can reduce oxidative stress and thereby prevent cellular damage (Chandra and Arora 2017). Oxidative stress is a common process that occurs in biological systems when there is an equilibrium between the production of reactive oxygen species (FR) and the body's capacity to remove oxidative stress by using both intrinsic and extrinsic antioxidants. Consumption of antioxidants has been shown to lessen the risk of heart disease and other ailments, according to epidemiologists. Many antioxidants come from natural

plant sources such as fruits, vegetables, and herbs that are high in phenolic compounds, vitamins, and carotenoids. Antioxidants protect cells by a variety of ways, including converting reactive oxygen species (ROS) to non-radical species, stopping the auto-oxidative chain reaction started by reactive oxygen species (ROS), and lowering localized oxygen concentrations (Lourenço, Moldão-Martins et al. 2019). Antioxidants can activate the defense system in three ways: by blocking proteins from binding to free radicals and so reducing their harmful effects, by making tiny molecules that can scavenge free radicals, and by establishing a mechanism to rectify ROS-induced DNA damage (Ifeanyi 2018). Although antioxidant defenses differ between animals, antioxidant defense is ubiquitous. In the intracellular and extracellular environment, antioxidants exist in both enzymatic and non-enzymatic forms (Nimse and Pal 2015).

Antioxidants are classified into two basic types based on their activity: enzymatic and non-enzymatic antioxidants. Free radicals can be broken down and removed by enzyme antioxidants. In the presence of cofactors like copper, zinc, manganese, and iron, it can also transition from hazardous oxidative products to hydrogen peroxide (H_2O_2) and then back to water. Non-enzymatic antioxidants can cause free radical chain processes to be disrupted. Vitamin C, vitamin E, plant polyphenols, carotenoids, and glutathione are examples of non-enzymatic antioxidants. Antioxidants are classified as either water-soluble or lipid-soluble, depending on their solubility in water or lipids. Vitamin C and other water-soluble antioxidants are found in cellular fluids such as cytosol and cytoplasmic matrix. Vitamin E, carotenoids, and lipoic acid are lipid-soluble antioxidants that are generally found in cell membranes. Moreover, depending on their size, they might be classed as small-molecule antioxidants or large-molecule antioxidants. In the radical scavenging process, small-molecule antioxidants (such as vitamin C, vitamin E, carotenoids, and glutathione) can neutralize ROS. Enzymes and sacrificial proteins (albumin) are large-molecule antioxidants that can absorb ROS and stop them from interacting with the other components (Nimse and Pal 2015).

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2.2.1.3 Free Radicals

Free radicals are highly reactive compounds that have one electron unpaired. Reactive oxygen species (ROS) are the most common type of free radical, however

there is also a subset called reactive nitrogen species (RNS). Nitrogen-containing free radicals with a strong oxidizing capacity that increase oxidative stress are known as relative nitrogen species (RNS) (Aruoma 1998). In a variety of pathophysiological situations, ROS and RNS are linked to oxidative stress. Depending on the biological system, ROS and RNS can have both positive and detrimental effects. At low to moderate doses, the positive impact occurs, whereas at high concentrations, the negative effect may occur. Free radicals disrupt normal physiological processes at various stages, resulting in detrimental chain reactions and biological tissue molecular damage

ROS/RNS have the potential to generate oxidative stress and nitrosative stress, both of which cause cellular harm. An excess of reactive oxygen species (ROS) can degrade cellular lipids, proteins, or DNA, disrupting normal function. Singlet oxygen, superoxide anion (O^{2-}), hydrogen peroxide (H_2O_2), peroxyl radical (ROO), and the highly reactive hydroxyl radical are all reactive oxygen species (OH). Nitric oxide (NO) and peroxynitrite anion are also free radicals produced from reactive nitrogen (ONOO) (Ifeanyi 2018).

2.2.1.4 Analysis of antioxidant effects

2.2.1.4.1 2,2-diphenyl-1-picryhydrazyl (DPPH) radical scavenging assay

The DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical (Figure 10), which is based on the premise of an electron transfer that forms a violet solution in ethanol, is used to determine antioxidant activity. In the presence of antioxidant molecules, a free radical that is stable at room temperature can be reduced, resulting in a colorless ethanol solution. Using a spectrophotometer and an absorbance wavelength of 515 nm, the DPPH assay is a simple and quick approach to access antioxidant activity.

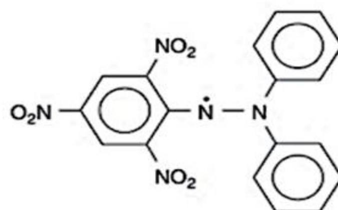

Figure 10 DPPH structure

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Source: (Proestos and Komaitis 2009)

When DPPH combines with a chemical that can donate a hydrogen atom or another radical (R·), it forms the reduced form of DPPH-H or DPPH-R, which loses color and results in a lower absorbance value (Figure 11). ArOH is an antioxidant that donates hydrogen atoms to form a stable molecular structure radical that can block and stop chain reactions. To obtain stable compounds, the new radical (ArO·) might react with another radical (DPPH-OAr, ArO-Oar).

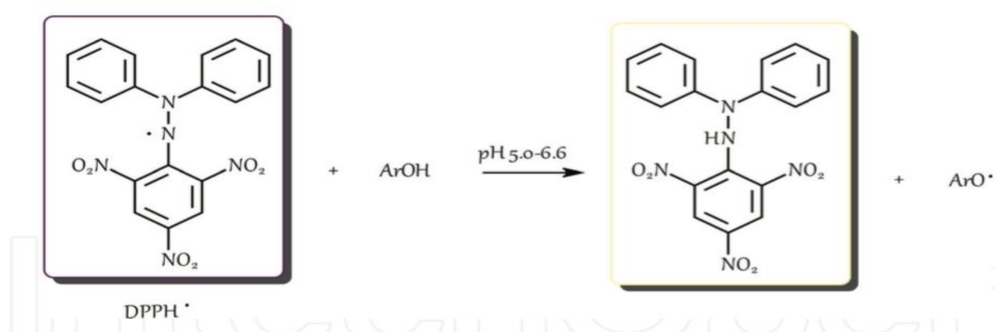

Figure 11 DPPH reduction by an antioxidant

Source : (Proestos and Komaitis 2009)

Because of its stability, the radical scavenging DPPH technique can be employed for practically any reaction with any type of antioxidant. To determine hydrophilic and lipophilic antioxidants, this approach can be employed with both polar and nonpolar organic solvents. The stability of DPPH varies depending on the presence of oxygen and solvents having Lewis's base characteristics. In methanol and acetone, the absorbance value of DPPH is lower than in other solvents (Santos-Sánchez, Salas-Coronado et al. 2019).

2.2.1.4.2 Ferric reducing power (FRAP) assay

Ferric reducing ability of plasma (FRAP) assay is a method to determine the antioxidant activity relies on the principle of reduction of ferric-tripyridyltriazine (Fe^{3+} - TPTZ) complex to ferrous tripyridyltriazine (Fe^{2+} - TPTZ) (Figure 12).

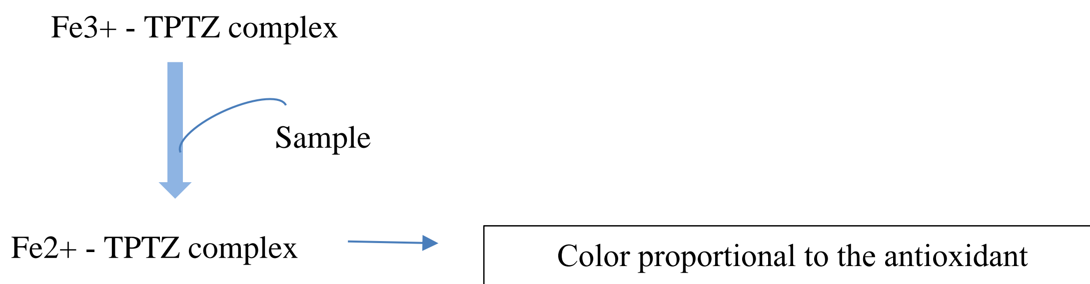

Figure 12 An overview of FRAP reaction

Source : (Rubio, Hernández-Ruiz et al. 2016)

Ferrous tripyridyltriazine (Fe^{2+} - TPTZ) has blue color with absorbance value of 593 nm and the changes in absorbance value depend on the antioxidant capacity of the plasma (Figure 13) (Rubio, Hernández-Ruiz et al. 2016). In most samples, FRAP assay needs an incubation time of 4 min at 37 °C for the antioxidant properties due to the redox reactions found in the incubation period (Santos-Sánchez, Salas-Coronado et al. 2019).

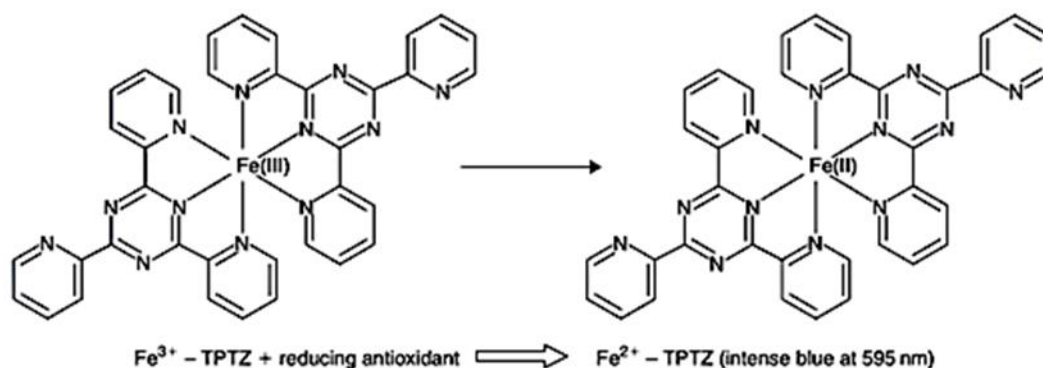

Figure 13 FRAP assay reaction

Source: (Proestos and Komaitis 2009)

2.2.1.5 Carotenoids

Carotenoids are hydrophobic compounds, lipophilic, water insoluble and soluble in solvents such as acetone, alcohol, and chloroform (Mezzomo and Ferreira 2016). Marigold flower (*Tagetes erecta* L.) is one of the common sources of carotenoids, mostly lutein diesters. Lutein possess biological activities such as cancer prevention, enhanced immune function, inhibition of auto-oxidation of cellular lipids, prevention of age-related molecular degeneration (Pratheesh, Benny et al. 2009). Two

types of carotenoids found in nature are: (a) the carotenes such as β-carotene containing linear hydrocarbons that can be attached at one side of the end or both side of the molecule and (b) Xanthophylls, the oxygenated derivatives of carotenes such as lutein, violaxanthin, neoxanthin and zeaxanthin (Figure 14) (Mezzomo and Ferreira 2016). Carotenoids have been widely used as safe chemicals for food supplementation and nutraceutical purposes because of their coloring abilities.

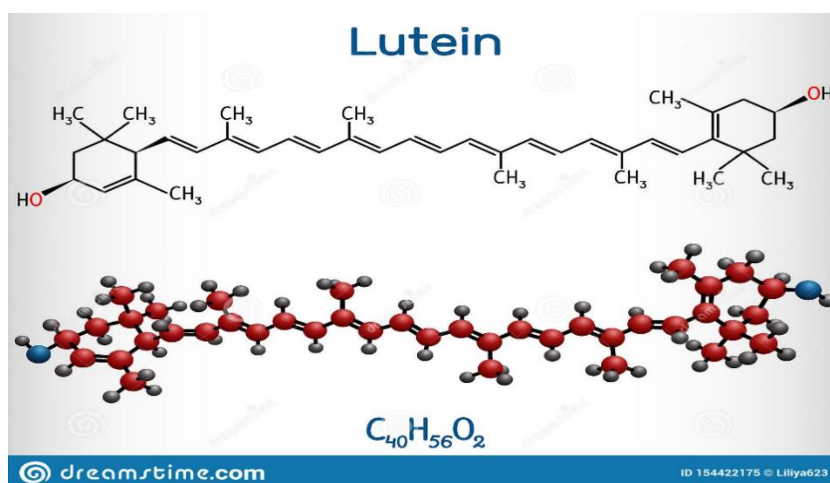

Figure 14 Structure of Lutein, Xanthophylls

Source : (Maoka 2020)

Carotenoids are composed of long chains of alternating double and single carbon-carbon bonds, with cyclic end groups and various keto-, hydroxyl-, and acid-functional groups in different places (Figure 15) (Tinoi, Rakariyatham et al. 2006).

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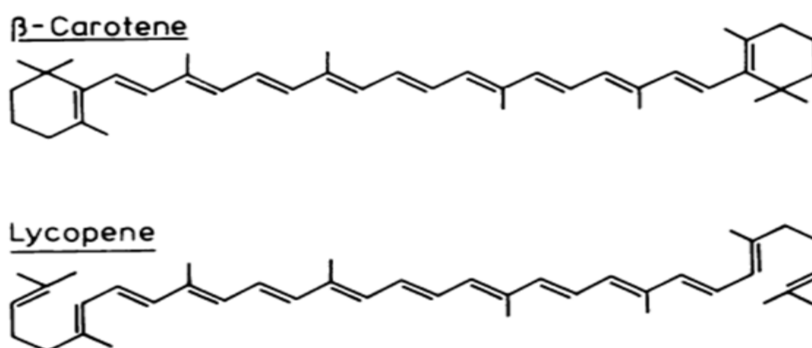

Figure 15 Structure of carotenoids

Source : (Maoka 2020)

Basic carotenoid biosynthetic pathways are indicated in Figure 16. Carotenoids are abundant and can be found in all sections of the plant (root, leaf, flower, fruit, and seed), but flowers are one of the most visible. Photosynthesis relies heavily on carotenoid pigments. Lutein is a yellow pigment that belongs to the xanthophyll group of oxygenated carotenoids. Because of its various industrial zones, such as poultry farming, cosmetics, food processing, and health-care industries, lutein has been called a natural bioactive chemical (Longa, Ahmedb et al.).

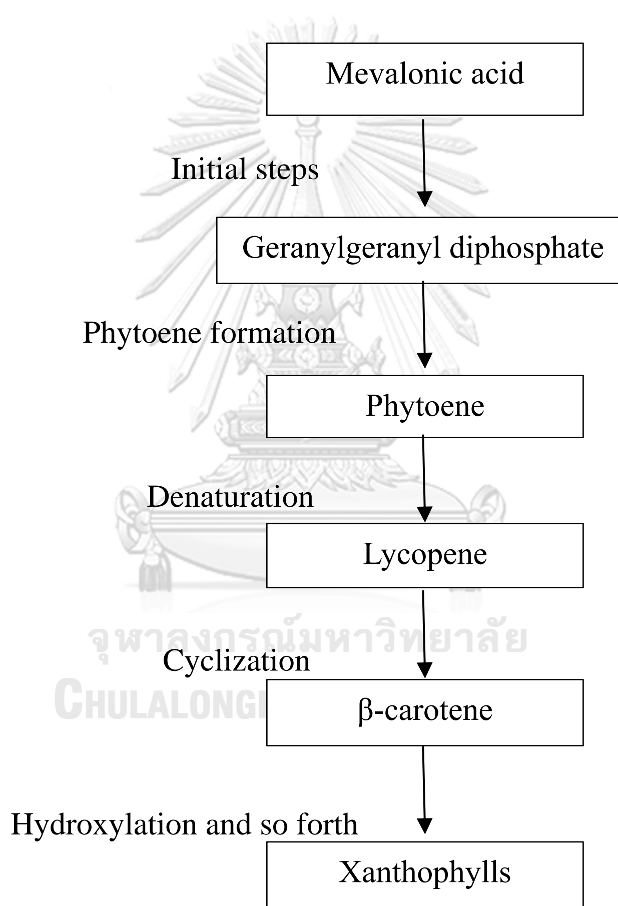

Figure 16 Flowchart of the biosynthesis stages of carotenoids

Source: (Maoka 2020)

Color is achieved using a variety of pigments. Carotenoids, on the other hand, are pigments found in yellow, orange, and red flowers (Tinoi, Rakariyatham et al. 2006). In addition, lutein has antioxidant capabilities, and carotenoids have biological qualities such as gap junction communication, cell growth regulation, gene expression

modulation, and immune response modulation. Because of their conjugated polyene chain, carotenoids are unsaturated. When carotenoids are stored in the presence of oxygen, they degrade quickly. Furthermore, temperature, light, and pH can alter the color of meals as well as their nutritional value (Spinelli 2016).

Furthermore, due of the activity of provitamin A, they are frequently employed as food additives. Vitamin A is essential for healthy growth, development, and immunological function. The lack of this chemical is a severe public health issue. It has the potential to cause significant illnesses such as growth problems and learning impairments, particularly in youngsters. Taking too much vitamin A, on the other hand, can induce toxicity and congenital deformity in pregnant women, as well as bone disease in persons with chronic renal failure (Mezzomo and Ferreira 2016). Carotenoids are extensively employed as a colorant in the food industry to standardize the color of products such as juices, beverages, candies, and margarines. It's also been used to standardize the hue of capsules, vitamins, and cosmetics in the cosmetics and pharmaceutical industries (Maoka 2020).

2.3 Introduction to Extraction Method

The first step in identifying bioactive chemicals from plant sources is to extract them using appropriate solvents. Extraction is the process of using appropriate solvents to separate bioactive chemicals from natural plant sources. The choice of solvent is an important role in the extraction method. The most used solvents for bioactive chemical extraction are ethanol and methanol. Furthermore, the choice of an appropriate extraction method unique to bioactive substances is critical since it impacts the bioactive characteristics' qualitative and quantitative features. Extraction is a critical stage in the food production of higher nutritional products such as health promoters, as bioactive chemicals must be obtained in adequate quantities and without causing damage to the raw materials. For additional classification, identification, and analysis of bioactive chemicals, a suitable extraction procedure must be used. Traditional methods such as maceration, Soxhlet extraction, hydro distillation, and solvent extraction, as well as non-conventional approaches such as ultrasound-assisted extraction and microwave extraction, can be used to extract biologically active

chemicals from organic matter. The right and appropriate extraction process is chosen to ensure a high extraction yield and extract quality. The following are the goals of extracting natural bioactive compounds: To extract the selected natural bioactive compounds from plant materials

1) To enhance the selectively of analytical methods by boosting the amount of specific molecules
2) To transform the bioactive substances for identification and characterization, and
3) To provide reproducible and efficient method

2.3.1 Hydro distillation

The classic method of extracting key bioactive components from natural plant sources is hydro distillation (Figure 17). Water distillation, water and steam distillation, and straight steam distillation are the three kinds of extraction. The plant materials must first be placed in the chamber, followed by the addition of water to the sample. After that, heat is used to bring the mixture to a boil. The key influencing elements for extracting bioactive chemicals from plant sources in this approach are hot water and steam. When the solvent is heated to its boiling point, vapor forms and condenses via the condenser. The condensed solvents are then passed through the separator, where the water is separated from the bioactive chemicals. The key physicochemical processes include hydro distillation, hydrolysis, and heat degradation. Some volatile chemicals may be degraded if the extraction temperature is too high. Furthermore, this approach is ineffective with thermally labile substances (Roopan and Madhumitha 2018).

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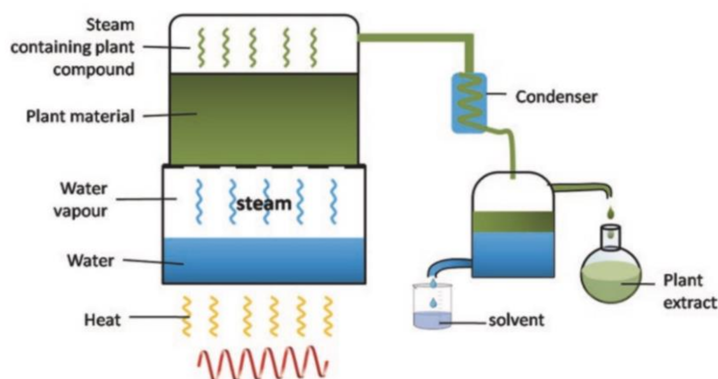

Figure 17 Hydro distillation

Source: (Roopan and Madhumitha 2018)

2.3.2 Maceration

Maceration is a low-cost way of extracting essential oils and bioactive substances from plants (Figure 18). The soluble substance is dissolved by maceration at a temperature of 15°C to 20°C. The method is broken down into several steps. To begin, plant materials must be grounded to maximize the surface area available for interaction with the solvent. In the second phase, a suitable solvent for extraction is added to the vessel and left to soften the plant tissue for 12-24 hr or more. After that, the liquid solution is filtered, and the solid residue is employed to recover the solvent once more. During processing, occasional shaking should be done to increase the rate of extraction by two ways; (a) increase diffusion, (b) remove concentrated solution to replace with the new solvent to get more extraction yield (Azmir, Zaidul et al. 2013).

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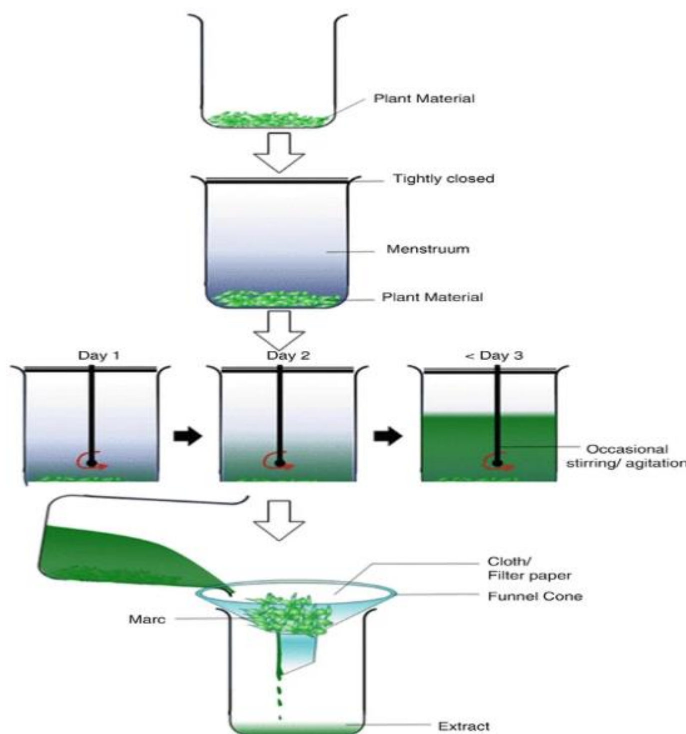

Figure 18 Maceration Extraction

Source: (Sasidharan, Jothy et al. 2018)

2.3.3 Soxhlet Extraction

A continuous solid/liquid extraction is known as Soxhlet extraction (Figure 19). This approach is often employed in the extraction of solid liquids, such as pharmaceuticals and food goods. To use, the solid material is placed in the thimble, which is then inserted into the extractor and fitted with a condenser. The organic solvent is heated in this apparatus, and the vapors are subsequently elevated and condensed into the thimble. The extract flows back into the chamber with the help of the condenser when the solvent reaches the level of siphoning.

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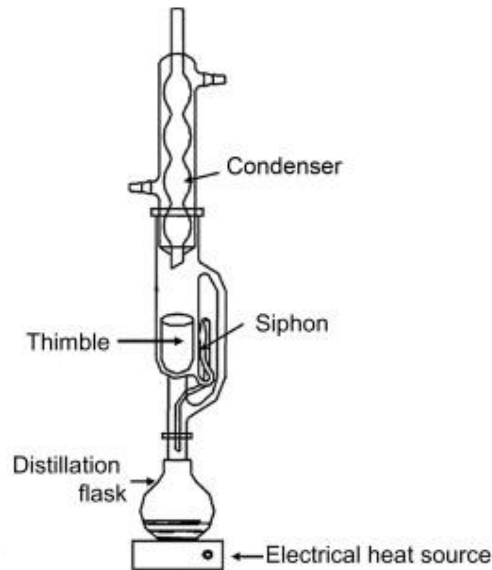

Figure 19 Soxhlet extractor

Source: (López-Bascón and De Castro 2020)

This approach is best for fine powder extracts that are heat resistant. It provides benefits such as high solvent recovery capacity, ease of use, and low cost. However, the Soxhlet extraction process has some disadvantages including a long extraction time, a significant amount of solvent required, and a lack of automation (Patel, Panchal et al. 2019).

2.3.4 Solvent Extraction

Solvent extraction is the process of combining two solvents (water + organic solvents) that do not dissolve uniformly. The feed solution is in one phase while the second solvent is in another during liquid extraction. The two solvents will be combined and separated in this extraction by touching each other (Figure 20).

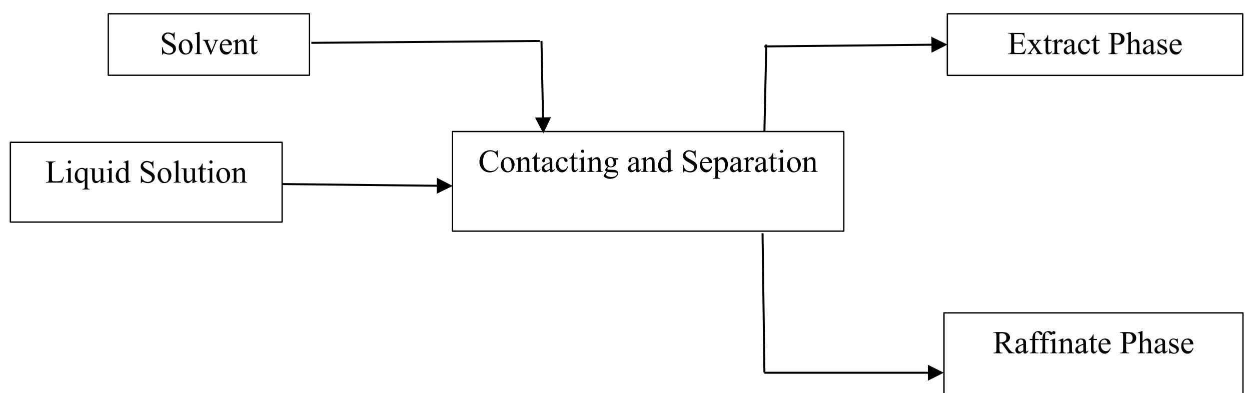

Figure 20 Block diagram for liquid-liquid extraction

Source: (Warade, Gaikwad et al. 2011)

Solid phase extraction is a sample preparation procedure that uses physical and chemical properties to isolate, enrich, and purify components from aqueous solutions as shown in Figure 21. This method describes an operation in which aqueous samples come into touch with a solid phase or sorbent and absorb on the solid phase's surface before being eluted. When compared to the size of the sample, the amount collected is insignificant (Patel, Panchal et al. 2019).

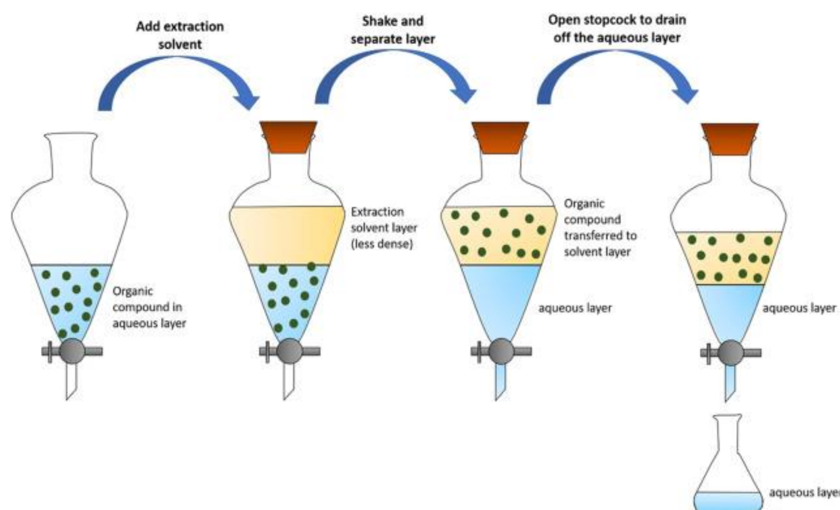

Figure 21 Solvent extraction diagram

Source: (Rudrapal, Kothawade et al. 2022)

2.3.5 Microwave Extraction

Microwave extraction is a type of non-ionizing electromagnetic radiation that differs from radio waves in that it is not ionizing as shown in Figure 22. Microwaves are classified by wavelength, which ranges from 1 mm to 1 m, frequency, which ranges from 300 GHz to 300 MHz, and photon energy, which ranges from 1.24 millielectron Volt (meV) to 1.24 microelectron Volt (eV). Due to their electromagnetic nature, can produce a perpendicular situation between electric and magnetic forces. Because of the arrangement of molecules, this electric field generates heat and induces dipolar rotation. The vibrating molecules collide with each other, releasing heat energy.

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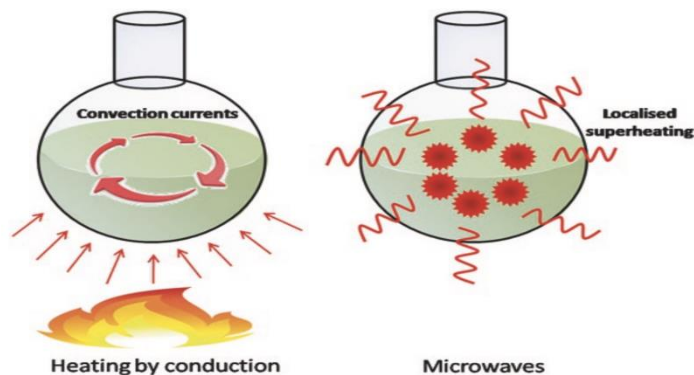

Figure 22 Heating process by conduction and microwaves
Source: (Roopan and Madhumitha 2018)

The dipole rotation of the molecule during microwave heating fractures the hydrogen bond, allowing dissolved ions to diffuse. There are numerous factors that influence the extraction method. Solvent type, solvent volume, extraction time, microwave power, matrix properties, and temperature are all factors to consider. Because of the reduced solvent usage, cost-effective and time-saving extraction, and high extraction rate, this approach is usually referred to as a green extraction (green technology). However, there are several disadvantages to this strategy. Bioactive chemicals, particularly tannins and anthocyanins, may be degraded by heat. Microwave extraction is a quick and effective approach; however, it has a low selectivity for bioactive chemicals.

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2.4 Ultrasound-assisted Extraction

Ultrasounds are electromagnetic radiations that have frequencies above human hearing but below microwave frequencies (from 20 kHz to 100 kHz). There are two categories of ultrasound equipment: named ultrasound (sonication bath) and probe-type ultrasound equipment (sonicator). Both devices employ a transducer to generate ultrasonic power. Sonication baths can be performed at a lesser cost and then used in a variety of procedures, including degassing and glassware cleaning (Koçak and Pazır 2018). The ultrasound assisted extraction (UAE) machine is made up of many parts: (a) an ultrasonic electric generator that generates a signal (typically around 20 kHz) that drives the transducer, and (b) a transducer that converts energy into a different form. This device resembles a probe and converts electrical energy into ultrasonic

energy. The transducer converts electrical energy into mechanical vibrations using piezoelectric crystals. (c) The sonicator, which amplifies the vibrations till they pass through the probe, and (d) The probe, which can transmit the vibration to the sonicated solution. The application of ultrasound waves in the extraction of bioactive compounds with two types of ultrasound-assisted extraction machine can be seen in Table 5.

Table 5 Application of ultrasound waves in the extraction of bioactive compounds from various plants

Plant	Bioactive compounds	Machine	Extraction Conditions	Reference
Mangrove leaves	Antioxidant	Ultrasonic bath	T= (5-60) min, 70% ethanol, 400 W	(Audah, Manuella et al. 2018)
Nephelium lappaceum L.fruit peel	Anthocyanin	Ultrasonic bath	T = (30-50) °C, time= (10-30) min, solid-liquid ratio (1:10)	(Maran, Manikandan et al. 2017)
Palm pressed fiber	Antioxidant	Ultrasound probe	T = 20±2 °C, intensity= (36-204) W/cm^2	(Dal Prá, Lunelli et al. 2017)
Garlic	Odor Additives	Ultrasonic bath	T =25 °C, t=30 min, solvent: ethyl acetate	(Kimbaris, Siatis et al. 2006)
Orange (peel)	Flavanone Glycoside	Ultrasound probe	ethanol: water (4:1), T = 40 °C, P=150 W	(Khan, Abert-Vian et al. 2010)

UAE uses ultrasound waves which cause contraction and expansion of gas bubbles to enhance the extraction process with the induction of cavitation damage in the cells containing organelles and cytoplasm. High shear forces can enhance the mass transfer rate for the soluble constituents into the cells. As the gas bubbles expand, the substances or compounds inside the cells can be dissolved into the

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solvent. Moreover, ultrasound can operate the process with enlarging the pores of the cell wall and enhancing the index for the swelling within sonication process. As a result, the cells break down and it will make easier to extract the compounds inside the cells (Figure 23) (Roopan and Madhumitha 2018).

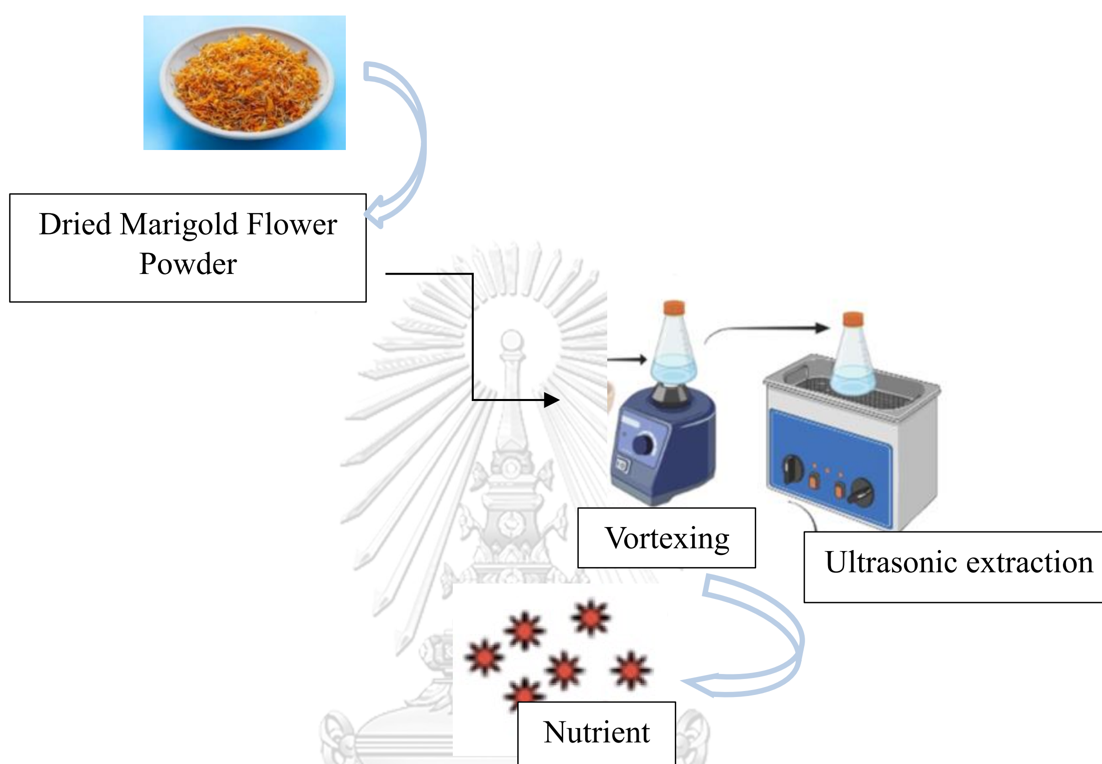

Figure 23 Steps to extract bioactive compounds using ultrasound-assisted extraction

Source: (Wen, Zhang et al. 2018)

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The following is how energy is converted in an ultrasonic machine: high voltage electrical energy and electric current are fed into the transducer, and this energy is converted to mechanical energy. The transducer then generates acoustic waves, which start the cavitation process, which involves the production of bubbles inside the liquid, which then crumple against one other as shown in Figure 24. High temperatures and pressures result from cavitation bubbles collapsing, which can create high shear energy waves and turbulence in the process. Temperature, viscosity, and treatment time are all essential processing parameters in the UAE. The viscosity of the sample decreases as the temperature rises, while the number of cavitation bubbles increases, resulting in a less viscous environment. The flow rate into the

ultrasound machine is likewise linked to the exposure time. When the energy input is increased, the flow rate decreases (Koçak and Pazır 2018).

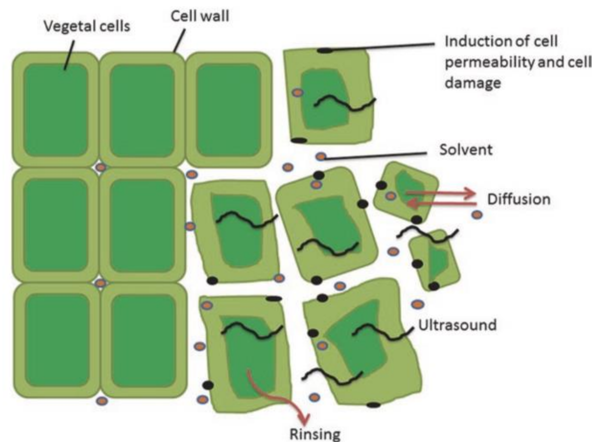

Figure 24 Schematic diagram for plant cell structure

Source: (Roopan and Madhumitha 2018)

Mechanisms of UAE can be categorized into two steps.

(1) Ultrasound waves can develop and cause cavitation bubbles that have large surface area near the surface of plant material. When the shape of the bubbles reaches the critical state, they crumble each other and generate huge amounts of energy.

(2) The generated temperature and pressure caused microjets which led towards the plant surface and broke up the cell membranes. As a result, antioxidants can be generated from plant matter as shown in Figure 25.

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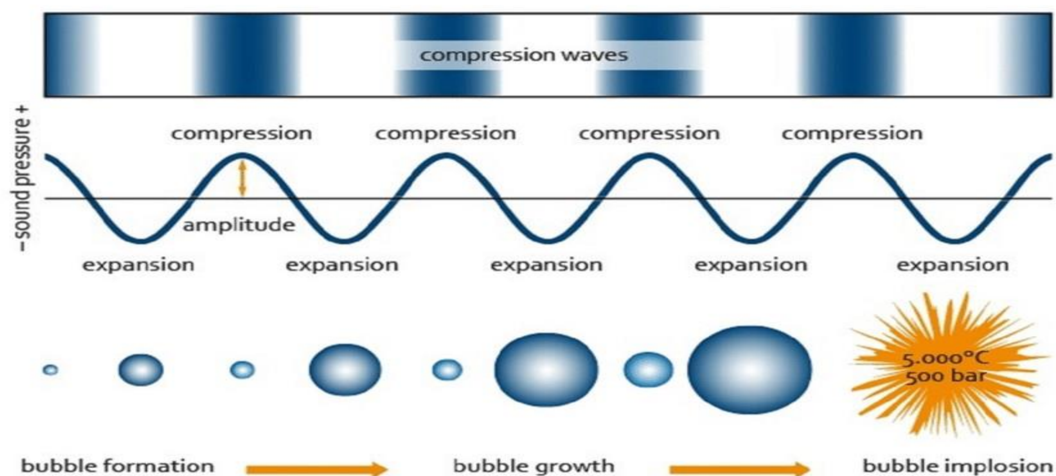

Figure 25 Mechanism of ultrasound-assisted extraction

Source: (Johansson, Pamidi et al. 2017)

Sonication has a number of advantages, including a faster extraction time and less energy and solvent use. The operation is inexpensive, readily available, and can handle a large number of samples at once. In comparison to the traditional method, ultrasound-assisted extraction is a green, environmentally friendly technique for the extraction of bioactive compounds (Roopan and Madhumitha 2018). The application of ultrasound energy in the extraction process can aid in efficient blending, improved energy transmission, temperature fluctuations and thermal treatment, and an improvement in production rate (Azmir, Zaidul et al. 2013).

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2.4.1 Cavitation

Ultrasound or ultrasonic waves are sound waves which have frequencies ranging from 20-100 kHz. Cavitation is the formation of small bubbles in the liquid medium due to not only the extreme acoustic pressure but also by the others such as hydrodynamic cavitation. Cavitation occurs in liquids when the pressure in the liquid medium falls until the critical point (Figure 26).

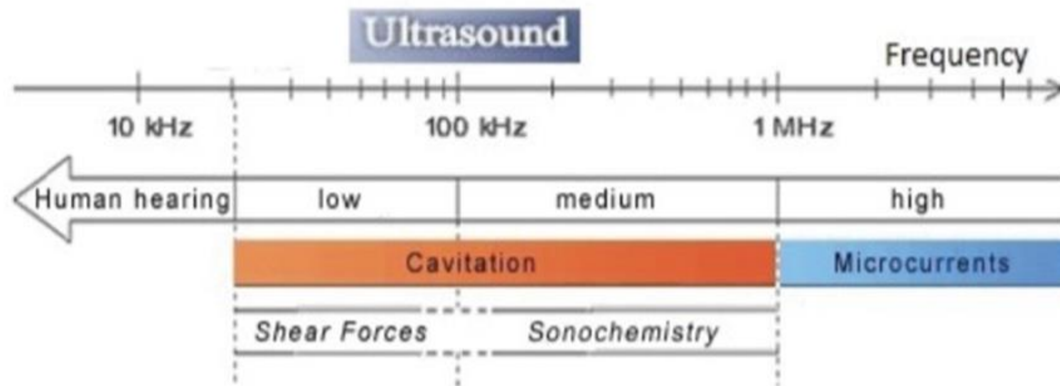

Figure 26 High frequency ultrasound wave

Source: (Vijigiri and Pamidi 2014)

Using high intensity ultrasound causes compression and rarefactions as shown in Figure 27. Cavitation can be separated into two categories: stable (non-inertial) cavitation and transient (inertial) cavitation. Stable (non-inertial) cavitation is the process formed when the energy is used as the acoustic field. This process occurs during the sub harmonic and ultra-hormonic frequencies of the main excitation frequency. Transient (inertial) cavitation occurs when violent implosion happens and results in the formation of shock wave ranging between 20 – 350 kHz (Vijigiri and Pamidi 2014).

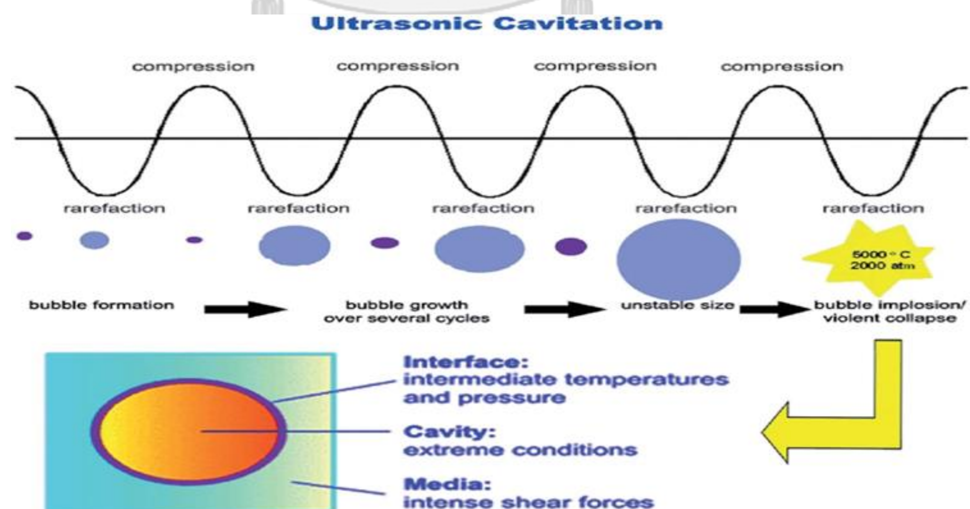

Figure 27 Ultrasound propagation and cavitation bubbles

Source: (Ashiri 2015)

Cavitation occurs when the ultrasonic waves produce bubbles. When the ultrasonic waves produce into the liquid, compression and expansion cycles are formed and lead to the formation of bubbles. The formation and development of bubbles, and implosive disruption is called cavitation (Naik, Suryawanshi et al. 2021). In compression phase, high acoustic pressure causes expansion of the bubbles. When the bubble ruptures, the shape of the bubbles is varied and generated microjets of the solvents and improves the penetration of the solvent into the cell wall. It improves the interaction between solid and liquid as shown in Figure 28.

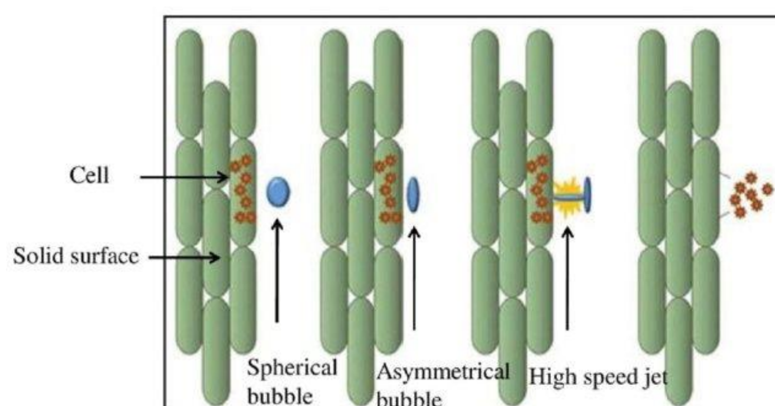

Figure 28 Disruption of cavitation bubbles and discharge of plant extract

Source: (Vardanega, Santos et al. 2014)

Additionally, for solid material, ultrasonic waves can cause swelling, hydration, and expansion of the pores of the cell wall and results in increasing diffusion and enhance mass transfer rate.

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2.4.2 Factors affecting the extraction

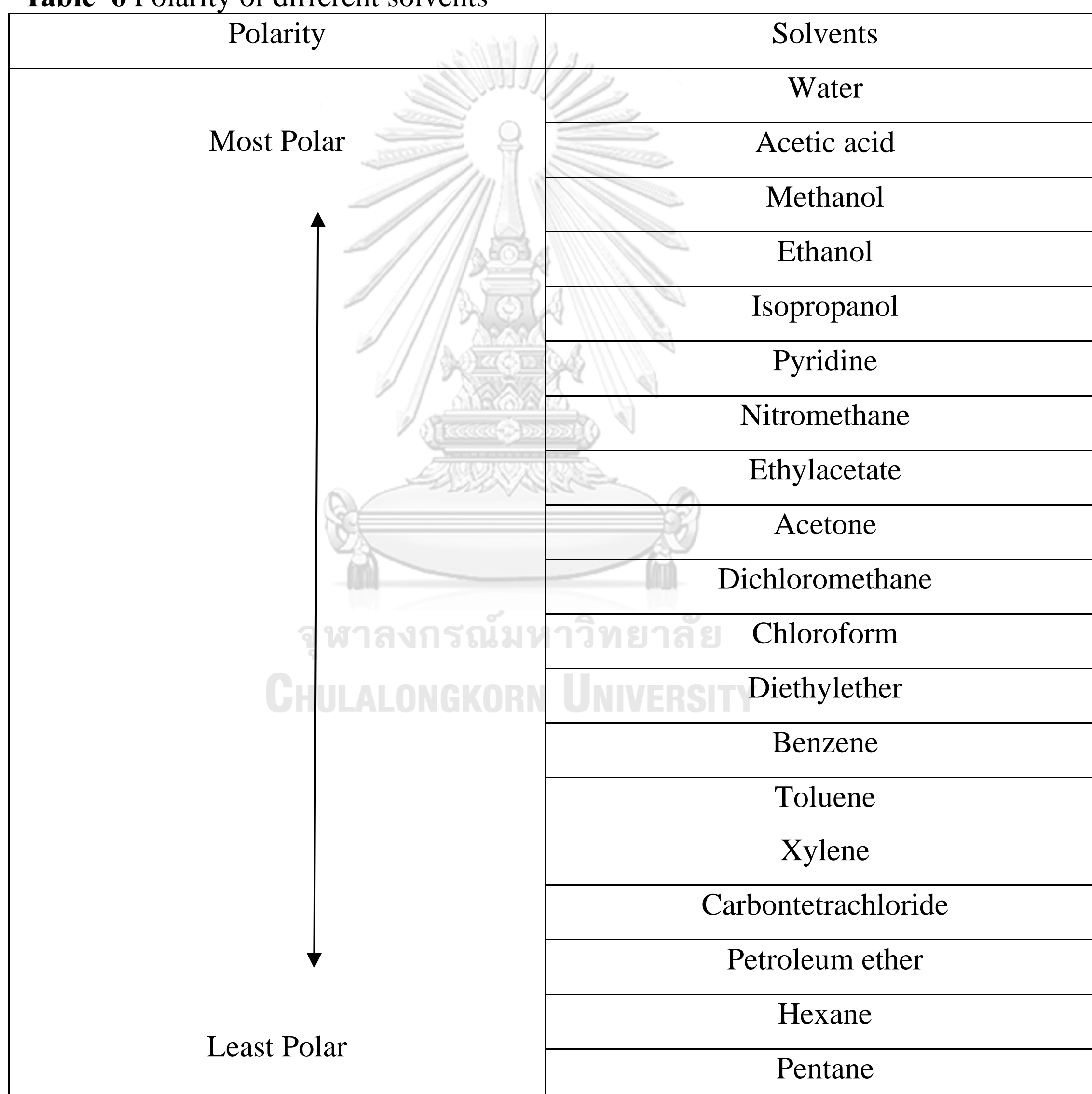
Factors related to high frequency sound wave extraction method are one of the major important because of the higher extraction efficiency. There are various factors affecting the extraction efficiency such as extraction method, solvent type, extraction temperature and time depending on the type of extracted compound (Chew, Khoo et al. 2011).

2.4.2.1 Solvent type

Different types of organic solvents starting from low polar organic solvents to high polar organic solvents are widely used in the extraction. Among solvents, ethanol

is the most used solvent compared to pure water, and others. The combination of water and ethanol enhances the recovery of the extracted phenolic compounds, since polar compounds are dissolved in the water whereas the compounds with less polarity are dissolved in the ethanol (Borrás-Enríquez, Reyes-Ventura et al. 2021). The efficiency of the extraction on bioactive compounds mainly depends on the nature of the extracted compounds, degree of polymerization and the selected suitable solvents with different polarities shown in Table 6:

Table 6 Polarity of different solvents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Polarity	Solvents
Most Polar	Water
↑	Acetic acid
	Methanol
	Ethanol
	Isopropanol
	Pyridine
	Nitromethane
	Ethylacetate
	Acetone
	Dichloromethane
	Chloroform
	Diethylether
	Benzene
	Toluene Xylene
	Carbontetrachloride
↓	Petroleum ether
	Hexane
Least Polar	Pentane

Source: Prasert Sripiroj (1985)

Polar organic solvents including methanol, ethanol, ethyl acetate, acetone, and combination with water are commonly used for the extraction of bioactive compounds.

2.4.2.2 Temperature

Temperature is an important factor that affected the extraction efficiency and extraction conditions of bioactive compounds. (Ghafoor, Choi et al. 2009) studied the extraction of polyphenols, and antioxidant form grape seeds with temperature ranging from 40 – 60 °C. The results revealed that increasing temperature results in an increase in phenolic compounds. For antioxidant properties, the highest amount is obtained at the highest temperature. It means that when the temperature increases, the antioxidant activity will also increase. The percentage yield of bioactive compound improved due to increasing extraction temperature. The effect of increasing temperature makes to enhance the number of cavitation bubbles and release more bioactive compounds into the solvent form the plant cells (Febriana, Kusuma et al. 2016). (Palupi, Serang et al. 2018) studied the extraction of antioxidant compounds from Petai (*Parkia speciosa* Hassk) leaves with extraction temperature of ranging from 40 – 70 °C. The highest temperature for the highest antioxidant compound was 65 °C. It showed that the increasing the region of interaction between solid and liquid phase can make the solute distribute quickly into the solvent due to the mechanical effects of compression. But for phenolic compounds, it is not stable for a long time at high temperature. The results from the previous studies revealed that ultrasound induction can produce the extract during temperature 60 – 80 °C. According to the results from the studies, the percent yield increases when temperature increases due to rising temperature can reduce velocity and make to increase solubility, diffusion rate and vapor pressure of the solvent and finally leads to releasing high quality of the extract compounds. However, there is also decreasing of yield when increasing temperature. This is due to the fact that the extracted compounds is also dependent on the chemical properties of the extracted compounds (Corbin, Byrt et al. 2015).

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2.4.2.3 Time

Time is one of the important factors which has effects on the extraction because it has the ability to contact the solutes with the solvent. (Borrás-Enríquez,

Reyes-Ventura et al. 2021) studied that the extraction of phenolic and antioxidant activity from mango residues with ultrasound-assisted extraction. In previous studies, the extraction was done at various extraction times (10, 20 and 30 min). The extraction efficiency has been affected by the interaction of the sonication time when the solutes are mixed with solvent during extraction. The results from the study revealed that the total amount of phenolic compounds increase when the extraction time increases. The amount of total phenolic compounds increases when the time increases from 10 to 20 min. However, when the extraction time is over 20 min, the amount of total phenolic compound decreases in concentration. This result was the same with the research done by (Zou, Xia et al. 2014) and showed similar effect on the bioactive compounds. It was identified that increasing solvent ratio and extraction time (sonication time) to 20 min gives the highest number of bioactive compounds because sonication can facilitate the hydration process and enhance the mass transfer into the solvent. Therefore, extraction time is an important factor in the extraction of phenolic compounds using UAE. Long extraction (sonication) time allows greater contact time to cause cavitation which ruptures the cell walls and results in releasing of bioactive compound especially phenolic compounds. However, using too high extraction time can cause decomposition of all phenolic compounds because of increased amount of heat.

2.5 Antimicrobial Activity

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2.5.1 Disc Diffusion Method

The disc diffusion test, a culture-based microbiology assay used in diagnostic and drug laboratories, can be considered as Kirby-Bauer test, disc diffusion antibiotics susceptibility test. This method is one of the most suitable antibiotic tests for antibacterial activity.

In diagnostic laboratories, the test is processed with the agar plate inoculated with the bacteria. In this test, antibiotic paper disks are used in the agar plate and then incubated for 18-24 hr. When the antibiotic has the ability to stop or kill the growth of bacteria, it will show a clear area around the disk where there is no bacterial growth. This area is known as the inhibition zone. The susceptibility of the bacteria to the antibiotics can be categorized into susceptible, moderately susceptible, and resistant

bacteria by comparing the diameter of the inhibition zone. According to this way, it can be chosen the most appropriate antibiotic relating to bacteria. For drug discovery laboratories, it is slightly different from the diagnostic laboratories. In this method, sample extract (plant extract or microbial extract) will be used to perform. The disks with the natural product extract are placed on to the agar plate inoculated with the selected bacterial strain and then inoculated. The sample extract with organic solvents such as petroleum ether, chloroform, ethanol and acetone will be determined for inhibition zone.

2.5.2 Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) is considered as the lowest concentration of the antimicrobial agent that will prevent the growth of the bacterial strain after overnight incubation. There are two methods for MIC determination:

1. Dilution methods
    - ➢ In agar
    - ➢ In a liquid medium
        - ✓ Microdilution
        - ✓ Macrodilution
2. Gradient methods
    - ➢ Strips impregnated with a predefined concentration gradient of antibiotic

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2.5.2.1 Dilution Methods

Broth microdilution is one of the dilution methods and mostly recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST). To determine MIC, Mueller-Hinton (MH) medium can be used in the form of agar (MHA) or broth (MHB). However, in some cases, 5% lysed horse blood or other compounds can be added as a supplement based on the selected bacteria or antibiotic type.

One of the practical and popular antimicrobial susceptibility tests is the micro-dilution method. To determine MIC by dilution methods, the used antibiotics are needed to do preliminary dissolution for the stock solution and then make dilution to

obtain the desired concentration (Kowalska-Krochmal and Dudek-Wicher 2021). Two-fold dilutions of antibiotics in the liquid medium will involve (Reller, Weinstein et al. 2009). Water can be used as solvent and dilution solvent especially for beta-lactams, fluroquinolones, and aminoglycosides. While others use phosphate buffer or dimethyl sulfoxide (DMSO), macrolides, chloramphenicol, and rifampicin use alcohol as the solvent. Standard trays contain 96 wells and each well contains 0.1mL. In broth microdilution method, the solution containing the dilution of antibiotics are transferred into the wells of the microplates. The plates with antibiotics were inoculated with the bacteria (1-5* 10^5 CFU/mL) and then incubated at 37 °C for 18-24 hr. After that, MIC was determined according to the turbidity of the plates as shown in Figure 29.

Figure 29 96 reagent wells plate and disposable tray inoculator

Source : (Reller, Weinstein et al. 2009)

2.5.2.2 Gradient Method

The gradient diffusion method employs the antimicrobial concentration gradient in the agar medium to determine the antimicrobial susceptibility. E-test strips impregnated on the underside with the dried gradient of antibiotic concentration and are made the signature on the upper surface with the concentration scale. The application of E-test strips (thin plastic test strips) is simple, fast, and suitable in routine microbiological diagnostics. The test strips were placed on the surface of the

agar plate that had been made inoculation with the selected bacteria and then incubated overnight. After incubation, MIC was determined by seeing the strips from the top and the ellipse shaped that showing the growth inhibition area with the test strip (Figure 30) (Reller, Weinstein et al. 2009).

Figure 30 E-test gradient diffusion method with vancomycin (VA), daptomycin (DM), and linezolid (LZ) using Mueller-Hinton agar

Source: (Reller, Weinstein et al. 2009)

2.6 Encapsulation Using Spray Drying

Bioactive compounds are easily destroyed, losing their activity, and posing a risk when they combine with other compounds in food, resulting in a change in the color or taste of the meal. Encapsulation is a process in which small particles or bioactive components are encapsulated within a covering material to improve phytochemical stability and protect chemicals from sensitive environmental elements such heat, moisture, oxygen, light, and interactions with other sensitive compounds (Stajčić, Ćetković et al. 2020). The substance that is used in the encapsulating may be expressed as the coating, membrane, shell, carrier material, wall material, external phase, or matrix. Two main types of encapsulates are expressed i.e., reservoir type and matrix type in Figure 31.

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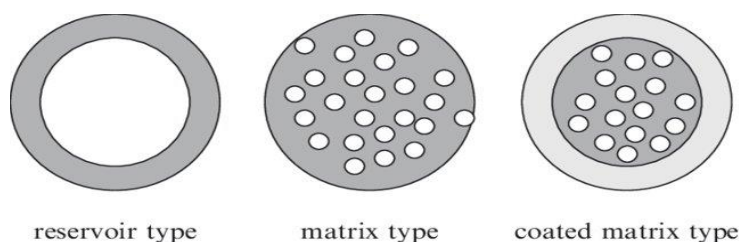

Figure 31 Structure of encapsulates

Source: (Zuidam and Shimoni 2010)

The reservoir type has a shell around the active agent and is called capsule, single-core, mono-core, or core-shell type. The active ingredient in the matrix type is dispersed considerably more over the carrier material; it can be in the form of tiny droplets or scattered more uniformly throughout the encapsulation. In contrast to reservoir encapsulates, active agents in matrix encapsulates are generally also present at the surface. Material inside the microcapsule is called core while the wall material refers to shell, coating, or membrane. The shape and type of the microcapsule can be chosen according to the properties of the encapsulation method, type of coating and core. Microencapsulation has been widely used not only for the food industry but also for pharmaceuticals as shown in Figure 32. One of the reasons for doing encapsulation is to enhance the stability of the products. Most of the bioactive compounds such as omega- 3 oils, carotenes and polyphenols can be degraded very easily and need to be protected from oxidation which can lead to off-flavors and off-odors. Moreover, microencapsulation can reduce evaporation, degradation of volatile compounds including aroma and it can help to improve the handling capacity of the ingredients during processing (Spinelli 2016).

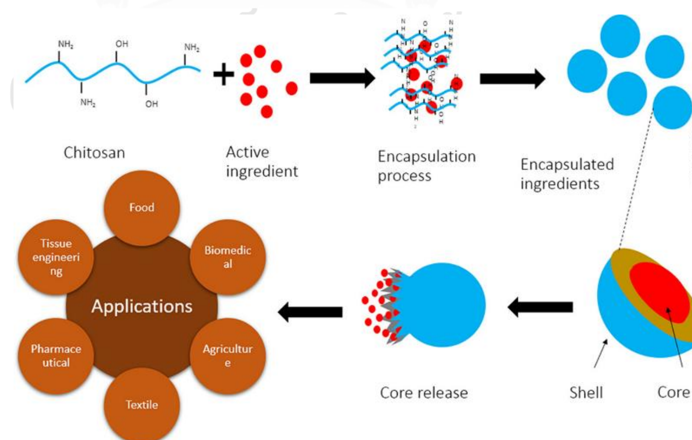

Figure 32 Process and application of encapsulation

Source: (Raza, Khalil et al. 2020)

Different techniques and coating/wall materials can be used in the encapsulation process. Spray drying, spray chilling, air suspension coating, extrusion, spray cooling, fluidized bed coating, centrifugal extrusion, freeze drying, coacervation, co crystallization, lipase entrapment, interfacial polymerization, and molecular inclusion are some of the techniques used in the encapsulation process (Kandansamy and Somasundaram 2012). Furthermore, the selection of encapsulation methodology and coating material is a critical phase in the encapsulation process since the microcapsule's encapsulation effectiveness and stability are reliant on the method and wall material used. Microencapsulation begins with the selection of an appropriate covering material. Furthermore, the selection of coating material is depend on the physicochemical parameters such as solubility, emulsifying, and drying properties, nature of the selected materials, desired characteristics of the final product and encapsulation process (Figure 33) (Stajčić, Ćetković et al. 2020).

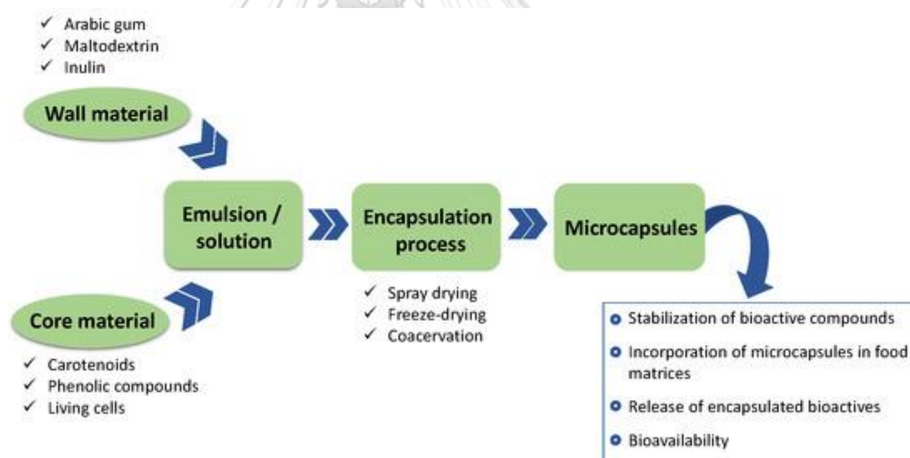

Figure 33 Encapsulation process with drying method
Source: (Corrêa-Filho, Moldão-Martins et al. 2019)

Gum Arabic, maltodextrin, starch, sodium caseinate, and gelatin are the most utilized coating materials. The wall materials must be water soluble throughout the spray drying procedure. Spray drying and freeze drying are prominent encapsulation techniques in the meat industry. Spray drying is generally used because of its speed, adaptability, and cheap, whereas freeze drying is mostly employed for substances that

deteriorate at extreme temperatures. Freeze drying requires more time to complete than spray drying and has a higher operational cost (Šeregelj, Ćetković et al. 2020).

(Shahidi and Han 1993) revealed the reasons for using microencapsulation in food applications:

a. to protect and reduce the core reactivity from external conditions
b. to minimize and manage the rate at which the solid material is transferred to the surroundings
c. to enhance the capacity of handling
d. to cover up the core taste
e. to dilute the core material when utilized in smaller doses.

2.7 Spray drying

Spray drying is mostly used for heat-labile products because of the lower temperature. Spray drying is one of the earliest encapsulation techniques used since the 1930's to encapsulate flavors with gum acacia. Spray drying is a process of making powder with atomizing liquid product by hot gas. Spray drying has been extensively employed for food and drug sectors. There are 3 steps in spray-drying process:

a. **Preparation of the dispersion**. In this stage, stable emulsion will be formed. The hydrophobic core material is dispersed into the immiscible coating agent solution to create the paste combination that will be atomized.
b. **Homogenization of the dispersion.** The prepared dispersion involves heating and blended with or without using a surfactant according to the condition of rheological behavior. The stability of the emulsion is also important and depends on the period of time. (Rosenberg, Kopelman et al. 1990) reported that viscosity and particle size can affect the efficiency of spray drying. High viscosity can disrupt the atomization and cause the formation of long and big droplets that slow the rate of drying.
c. **Atomization of the mass.** The atomization process can be done by atomizers which contain rotary atomizers, pressure nozzles, pneumatic nozzles, and sonic nozzles. In this step, the solvent mixture is atomized into heated air stream with drying chamber in which evaporation process occurs and then

leads to microencapsulation process. Decreasing the particle size and dispersion of the particles can increase the surface area of the particles which can help to dry the feed for a while. Finally, the granules, powders and agglomerates can be obtained (Spinelli 2016) (Figure 34).

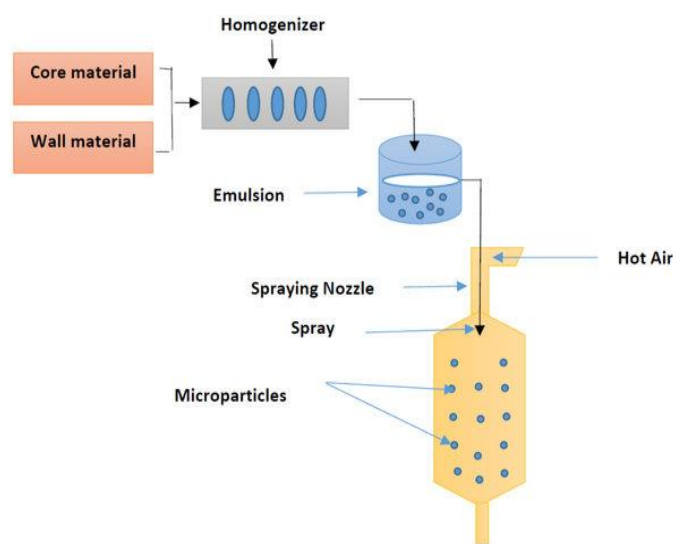

Figure 34 Typical spray-drying process flow chart
Source: (Mohammed, Tan et al. 2020)

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The operating procedure for this method is to dissolve the core materials in water to get emulsion (liquid form) and then add into the hot medium (100-300 ℃) to remove the water. Using high temperature in the drying machine helps to evaporate the water from the feed. Finally, regardless of the type of components and the working circumstances, the dehydrated particle could be produced as granules or encapsulated particles.

Drying efficiency and properties of the final product depend on the phases and operational parameters such as feed temperature, inlet air temperature and outlet temperature. In general, viscosity of the emulsion and fluidity are influenced by feed temperature. Using too high a temperature can cause volatilization or degradation of thermolabile materials. Inlet air temperature is also relatively proportional to the microcapsule-drying rate and final water content. However, high temperature may lead to evaporation which can cause some cracks in the membrane, therefore.

Moreover, flavoring agents can be released through volatilization and thermal degradation. For the outlet air temperature, it cannot be controlled but it depends on the inlet air temperature. Temperature for the microencapsulation process should be around 50 – 80 °C. In addition, some limitation for spray drying is that wall coating material should have good solubility properties (Spinelli 2016).

2.8 Wall materials for microencapsulation

Microencapsulating agents are biomolecules obtained from various plants and animals. Desai (2005) studied that coating materials must have good rheological properties at high concentration, and it should have the ability to disperse or emulsify the active material and stabilize the emulsion. The wall materials have the effect on the emulsion stability and the properties of the generated microparticles as shown in Table 7. Various types of wall materials have been utilized in the encapsulation of the flavors and oils, containing low molecular weight polysaccharides. Combination of two or more coating agents can increase the encapsulation effectiveness of spray-dried powders and improve the consistency and distribution of the size dispersion.

Table 7 The majority of the substances for walls being used spray drying

Wall material	Interest
Maltodextrin (DE < 20)	Film forming
Corn syrup solid (DE > 20)	Film forming, reducibility
Modified starch	Very good emulsifier
Gum Arabic	Emulsifier, film forming
Modified cellulose	Film forming
Gelatin	Emulsifier, film forming
Cyclodextrin	Encapsulant, emulsifier
Lecithin	Emulsifier
Whey protein	Good emulsifier
Hydrogenated fat	Barrier to oxygen and water
Chitosan	Carrier of drug delivery

Source: (Mohammed, Tan et al. 2020)

The coating materials can be categorized into 3 groups:

- Protein is amphiphilic that has physicochemical and functional properties for encapsulation with hydrophobic core materials. The most widely used proteins are whey proteins and gelatin. Whey proteins can be used for anhydrous milk fat to encapsulate with the yield over 90%. However, at high temperatures, the effect of spray drying on the stability of wall materials is not easy to predict. In addition, gelatin, effective for entrapping agent, has emulsifying activity, high stabilizing activity. However, using protein as an encapsulating agent has some limitations. When microcapsules are mixed with the product with pH near isoelectric point, allergy or protein precipitation may occur.
- Lipids, secondary coating materials, are used to enhance the properties of moisture barrier. Lipids can be used by combination in the emulsion to get the matrix from or film.
- Carbohydrates containing starch, maltodextrins and gums are also encapsulating agents with low viscosity and good solubility. Among hydrolyzed starch, maltodextrins are one of the coating agents having low cost and good flavor protection but with emulsifying properties deficiency. When used as coating materials, it needs to be mixed with other wall materials such as gelating agent, whey proteins to enhance the emulsifying properties. Gum Arabic is also a natural product from acacia and one of the effective wall materials for microencapsulation which has low viscosity in aqueous solution and excellent in volatile retention.

2.8.1 Maltodextrin

Maltodextrin (MDs) are a type of carbohydrates (CHOs) extracted from plant sources produced by enzymatic or acid hydrolysis of the starch, then lead to purification and spray drying (Hofman, Van Buul et al. 2016). Maltodextrin has been used as an alternative for acacia gum to improve the oxidative stability of encapsulated products. It can be considered as a polymer of D-glucose chains connected with glycoside α- (1-4) and α- (1-6) bonds and resulting in the formation of linear (amylase) and branched (amylopectin) carbohydrates with dextrose (DE). Maltodextrin has DE values within 3-20 ranges while those have higher than 20 refers to glucose syrups. Maltodextrin with low DE value is viscous (Figure 35).

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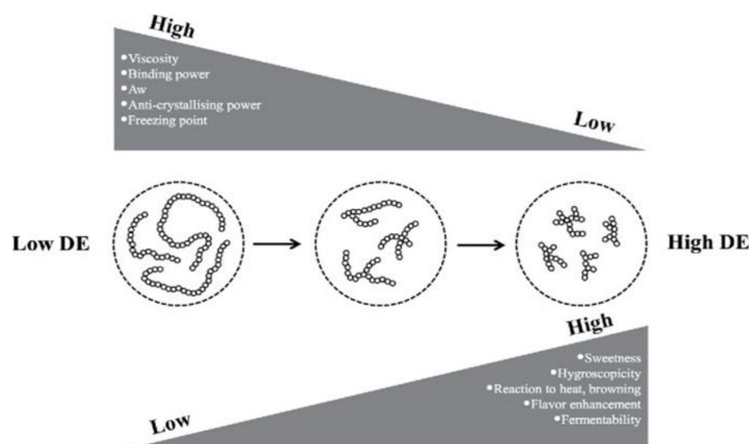

Figure 35 Maltodextrin related to the DE equivalent

Source: (Hofman, Van Buul et al. 2016)

Generally, maltodextrin with greater DE value has smaller molecular weight and greater solubility. However, maltodextrin with high DE value has high humidity due to the presence of more water (Tonon, Brabet et al. 2009). The physicochemical and functional properties of MXs depend on the DE value. For example, powder can be considered as highly hygroscopic powder or as a concentrated liquid solution.

2.8.1.1 Production of Maltodextrins

Maltodextrin was invented by Japanese scientists and manufactured under Matsutani Chemical Industry. The process of maltodextrin production (Figure 36) consists of an acid digestion process. During the process, the starch is heated at 140-160 ℃ with water, resulting in the swallowing of the crystalline structure of starch granules and then broken. After degradation, the length and appearance of the D-glucose will be varied. Digestible MDs $(C_6H_{10}O_5)$ nH_2O, saccharide polymers, have short chain length.

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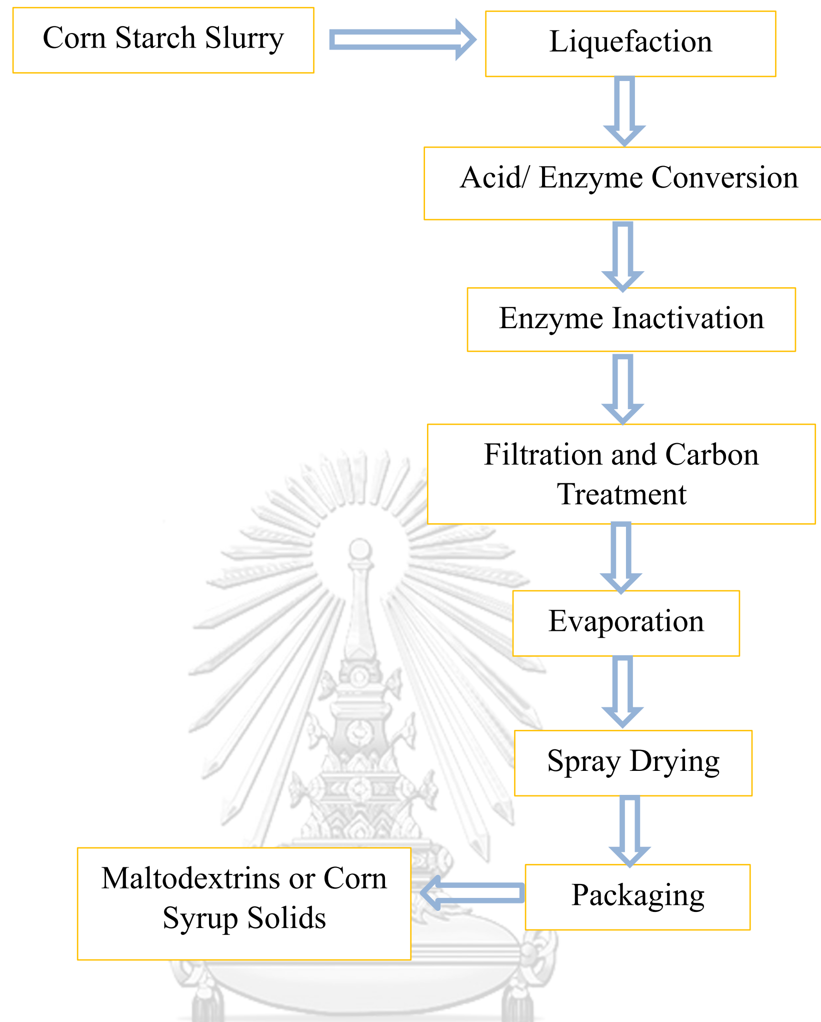

Figure 36 Maltodextrin production process

Source: (Hashizume and Okuma 2009)

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The final product is white powder with high purity and microbiological safety and mostly utilized in food and beverage products such as baked foods and in carbonated beverages. Moreover, MDs have been widely utilized in numerous applications including medical food products such as tablets and powder. MDs are mostly soluble in water and it has important properties such as gelling or freeze control (Hofman, Van Buul et al. 2016). In the food manufacturing process, MXs have been employed to enhance the smoothness, reduce the floury taste, manage non-enzymatic browning, decrease the freezing point in mixes, and can modify sweetness. The most important beneficial effect of using MXs is to increase T_g of the food

product and to preserve the physicochemical properties (Saavedra-Leos, Leyva-Porras et al. 2015).

2.8.2 Gums

Gums have been used in encapsulation process for the film formation because of the stability of the emulsion. The most used gum is acacia gum, known as gum Arabic (GA) due to its emulsification properties. GA is a polymer containing D-glucuronic acid, L-rhamnose, D-galactose, and L-arabinose, with almost 2% protein. GA is a useful substance that can be used as an emulsifier, flavor enhancer, emollients, thickening, surface-finishing agent, and to delay the crystallization of sucrose (Mohammed, Tan et al. 2020).

Gum Arabic is obtained from *Acacia* trees and the Joint Expert Committee on Food Additives of the Food and Agricultural organization (JECFA/FAO) in 1999 showed that the dried exudate is obtained from the stems and branches of *Acacia senegal* (L.) Wildenow or *Acacia seyal* (Family *Leguminosae*) (Figure 37).

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Figure 37 Arabic gum from the acacia tree's holly bushes
Source: (Musa, Ahmed et al. 2018)

Gum obtained from *A. senegal* is considered as Hashab, while the other obtained from *A. seyal* is referred to as Talha. Gum Arabic is known as an excellent reputation and earliest natural gum since it has been used at the time of the ancient Egyptians over 5,000 years ago. *Acacia senegal* is considered as the common origin of gum Arabic due to its water-resistant ability and tolerance of environmental conditions such as variation in temperature.

Gum exudates extracted from plants are considered water soluble, complex acidic polysaccharides and have viscosity or gelling properties. In general, it can be used in the mummification processes because its composition can promote the preservation of the bodies, changing the odor of the decomposition. Moreover, it can also be utilized as a thickening agent in various industries such as pharmaceutical, cosmetic, food and textile industries because of its emulsion stability and emulsifying properties.

To produce food industry, gum Arabic (Source: *Acacia senegal* and *Acacia seyal*), Tragacanth gum (Source: *Astrangalus gummifer*), gum Ghatti (Source: *Anogeissus latifolia*), gum Karaya (Source: *Sterclia urens*), Grewia gum (Sources: *Grewia mollis*) and Mesquite gum (Source: *Prosopis* spp.) are the most commercial used. Among them, gum Arabic is the most widely used in commercials. It is widely used in encapsulation process and stabilizing of oil emulsion in soft drinks.

Physical properties of gum Arabic observed are moisture, ash, volatile matter, and internal energy as shown in Table 8. Generally, gum Arabic contains a water-loving part (carbohydrate) and non-water loving part (protein).

Table 8 Quality parameters observed in gum Arabic

Property	Range		
Moisture content (%)	13 – 15		
Ash content (%)	2 – 4		
Internal energy (%)	30 – 39		
Volatile matter (%)	51 – 65		
Optical rotation (degrees)	-26 - -34		
Nitrogen content (%)	0.26 – 0.39		
Cationic composition of total ash (550 °C)			
Copper (ppm)	Iron (ppm)	Manganese (ppm)	Zinc (ppm)
52 – 66	730 – 2490	69 - 117	45 - 11

Source: (Dauqan and Abdullah 2013)

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Water content improves the absorption of carbohydrates with water loving capacity and water free proteins in gum Arabic. Total ash content is utilized to reveal the amount of insoluble products in acid, calcium salt, potassium and magnesium. The composition of cation in ash is used to indicate the number of heavy metals in gum Arabic.

Chemical composition of gum Arabic is a slightly acidic compound which is made up of polysaccharides, glycoproteins and calcium, magnesium, and potassium. Many researchers have been analyzed the chemical composition of gum Arabic harvested from *Acacia senegal* and *Acacia seyal* trees. It was discovered that gum Arabic derived from both species had the same composition of amino acids but for protein *Acacia senegal* has 2.7% while *Acacia seyal* has 1.0% as shown in Table 9.

Table 9 Gum from *A. sengal* and *A. seyal* characteristics

	Acacia Senegal	*Acacia seyal*
% galactose	44.00	38.00
% arabinose	27.00	46.00
% rhamnose	13.00	4.00
% glucuronic acid	14.50	6.50
4-O-methyl glucuronic acid	1.50	5.50
% Nitrogen	0.36	0.15
Protein	27.00	10.00
Specific rotation/degrees	-30.00	51.00
Average molecular mass (mw)	380,000	850,000

Source: (Dauqan and Abdullah 2013)

Many researchers studied that gum Arabic contains three fractions. The structure of gum Arabic (Dauqan and Abdullah 2013):

- The major fraction is a branched polysaccharide linking D-galactose with branches of L-arabinose, L-rhamnose and D-glucuronic acids.

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- The smaller fraction has a higher molecular weight arabinogalactan-protein complex which is covalently linked with a protein chain. Glucoronic acid is observed in the attached arabinogalactan.
- The smallest one with highest protein content is a glycoprotein but it has different structure in amino acids composition as shown in Figure 38.

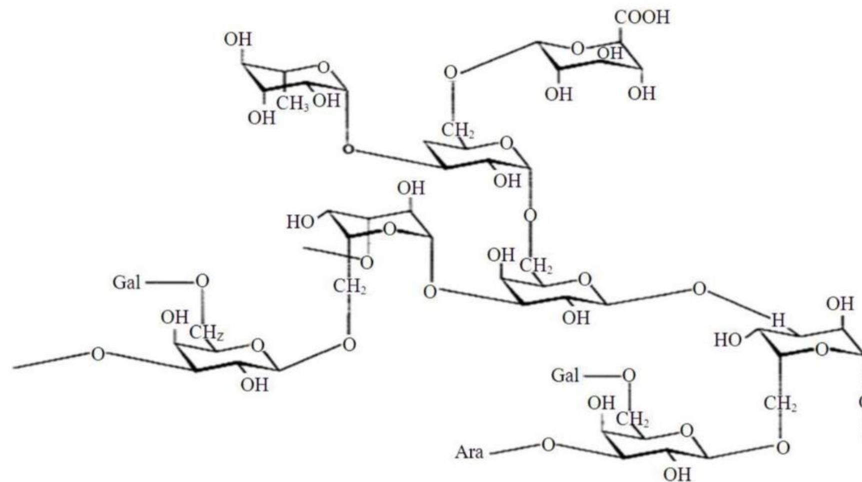

Figure 38 Structure of Gum Arabic
Source: (Dauqan and Abdullah 2013)

In addition, research done by (Sakulnarmrat, Wongsrikaew et al. 2021) revealed that gum Arabic has better encapsulation properties for the microcapsules from red cabbage compared to the products with maltodextrin because maltodextrin cannot serve to form microsphere but can act as wall material. Gums obtained from plants are low cost, nontoxic, odorless, colorless, tasteless and completely soluble in water and does not significantly affect the flavor, odor and color of the food products (Gashua 2016).

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Chapter 3

Research Operations

Raw Materials

Marigold Flower

Resistant Maltodextrin DE 10-12 (Zhucheng dongxiao, China)

Gum Arabic (Agrigum, UK)

Chemicals and reagents

2,2-diphenyl-1-picrylhydrazyl (DPPH) (Fluka, U.S.A)

6-hydroxy-2,5,7,8-tretamethylchroman-2-carboxylic acid (Trolox) (Fluka, Denmark)

Aluminium chloride (Ajax Finechem, New Zealand)

Ethanol (EMSURE, Germany)

Ferric chloride (Fisher Scientific, UK)

Folin-Ciocalteu reagent (Carlo Erba, France)

Gallic acid (Fluka, Spain)

Glacial acetic acid (A.R. grade, QReC, New Zealand)

Hydrochloric acid 0.1 M (A.R. grade, Ajax Finechem, Australia)

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Methanol (Fisher Scientific, UK)

Quercetin (Sigma-Aldich, Germany), and acetone

Sodium acetate tetra-hydrate ($CH_3COONa.3H_20$) (A.R. grade KemAus, Australia)

Sodium carbonate (Na_2CO_3) (A.R. grade, Ajax Finechem, Australia)

Sodium nitrite ($NaNO_2$) (Ajax Finechem, New Zealand)

Tripyridyltriazine (TPTZ) (Merck, Germany)

Acetone (Barentz. U.S)

Tools

X-ray energy dispersive spectroscopy and scanning electron microscopy

Spectrometer (JEOL, JSM-IT300, Japan)

Rotary Evaporator (BÜCHI, R-114, Switzerland)

Slurry Mixer with Hotplate Stirrer (SCILOGEX, SCI550-S, U.S.A)

Ultrasonic processor (Hielscher, MODEL UP400S, Germany)

Vortex Mixer (Scientific Industries, GENIE 2, U.S.A)

Analytical Balance (Mettler Toledo, New Classic MF, Switzerland)

Semi Auto Impulse Sealer (TUPACK, PHS 450/10D, Thailand)

Vacuum Sealer (Multivac, Model A300/16, Germany)

Spray Dryer (BUCHI, B-290, Switzerland)

Laboratory Blender (Warning 8010BU, U.S.A)

Homogenizer Laboratory (Ystral, X10, Germany)

Centrifuge (Kubota, 6000, Japan)

Aqualab, series 3 TE, U.S.A

UV-Visible Spectrometer (Thermo Fisher Scientific, GENESYSTM 20 Visible, U.S.A)

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Differential Scanning Calorimeter (NETZSCH, 204 F1 Phoenix, Germany)

Chroma meter (Konica Minolta, CR-400, Japan)

Hot air oven (Memmert, DO 6062, Germany)

Laminated aluminum foil bag

Shaking water bath (Julabo, SW33, Germany)

Procedures for conducting research experiments

3.1 Marigold flower sample preparation

Marigold flowers were picked in Thailand, and transferred to Chulalongkorn University's Department of Food Technology, Faculty of Science. Before being used in the lab, the flowers were cleaned with tap water to eliminate dust. The flowers were then dried for 24 h at 60 °C in a hot air oven (Memmert, DO 6062, Germany) until the moisture content was less than 5%. The dried marigold flowers were ground and sieved through a 50-mesh sieve before being vacuum sealed in an aluminum alloys foil package and stored at -20 °C for further investigation from the UAE experiment.

3.2 Ultrasound-assisted extraction (UAE)

3 g of marigold flower extract was weighed and combined with 100 mL (60 %, 80 %, and 100 % ethanol) (v/v) of solvent in a beaker (250mL) extraction. UAE was then operated on using an ultrasound processor at various temperatures (30, 40, 50 °C) and times (5, 10, 15 min) as shown in Table 10. After extraction, the samples were centrifuged at 10,000 rpm for 10 min at room temperature (Centrifuge Kubota, series 6000, Japan), and the supernatant was filtered and evaporated under vacuum at 45 °C using a rotary evaporator (Oilbath B-485, BUCHI, Switzerland). The resultant sample extract was diluted to 10 mL with distilled water after evaporation and stored in amber glass bottles at 4 °C before further analysis.

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Table 10 Levels of factors in the extraction using Ultrasound-assisted extraction method

Factors	**Levels**		
Ethanol concentration (%)	60	80	100
Extraction time (min)	5	10	15
Extraction temperature (°C)	30	40	50

Marigold flower extract was further determined for antioxidant and antioxidant activity as follows.

3.2.1 Total phenolic compounds by Folin-Ciocalteau colorimetry (Appendix A.1)

3.2.2 Total flavonoid content by aluminum chloride colorimetry (Appendix A.2)

3.2.3 2,2-diphenyl-1-picrylhydrazyl's antioxidant activity (DPPH) (Appendix A.3)

3.2.4 Antioxidant activity by ferric ion reducing antioxidant power (FRAP) (Appendix A.4)

3.2.5 Total carotenoid content (Appendix A.5)

3.3 Optimization of ultrasound-assisted extraction in marigold flower

Response surface methodology (RSM) with Box-Behnken Design (BBD) was used to evaluate the optimum condition of the three independent variables (A, ethanol concentration, B, temperature, C, time) and three levels (-1, 0, 1) each were utilized to analyze the ideal conditions for five responses—total polyphenol compound (TPC), total flavonoid content (TFC), antioxidant activity as measured by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assay, antioxidant activity as measured by the ferric reducing antioxidant power (FRAP) assay and total carotenoid content. The independent variable and its levels were displayed in Table 11.

Table 11 Actual independent variable levels at coded factor levels utilized in the RSM

Symbol	Independent variable	Actual levels at coded factor levels		
		-1	0	1
A	Ethanol Concentration (%)	60	80	100
B	Temperature (°C)	30	40	50
C	Time (min)	5	10	15

The variation of TPC, TFC, antioxidant activities by DPPH radical scavenging activity and FRAP and carotenoid content relating to the three variables A, B, and C were evaluated using a second-order polynomial equation:

$$Y = \beta_0 + \sum\beta_i X_i + \sum\beta_{ii} X_i^2 + \sum\beta_{ij} X_i X_j$$

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where Y is response (total phenolic compound, total flavonoid content, antioxidant activity by DPPH and FRAP assay, and total carotenoid content, respectively), β_0, is the constant coefficient of intercept, β_i, β_{ii}, β_{ij} are linear, quadratic and interaction coefficients, respectively. While X_i and X_j are the levels of three independent variables (ethanol concentration, temperature, and time). To visualize the adjustment between the values of each independent variable and the dependent variables, three - dimensional surface plots were created using polynomial equations.

To determine the ideal operational conditions for the ultrasound-assisted extraction of the marigold flower, the following factors were considered: ethanol concentration (A: 60, 80, 100 % v/v), extraction temperature (B: 30, 40, 50 °C), and duration (C: 5, 10, 15 min). (Table 12).

Table 12 Designing an experiment to extract a bioactive component using marigold flower extract using response surface technique

Run	Ethanol concentration (%)	Temperature (°C)	Time (min)
1	80	50	15
2	100	30	10
3	80	40	10
4	80	40	10
5	100	40	15
6	80	40	10
7	80	30	5
8	80	40	10
9	80	50	5
10	100	50	10
11	60	40	5

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12	100	40	5
13	80	40	10
14	60	50	10
15	60	40	15
16	60	30	10
17	80	30	15

Then the optimal condition was chosen for the next steps of the experiment according to RSM with BBD design. In order to compare experimental data with model predictions, the experiment was conducted in ideal conditions. To determine the model validity, % Difference was calculated as

% Difference = (predicted value – experimental value) / predicted value*100

Optimized marigold flower extract was used for antimicrobial activity against Gram positive (*Staphylococcus aureus*) and Gram negative (*Escherichia coli*) as follows.

3.2.6 Antimicrobial activity by Disc Diffusion Method (Appendix A.6)

3.2.7 Minimum inhibitory concentration (MIC) by Broth Microdilution Method (Appendix A.7)

3.4 Microencapsulation Experiment of marigold flower extract

The optimized marigold flower extract from previous experiment was used in microencapsulation experiment. To study the effect of coating material and extract and coating material ratio in encapsulation process by spray drying on microcapsule properties, maltodextrin and gum Arabic was used as coating material with extract and coating material ratio of 1:1 and 1:2 for maltodextrin and 1:2 and 1:3 for gum Arabic. The concentration of maltodextrin was 45% (w/v) and gum Arabic was 20% (w/v). Sample was prepared by mixing optimized marigold flower extract with the coating material at different ratios (Table 13). Solution was stirred (SCILOGEX, model SCI550-S, USA) for 5 min and homogenized by using a high-speed blender

(Ystral, model X10, Germany) at 11,000 rpm for 10 min. The spray drying process was conducted to a spray dryer with a standard 0-5 mm nozzle. The solutions were fed into the spray dryer and the marigold flower extract emulsions were operated under the inlet air temperature of 155 ℃ and the outlet air temperature was 90 ℃. The powders were collected and kept at -20 ℃ in aluminum-laminated bag until further analysis

Table 13 Ratio of extracts and encapsulation in the production of marigold flower microcapsules by spray drying

Encapsulation type	Concentration percentage of the encapsulation (w/v)	Extract ratio: encapsulated substances (w/w)	
Maltodextrin DE 10-12	45	1:1	1:2
Gum Arabic	20	1:2	1:3

After the microencapsulation process by spray drying, the marigold flower extract encapsulated powder was collected to analyze the chemical, physical and antioxidant properties of marigold flower microcapsules as follows.

3.4.1 Physiochemical properties of microcapsules in accordance with clause 3.2.1 – 3.2.5

3.4.2 Moisture content % (appendix B.1)

3.4.3 Water activity (a_w) (appendix B.2)

3.4.4 Color value CIE LAB system with chroma meter (appendix B.3)

3.4.5 Encapsulation yield (appendix B.4)

3.4.6 Encapsulation efficiency (appendix B.5)

3.4.7 External shape characteristics of microcaps with electron microscope Scanning Electron Microscope (SEM) (appendix B.6)

3.4.8 Water solubility index (WSI) (appendix B.7)

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3.5 Statistical analysis

Design Expert software was used to optimize the UAE experiment using response surface methodology (RSM), and three-dimensional (3D) graphs of the models were evaluated using Design Expert statistical tools. Variance analysis (F test) was utilized to determine the models' significance. Furthermore, the significance variations between the average scores of the treatment methods were tested using analysis of variance (one-way ANOVA) with SPSS (statistical package for social sciences) software and Tukey's HSD (honestly significant difference) test at a significance level of $p \leq 0.05$ for the encapsulation experiment. All of the data was collected in triplicate, with the results shown as mean and standard error.

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Chapter 4

Results and Discussion

4.1 Optimization of total phenolic compound, total flavonoid content, antioxidant activity and total carotenoid content using response surface methodology

4.1.1 Total phenolic compounds of marigold flower extract with ultrasound-assisted extraction method

Fruits, vegetables, and plants, including marigold flowers and extracts, contain phenolic compounds that have antioxidant properties. Because of their antioxidant properties, phenolic compounds can reduce free radicals, act as hydrogen donors, and have biological effects that are beneficial to human health Air (2018). Table 14 shows that the lowest TPC value was revealed for the extraction condition of 10 min at 30 °C and 60% ethanol concentration, while the highest TPC value was identified with the condition of 5 min at 40 °C and 100% ethanol concentration.

Table 14 Experimental variables and response measurement values. The extracting parameters were ethanol concentration % (A), temperature in °C (B), and time in min (C). The responses were measured in duplicate as total phenolic content (TPC) expressed as mg GAE/100g db, total flavonoid content (TFC) expressed as mg QCE/100g db, antioxidant activity by DPPH and FRAP methods expressed as mM Trolox equivalent/100g, and carotenoid content expressed as mg carotenoid/100g db

Treatment	Independent Variables			Responses				
	Ethanol concentration % (A)	Temp °C (B)	Time min (C)	TPC	TFC	DPPH	FRAP	Carotenoid
1	80	50	15	72.3846	95.7328	610.5	4627.37	368.228
2	100	30	10	33.5934	37.8321	606.75	750.737	49.508
3	80	40	10	71.2857	66.3053	624.25	3364.21	288.617
4	80	40	10	70.2967	47.6794	625.5	2995.79	266.226
5	100	40	15	69.7473	76.1908	621.438	1722.11	137.964
6	80	40	10	74.1429	49.3588	643.625	2501.05	323.17
7	80	30	5	34.6923	17.4504	609.25	4585.26	414.667
8	80	40	10	72.7143	57.8321	644.875	2869.47	294.698
9	80	50	5	72.7143	56.7252	622.375	1869.47	312.942
10	100	50	10	74.1429	55.7328	627.375	7364.21	79.362

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11	60	40	5	79.1978	48.3664	634.25	2880	292.487
12	100	40	5	91.9451	92.145	623.313	1995.79	231.12
13	80	40	10	75.2019	64.3053	634.563	2932.63	266.226
14	60	50	10	69.3077	55.2366	615.813	9048.42	95.1183
15	60	40	15	70.4615	50.8473	633.625	2427.37	55.0365
16	60	30	10	31.3956	14.7786	581.125	1039.16	184.68
17	80	30	15	34.1429	18.3282	607.688	2422.32	319.3

The linear effects on the TPC values of the temperature, time and ethanol concentration applied to the MFE were found to be statistically significant ($P \leq 0.05$). The significant terms were added in the final models. The equilibrium of the second-order polynomial model which specifies the effect of temperature, time, and ethanol concentration on the value of the TPC in the sample extract according to the result of the response surface analysis was as follows:

TPC = -339.07602 – 0.423366 A+ 19.64846 B– 0.740277 C+ 0.003297 A* B-0.033654 A*C+ 0.001099 B* C+ 0.004670 A^2 – 0.224864 B^2 + 0.129665 C2

In Table 15, the high coefficient of determination ($R^2 = 97.58$) showed that the quadratic model was adequate for predicting experimental results, and the high degree of fit and adjusted coefficient of determination ($R^2_{Adj} = 94.48$) indicated that the model's predicted and experimental TPC values were in good agreement (Ykmş 2019).

Table 15 Analysis of variance (ANOVA) of responses for total phenolic compounds

	Total phenolic compound (mg GAE/100g db)				
Source	df	SS	MS	F-Value	P- Value
Model	9	5361.08	595.68	31.41	<0.0001
A	1	45.44	45.44	2.40	0.1656

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B	1	2992.49	2992.49	157.79	<0.0001
C	1	126.51	126.51	6.67	0.0363
AB	1	1.74	1.74	0.0917	0.7708
AC	1	45.30	45.30	2.39	0.1661
BC	1	0.0121	0.0121	0.0006	0.9806
A^2	1	14.69	14.69	0.7747	0.4080
B^2	1	2129.00	2129.00	112.26	<0.0001
C^2	1	44.24	44.24	2.33	0.1705
Residual	7	132.75	18.96		
Lack of fit	3	116.64	38.88	9.65	0.0265
Pure Error	4	16.11	4.03		
Total	16	5493.84			
R^2			0.9758		
Adj R^2			0.9448		
Pre R^2			0.6557		

Figure 39 demonstrates the surface plots of three-dimensional responses for the TPC of MFE samples. In this Figure 39a, the effects of temperature and time on the TPC values of the MFE samples indicated that the TPC values of the MFE sample were lowest at 30 °C. As the extracting duration was extended, the TPC values increased to rise. This procedure was carried out because the TPC values were greater and the ultrasound-assisted extraction time was longer. Moreover, TPC values become higher when the temperature increased. Kobus-Cisowska, Szczepaniak et al. (2019) reported that temperature has been shown to alter extraction efficiency by changing diffusion efficiency and solvent solubility. Consequently, temperature goes

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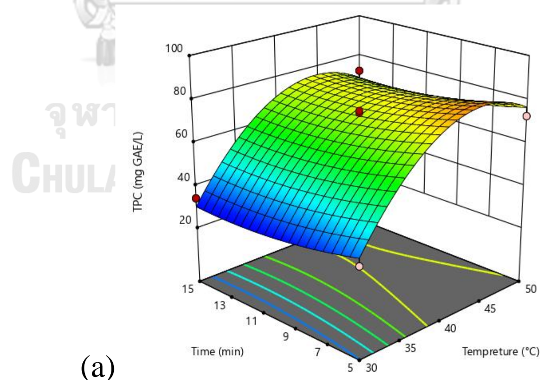
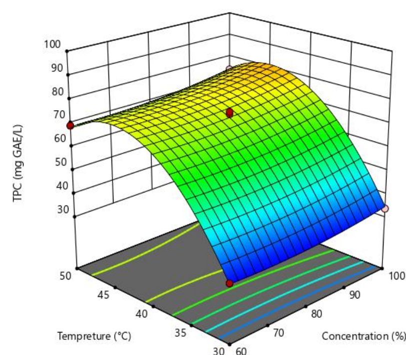
up the diffusion coefficient, which accelerates the rate of diffusion and the TPC values. Figure 39b shows the effect of temperature and ethanol concentration on the TPC values of MFE samples. The experimental results from the surface plot demonstrated that increasing ethanol concentration from 60% to 100% (v/v) tended to slowly decrease the TPC values. Furthermore, Figure 39c shows the effects of time and ethanol concentration on the TPC values of MFE samples, showing that longer extraction times resulted in higher TPC extraction efficiencies. Ultrasound-assisted extraction is a quick and effective way to extract high-quality intracellular compounds Wen, Zhang et al. (2018). As a result, it may be better to improve the extraction of bioactive components from natural plant sources, such as phenolic compounds. The ratio of water and ethanol in the extraction of phenolic compounds was an important component to consider, according to (Rostagno, Palma et al. 2004). They also observed that when the solvent content was greater than 60% (v/v), the phenolic compounds in the extracts decreased. The use of 60 % (v/v) ethanol in water for 10 min was shown to be the best conditions for extracting phenolic components from marigold flowers using RSM (Rostagno, Palma et al. 2004).

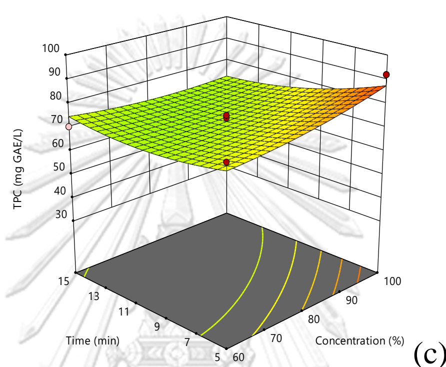
Surface plot of TPC *vs* Time, Temperature and ethanol concentration



(a)



(b)



(c)

Figure 39 Response surface plots (3D) of Total phenolic content (TPC) as function of (a) temperature and time; (b) temperature and ethanol concentration; (c) time and ethanol concentration of marigold flower extract

4.1.2 Total flavonoid content of marigold flower extract with ultrasound-assisted extraction method

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Fruits, vegetables, cereals, bark, roots, stems, flowers, tea, and wine all contain flavonoids, a class of organic chemicals with diverse phenolic structures. In the past, several organic substances have been linked to better health outcomes. Pharmaceuticals, medical equipment, cosmetics, and nutraceuticals all contain flavonoids in significant quantities. The potential of substances with anti-inflammatory, anti-carcinogenic, anti-mutagenic, and antioxidant capabilities to affect key cellular enzyme functions Panche, Diwan et al. (2016). The results in Table 14 revealed that the lowest TFC value was identified for 10 min, 30 °C and 60% ethanol concentration, while the highest TFC value was obtained at a condition of 80% ethanol concentration (v/v), 50 °C and 15 min. Temperature, time, and ethanol content all had statistically significant linear impacts on TFC values in MFE samples ($p<0.05$)

(Table 14). The linear effect of ethanol concentration and temperature and the quadratic effect of temperature had significant effect on TFC value. The equilibrium of the second-order polynomial model, indicating the effect of temperature, time, and ethanol concentration on TFC value using RSM analysis, was characterized as follows according to the analysis design:

$$TFC = -361.50901 + 1.44252 A + 16.94897 B - 9.66077 C - 0.028197 A*B - 0.046088 A*C + 0.190649 B*C + 0.004534 A^2 - 0.180147 B^2 + 0.319107 C^2$$

TFC's lack of fit test was insignificant ($P>0.05$), indicating that the proposed regression equation generated less errors when comparing experimental findings and that independent variables had substantial effects on the outcomes. The coefficient of determination ($R^2 = 84.35$), as well as the adjusted coefficient of determination ($R^2_{Adj} = 64.22$) (Table 16), showed a high degree of fit and appropriateness in predicting experimental results.

Table 16 Analysis of variance (ANOVA) of responses for total flavonoid compounds

Source	Total flavonoid compound (mg QCE/100g db)				
	df	SS	MS	F-Value	P- Value
Model	9	7147.90	794.21	4.19	0.0360
A	1	1073.51	1073.51	5.66	0.0489
B	1	3829.80	3829.80	20.21	0.0028
C	1	87.20	87.20	0.4602	0.5193
AB	1	127.21	127.21	0.6713	0.4396
AC	1	84.96	84.96	0.4483	0.5246
BC	1	363.47	363.47	1.92	0.2086

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A^2	1	13.85	13.85	0.0731	0.7947
B^2	1	1366.44	1366.44	7.21	0.0313
C^2	1	267.97	267.97	1.41	0.2732
Residual	7	1326.53	1326.53		
Lack of fit	3	1040.66	346.89	4.85	0.0805
Pure Error	4	285.86	71.47		
Total	16	8474.43			
R^2			0.8435		
Adj R^2			0.6422		
Pre R^2			0.0175		

In Figure 40a, the effects of temperature and time on the TFC values of the MFE samples indicated that the TFC values of the MFE sample were lowest at 30 °C. TFC values increase as the extraction time increases because longer extraction time can increase the mass transfer velocity and then release the bioactive compounds from plant matrix by destroying the plant cells. Figure 40b showed that TFC values slowly increased until 100% ethanol concentration (v/v). In Figure 40c, it showed that the lowest extraction time for TFC value was 10 min then it increased continuously, and the maximum value was at 15 min. Ethanol is a polar solvent that is used to extract bioactive chemicals from plant matter. Factors that affect the extraction efficiency of phenolic compounds also have an impact on the extraction of flavonoid compounds, according to (Rad et al. 2019). Combining water and ethanol in the extraction procedure can improve flavonoid extraction performance. Flavonoids are secondary metabolites with a polyphenolic structure. Flavonoids are found in a number of nutraceuticals, pharmaceuticals, medical, cosmetic, and dietary items and have been linked to human health benefits. They're popular because of their antioxidant, anti-

inflammatory, anti-mutagenic, and anti-carcinogenic effects. Panche, Diwan et al. (2016).

Surface plot of TFC *vs* Time, Temperature, and ethanol concentration

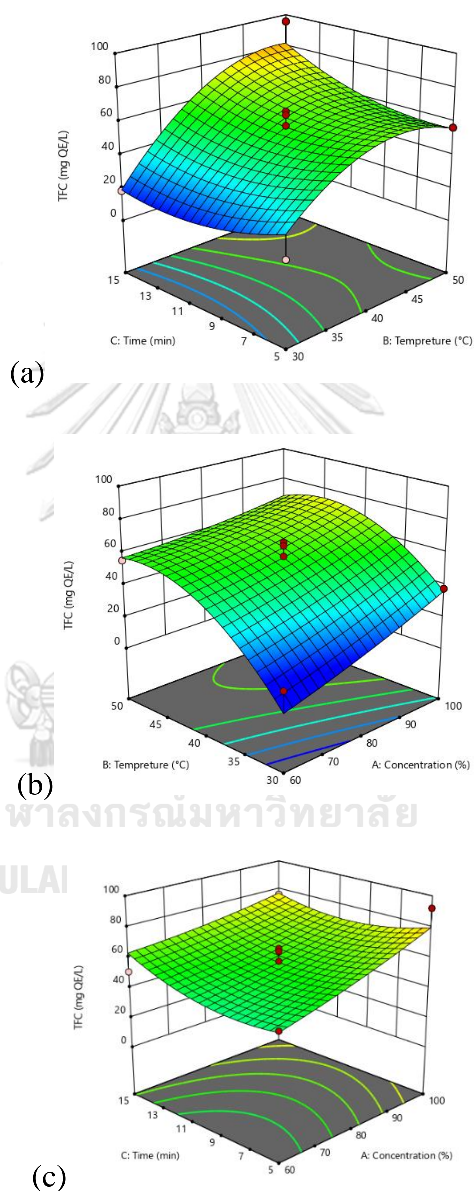

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Figure 40 Response surface plots (3D) of total flavonoid content (TFC) as a function of interaction between; (a) temperature and time; (b) temperature and ethanol concentration; (c) time and ethanol concentration of marigold flower extract

4.1.3 Antioxidant activity by DPPH method of marigold flower extract with ultrasound-assisted extraction method

Chemicals called antioxidants, which are included in most of our food, can assist to halt or lessen oxidative stress in the body. Free radicals, which are responsible for cell death, cardiovascular disease, diabetes, muscular degeneration, and cancer, are continuously produced by the body because of oxygen consumption. Free radical damage can be prevented and repaired by antioxidants, which are powerful free radical scavengers Rathor, Mishra et al. (2014). The equilibrium of the second-order polynomial model, describing the effect of temperature, time, and ethanol concentration on antioxidant activity by DPPH values of MFE using RSM analysis, was described as follows:

$$DPPH = +84.32812 + 3.04102\ A + 19.81250\ B + 2.60156\ C - 0.017578\ A*B - 0.003125\ A*C - 0.051563\ B*C - 0.013867\ A^2 - 0.212500\ B^2 - 0.034375\ C^2$$

Table 14 shows that the lowest antioxidant properties by DPPH were found at 60 % ethanol concentration (v/v), 30 °C, and 10 min, while the greatest value was found at 40 °C and 80 % ethanol concentration (v/v) for 10 min. According to analysis of variance, the quadratic effect of temperature applied to MFE samples on DPPH values were statistically significant ($P \leq 0.05$). Whereas the linear effects of temperature, time and ethanol concentration and the interaction effect of temperature and time, time and ethanol concentration, temperature and ethanol concentration and the quadratic effect of concentration and time applied to MFE samples on DPPH values was not statistically significant ($P > 0.05$). Because the lack of fit test for DPPH was insignificant ($P > 0.05$), the model was well fitted. The coefficient of determination ($R^2 = 73.46$) and adjusted coefficient of determination ($R^2_{Adj} = 39.34$) demonstrated a high degree of fit and appropriateness in predicting experimental results (Table 17). High R^2 values indicated that the quadratic model was very effective at fitting the data, and adjusted R^2 (R^2_{Adj}) indicated that the predicted and experimental results of the model were in good agreement.

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Table 17 Analysis of variance (ANOVA) of responses for antioxidant activity by DPPH

Source	**DPPH (mM Trolox/100g db)**				
	df	SS	MS	F-Value	P-Value
Model	9	2879.70	319.97	2.15	0.1623
A	1	24.72	24.72	0.1663	0.6956
B	1	634.57	634.57	4.27	0.0776
C	1	31.75	31.75	0.2136	0.6580
AB	1	49.44	49.44	0.3326	0.5822
AC	1	0.3906	0.3906	0.0026	0.9605
BC	1	26.59	26.59	0.1789	0.6850
A^2	1	129.55	129.55	0.8716	0.3816
B^2	1	1901.32	1901.32	12.79	0.0090
C^2	1	3.11	3.11	0.0209	0.8891
Residual	7	1040.41	148.63		
Lack of fit	3	663.45	221.15	2.35	0.2140
Pure Error	4	376.95	94.24		
Total	16	3920.11			
R^2			0.7346		
Adj R^2			0.3934		
Pre R^2			0.8581		

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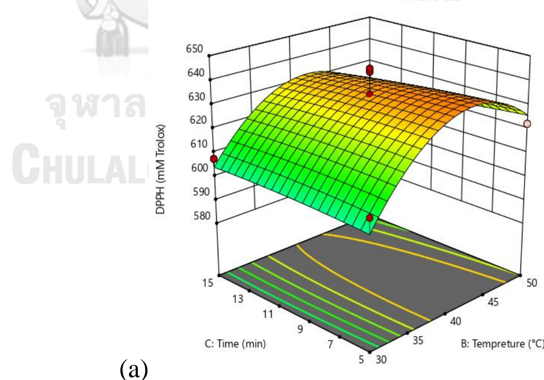
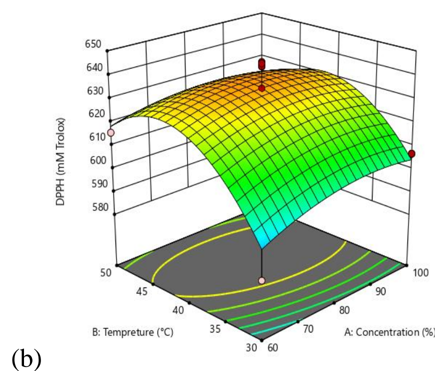
In addition, the surface plots of three-dimensional responses for the antioxidant activity by DPPH of MFE samples are demonstrated in Figure 41. In Figure 41a, the effect of extraction time and temperature on the antioxidant activity by DPPH, antioxidant activity increased with increasing time. The effect of ethanol concentration and temperature on the antioxidant activity by DPPH was shown in Figure 41b. The antioxidant activity by DPPH increased when the temperature increased to 40 °C whereas it decreased when the temperature increases from 40 °C to 50 °C. In Figure 41c, describing the effect of ethanol concentration and time, the ethanol concentration was increased more than 80% (v/v), the antioxidant activity by DPPH gradually decreases. This research is also in line with (Liyana-Pathirana and Shahidi 2006) who discovered that utilizing ethanol concentrations of 60 to 80 % (v/v) resulted in better antioxidant activity than using the same ethanol concentration with a longer extraction period using the DPPH approach . It's also approved that the extraction period must be long enough to avoid bioactive components degrading and resulting in reduced antioxidant activity Sungthong and Phadungkit (2015).

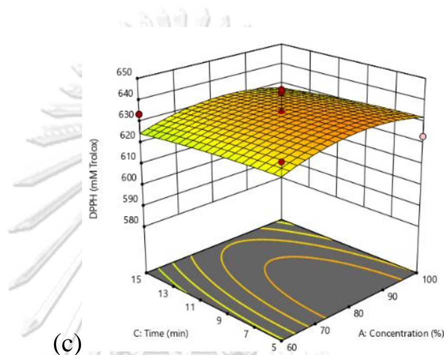
Surface plot of DPPH *vs* Time, Temperature and ethanol concentration



(a)



(b)



(c)

Figure 41 Response surface plots (3D) of antioxidant activity (DPPH) as a function of interaction between; (a) temperature and time; (b) temperature and ethanol concentration; (c) time and ethanol concentration of marigold flower

4.1.4 Antioxidant activity by FRAP assay of marigold flower extract with ultrasound-assisted extraction method

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Antioxidant activity by FRAP is a method of transferring electron from ferric (Fe^{3+})to ferrous (Fe^{2+}) ion reduction (Benzie and Strain 1996). The linear effects of temperature applied to MFE samples on FRAP values were statistically significant ($P \leq 0.05$) (Table 18). On the other hand, the quadratic effects and interaction of time, temperature and ethanol concentration applied to MFE samples on FRAP values were not statistically significant ($P > 0.05$) (Table 18).

Table 18 Analysis of variance (ANOVA) of responses for antioxidant activity by FRAP assay

Source	FRAP (mM Trolox/100g db)				
	df	SS	MS	F-Value	P-Value
Model	9	4.431 + 07	4.924 + 06	1.18	0.4237
A	1	1.586 + 06	1.586 + 06	0.3796	0.5573
B	1	2.489 + 07	2.489 + 07	5.96	0.0447
C	1	2157.21	2157.21	0.0005	0.9825
AB	1	4.871 + 05	4.871 + 05	0.1166	0.7428
AC	1	800.5 + 54	800.5 + 54	0.0019	0.9663
BC	1	6.054 + 06	6.054 + 06	1.45	0.2678
A^2	1	2.613 + 05	2.613 + 05	0.0625	0.8097
B^2	1	7.890 + 06	7.890 + 06	1.89	0.2117
C^2	1	3.606 + 06	3.606 + 06	0.8631	0.3838
Residual	7	2.924 + 07	4.178 + 06		
Lack of fit	3	2.886 + 07	9.621 + 06	101.15	0.0003
Pure Error	4	3.805 + 05	95124.65		
Total	16	7.356 + 07			
R^2	0.6024				
Adj R^2	0.0913				

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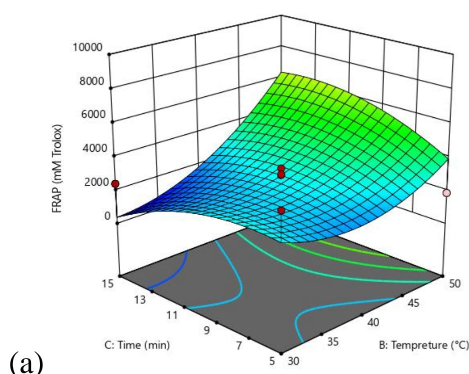
Pre R^2	0.2866

The equilibrium of the second-order polynomial model describing the effect of temperature, time and ethanol concentration on the antioxidant activity by FRAP values of MFE samples was as follows:

$$FRAP = +24493.26316 – 56.58947 A – 1025.17895 B – 282.90526 C – 1.74474 A^{*} B + 0.447368 A^{*} C + 24.60421 B^{*} C + 0.622763 A^2 + 13.68895 B^2 – 37.01684 C^2$$

The quadratic model's high R^2 values indicated that it was very good at fitting data under experimental conditions, and the modified coefficient of determination revealed the model's adequacy in predicting experimental findings as well as its high degree of fit. The lowest antioxidant activity measured by FRAP value was discovered at 100% ethanol concentration and 30 °C for 10 min, whereas the greatest FRAP value was obtained at 50 °C and 60% ethanol concentration for 10 min. Surface plots of three-dimensional responses for the effects of temperature and time, temperature and ethanol concentration, and ethanol concentration and time were shown in Figure 42. The results from Figure 42a demonstrated that antioxidant activity didn't show any significance changes when the temperature rises from 30 °C to 40 °C. However, when the temperature rises over 40 °C, the antioxidant activity by FRAP increased. Figure 42b demonstrated that increasing ethanol concentration caused a decrease of antioxidant activity by FRAP. In addition, in Figure 42c indicating the effect between ethanol concentration and time showed that the lowest antioxidant activity was at 10 min with 100% ethanol concentration (v/v).

Surface plot of FRAP *vs* Time, Temperature, and ethanol concentration



(a)

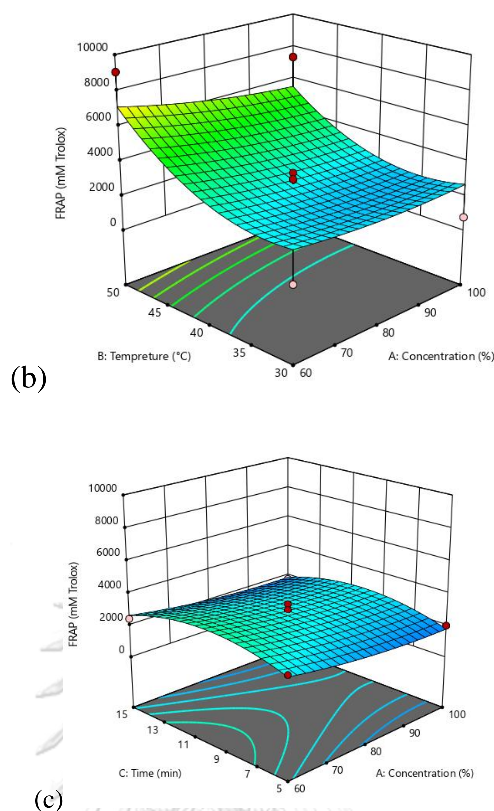


Figure 42 Response surface plots (3D) of antioxidant activity (FRAP) as a function of interaction between; (a) temperature and time; (b) temperature and ethanol concentration; (c) time and ethanol concentration of marigold flower extract

4.1.5 Total carotenoid content of marigold flower extract with ultrasound-assisted extraction method

Carotenoids, the pigments that give plants their colors of yellow, orange, and red, are found in nature. Carotenoids are precursors to plant growth regulators, and they can act as a photosynthetic device by quenching harmful ROS. They are phytochemicals that interact in certain processes and can be used to prevent disease and preserve health Stahl, Nicolai et al. (1997). According to the analysis, the linear effects of time and the quadratic effects of ethanol concentration and time applied to MFE samples on carotenoid were statistically significant ($P \leq 0.05$) (Table 19). The remaining quadratic and the interaction between temperature, time and ethanol concentration applied to MFE samples on carotenoid content were not statistically significant ($P > 0.05$) (Table 19).

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Table 19 Analysis of variance (ANOVA) of responses for carotenoid content

Source	**Total Carotenoid Content**				
	df	SS	MS	F-value	P-value
Model	9	1.887+05	20967.33	8.89	0.0044
A	1	2091.99	2091.99	0.8872	0.3776
B	1	1582.19	1582.19	0.6710	0.4397
C	1	17176.18	17176.18	7.28	0.0307
AB	1	3565.06	3565.06	1.51	0.2586
AC	1	5205.23	5205.23	2.21	0.1809
BC	1	5674.03	5674.03	2.41	0.1648
A^2	1	1.366+05	1.366+05	57.94	0.0001
B^2	1	127.09	127.09	0.0539	0.8231
C^2	1	21519.70	21519.70	9.13	0.0194
Residual	1	16505.75	2357.96		
Lack of fit	1	14275.60	4758.53	8.53	0.0326
Pure Error	1	2230.15	557.54		
Total	7	2.052+05			
R^2	3		0.9196		
Adj R^2	4		0.8162		
Pre R^2	16		0.1300		

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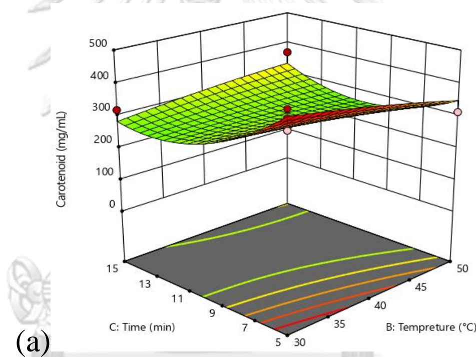
The equilibrium of the second-order polynomial model, describing the effect of temperature, time and ethanol concentration on total carotenoid content of MFE using RSM analysis, was described as follows:

$TCC = -1115.00719 + 61.66381\ A - 16.48538\ B - 125.44919\ C + 0.149270\ A*B + 0.360736\ A*C + 0.753262\ B*C - 0.450316\ A^2 - 0.054940\ B^2 + 2.85963\ C^2$

The lowest carotenoid value was discovered at 100% ethanol concentration and 30 °C for 10 min, while the greatest FRAP value was recorded for 5 min at 30 °C and 80% ethanol concentration. Surface plots of three-dimensional responses for the effects of temperature and time, temperature and ethanol concentration, and ethanol concentration and time were shown in Figure 43.

Surface plot of carotenoid *vs* Time, Temperature and ethanol concentration



(a)

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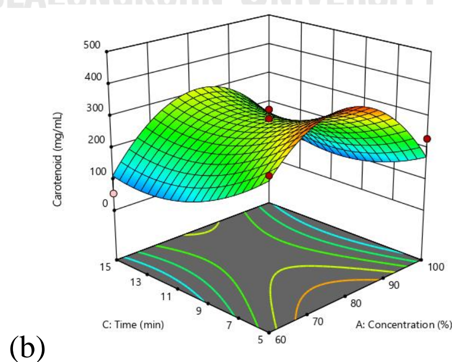
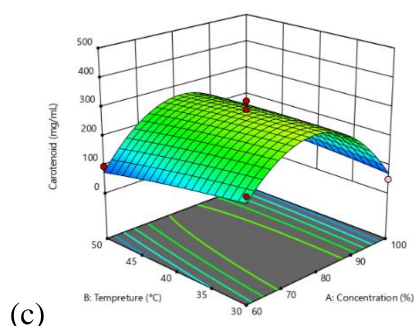


(b)



(c)

Figure 43 Response surface plots (3D) of carotenoid as a function of interaction between; (a) temperature and time; (b) temperature an ethanol concentration; (c) time and ethanol concentration of marigold flower extract

The effect of extraction time and temperature on carotenoid concentration is seen in Figure 43a, where the TCC value increased as the extraction period increased. This is due to the fact that longer extraction periods produce more cell wall breaking due to ultrasound. The duration of the extraction process is crucial in the extraction of carotenoids because prolonging the contact time of the solvent with the solids may promote the diffusion of the compounds and resulting in carotenoids being more easily discharged from the matrix into the extraction medium. Like that, ethanol concentration is crucial to getting the most carotenoids back. Figure 43b demonstrated that increasing ethanol concentration up to 80% results in increased carotenoid content and then decrease when increased ethanol concentration to 100%. This is possibly because ultrasound waves spread more widely in aqueous solutions, using a solvent with water can lead to more radicals' production because of the ultrasound-induced dissociation of water. The extraction efficiency of the target chemicals may reduce because the oxidative reaction and the extraction reaction can coexist (Umair, Jabbar et al. 2021)In Figure 43c, it showed that the lowest extraction time for TCC value was 10 min then it gradually increased until 15 min.

The results of the second-order polynomial equation and reaction surface plot clearly showed that the quadratic polynomial equation could be utilized to explain 3D response surface plots and predict TPC, TFC, antioxidant activity (DPPH and FRAP), and carotenoid concentration of MFE samples (Figure 39-43). Visually, the curvature of response surfaces can be noticed, which reflects the degree of effect of independent

variables in the study value. Temperature (B), time (C), and ethanol concentration (A) all had a substantial effect on the bioactive compounds and antioxidant activity of the MFE. Different shapes reflect various interactions between the factors being studied. If the contour plot was elliptical Yıkmış (2019), the interactions between the corresponding variables were very important; nevertheless, a circular contour plot suggested that there were no significant interactions between variables (Jiao, Li et al. 2020).

4.2 Optimal UAE condition and its validation

Following many statistical optimizations, the predicted optimal conditions were calculated to be 42.12 °C, 14.39 min, and 68.57 % (v/v) ethanol concentration. To experimentally confirm the model's validity, extraction was actually carried out under the following conditions: temperature 40 °C, time 15 min, and ethanol concentration 68 percent (v/v). To confirm the accuracy of the projected ideal condition, the yield of total phenolic compound, total flavonoid content, antioxidant activity by DPPH and FRAP assays, and total carotenoid content were measured. Table 20 displays the expected values and obtained from the experiment of each response derived from the ideal extraction condition. According to the validation results, it revealed that slight variation or % difference between the experimental response and predicted values from optimum condition confirmed the validity of the proposed optimum UAE condition. Therefore, expected models can be used.to optimize the process parameters for total phenolic compound, total flavonoid content, antioxidant activity by DPPH and FRAP assays and total carotenoid content.

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Table 20 Experimental values of the validation of predicted values

Parameters	Experimental values	Predicted values	% Difference	Unit
Total phenolic compound	1524.62 ± 45.42	1514.00	0.7	mg GAE/100g db
Total flavonoid content	664.58 ± 16.14	687.40	3.32	mg QE/100g db

Antioxidant activity by DPPH	2517.04 ± 13.20	2521.48	0.17	mM TE/100g db
Antioxidant activity by FRAP	3242.10 ± 11.26	3226.17	0.49	mM TE/100g db
Total carotenoid content	243.56 ± 6.17	234.74	3.76	mg carotenoid/100g db

Based on the optimum results, antimicrobial activity by disc diffusion method and minimum inhibitory concentration (MIC) on Gram positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*) were further investigated.

4.3 Antimicrobial activity by disc diffusion method and minimum inhibitory concentration (MIC)

Plants have traditionally been used as nutritional supplements and in the treatment of ailments by humans. Many plants are currently being studied for therapeutic characteristics all around the world on a yearly basis. Due to the rise of problems such as microbe resistance and antibiotic consequences, a portion of these studies focuses on assessing the antibacterial characteristics of medicinal plants Jafari and Ahmadizadeh (2017). Lauk et al. (2003) investigated the antibacterial activity of plant part extracts of *C. officinalis* against anaerobic and facultative aerobic bacteria. The plant components' activity against the test bacteria differs substantially. Secondary metabolites, such as tannins and other phenolic compounds, are also thought to be active antibacterial agents. As a result, the presence of these phytochemicals could help to explain some of the antibacterial activity discovered in this study Mahomoodally, Gurib-Fakim et al. (2005). Differences in antimicrobial activity of medicinal plants are obviously related to differences in their contents of active compounds.

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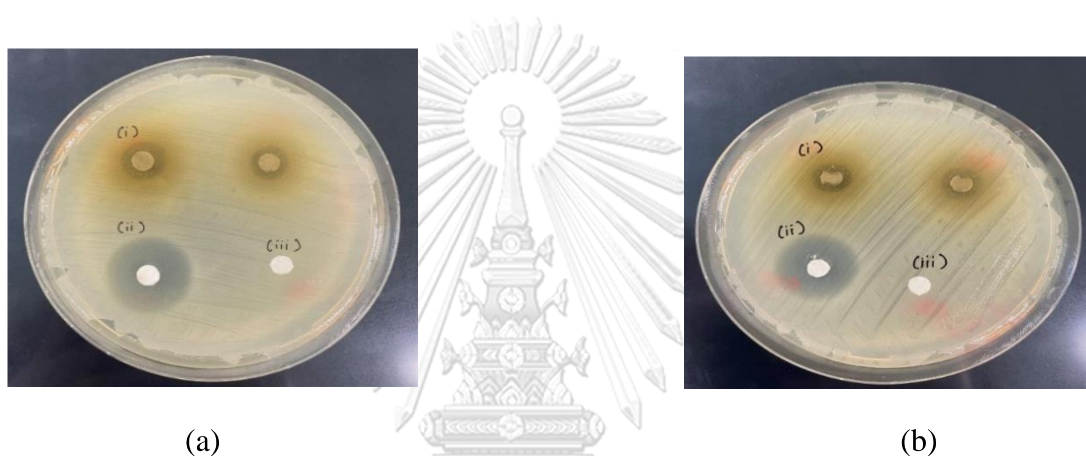
The ethanol extract of *T. erecta* was tested for their antimicrobial activity against Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*) in the current study. The diameter of the inhibitory zone of marigold flower extract for bacteria was determined. The ethanolic extract of marigold flower demonstrated antimicrobial activity by the growth of inhibition zones (Figure 44). The maximal inhibition zone was 9.66 mm, whereas the minimum inhibition zone measured 8.33 mm as shown in Figure 44.



(a) (b)

Figure 44 Antimicrobial activity, Zones of inhibition (a) Petridish with *E. coli* culture (b) Petridish with *S. aureus* culture, (i) absorbed in marigold flower extract (ii) absorbed in antibiotics, chloramphenicol (iii) absorbed in ethanol solvent

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The extract had an inhibitory effect on all bacteria with diameters of 9.66 and 8.33 mm against *S. aureus* and *E. coli*, respectively (Table 21). In the current investigation, *S. aureus* and *E. coli* both grew more slowly when exposed to an ethanolic extract of marigold flowers. It was because different antibacterial activities can penetrate in different ways and microbial structures vary. Gram-positive bacteria have less complex cell walls than Gram-negative bacteria, which makes *E. coli* more resistant to MFE. Consistently, the study of (De Zoysa, Rathnayake et al. 2019) demonstrated that the higher antimicrobial activity against *S. aureus* than *E. coli*.

Table 21 The diameter of the inhibition zone in mm and minimum inhibitory concentration of two bacteria strains of ethanol extract of marigold flower

Strain of bacteria	**Extract concentration**	**Inhibition Zone (mm)**	**MIC (mg/mL)**
Staphylococcus aureus	68%	9.66	25
Escherichia coli	68%	8.33	25

This is owing to the presence of polysaccharides in the cell membrane of Gram-negative bacteria, which have the potential to protect or inhibit the bacteria. Gram-negative bacteria have an outer membrane and periplasmic space and act as barrier to penetration of substances. Gram-positive bacteria have an outer peptidoglycan layer which makes the cell wall more permeable to antimicrobial substances than lipopolysaccharide layer. Changes in the outer membrane by Gram-negative bacteria like changing the hydrophobic properties or mutations in porins can create resistance (Breijyeh, Jubeh et al. 2020). Additionally, the extracts that found zones of inhibition against bacteria in disc diffusion method were subsequently determined MIC values. The lowest concentration that inhibits the growth of bacteria was 25 mg/mL. Therefore, ethanol extracts of marigold flower were used to evaluate a wide variety of antibacterial activity against Gram-positive (*S. aureus*) and Gram-negative bacteria (*E. coli*), and the results revealed antibacterial activity.

Because of the changes in concentration of the extracts, the efficiency of the plant extracts cannot be reliably assessed by comparing the different diameters acquired in the disc diffusion experiment. As a result, the minimum inhibitory concentration was employed to correctly measure the activity of the plant extracts De Zoysa, Rathnayake et al. (2019). The extracts that found significant zones of inhibition against indicator bacteria in a disc diffusion experiment were subsequently examined quantitatively to determine MIC values (Latifian, Cigdem et al. 2021). The lowest MIC value against all bacteria (*E. coli and S. aureus*) was 25 mg/mL.

4.3 Encapsulation study on marigold flower optimized extract

4.4.1 Condition for choosing the 45% concentration of maltodextrin and 20% concentration of gum Arabic

When the percentage of maltodextrin as the encapsulating agent was raised from 20% to 40% (w/v), the encapsulation efficiency of narinjin rose from 60% to 80% (w/v). In addition, it was found that increasing the coating material percentage increased the microcapsules' thickness, more effectively encapsulating the extract inside the core. When carrier (maltodextrin) concentrations were increased, encapsulation efficiency increased as a result of the greater protection provided by the higher concentration of carrier solids in the feed. Moreover, maltodextrin concentration of up to 70% is water soluble and has a good membrane-forming capability due to its medium molecular weight (Thuong Nhan, Tan Thanh et al. 2020). For gum Arabic concentration, moisture content decreases with increasing the amount of wall material in liquid feed solution for spray-drying: gum Arabic 10% was 7.6% and 20% was 6.7%. It was in line with the study of (Pudziuvelyte, Marksa et al. 2019). It has been demonstrated that the effectiveness of encapsulating rosmarinic acid (RA) rises with increasing concentrations (10% > 20% > 30%) of wall materials in the liquid feed solution for spray drying: gum Arabic at 24.89%, 62.47%, and 96.36%, respectively. According to the experiments from previous studies, 45% (w/v) concentration for maltodextrin and 20% (w/v) concentration for gum Arabic was chosen for microencapsulation by spray drying.

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4.4.2 Total amount of phenolic compounds of microcapsules

The vast majority of phenolic compounds have an aromatic ring with a hydroxyl substituent and are derived from plants. Phenolic compounds are helpful to people since they are natural antioxidants. These substances can protect against the biological environment by having antioxidant, antimutagenic, antiviral, antibacterial, algicidal, antifungal, and insecticidal properties. The radical-scavenging antioxidant prevents the oxidation of lipids, proteins, and DNA caused by free radicals, which is associated with disease. By inhibiting the enzymes that produce free radicals, phenolics can act as antioxidants Castellano, Tena et al. (2012). The total amount of phenolic compounds in microcapsules was investigated using a variety of coating materials and encapsulation ratios. The amount of total phenolic compounds was

discovered to be between 304.10 ± 7.06 and 627.91 ± 24.86 mg GAE/ 100 g db. The maximum number of phenolic compounds were found in the sample when gum Arabic was used in a 1:2 encapsulation ratio, followed by gum Arabic in a 1:3 encapsulation ratio, and finally maltodextrin in 1:2 and 1:3 encapsulation ratios, respectively. There were 304.10 ± 7.06, 435.24 ± 8.32, 508.13 ± 9.52, and 627.91 ± 24.86 mg GAE/100 g db of total phenolic compounds, respectively (Table 22).

Table 22 Total phenolic compound of marigold flower extract microcapsule from spray drying

Encapsulation type and Concentration (w/v)	Total phenolic compound (mg GAE/100g db)
45% Maltodextrin (MD) 1:1	435.24 ± 8.32^{c}
45% Maltodextrin (MD) 1:2	304.10 ± 7.06^{d}
20% gum Arabic ratio 1:2	627.91 ± 24.86^{a}
20% gum Arabic ratio 1:3	508.13 ± 9.52^{b}

*Average ± Standard Deviation

*Average with different characters (a, b, c, ….). There was significant statistical difference at a confidence level of 95 % (P≤0.05).

In this study, the high content of released polyphenol was produced using gum Arabic as a coating material, which may be a consequence of high polyphenols affinity for the polysaccharide type carrier. Gum Arabic is highly soluble material with good emulsification and film creation properties which can store phenolic compounds during processing Kalušević, Lević et al. (2017). As a result of their dissolved qualities, the samples displayed an increase in phenolic content and antioxidant chemicals. Phenolic chemicals are efficiently contained in the Gum Arabic used as the microcapsules' covering surface. Ćujić-Nikolić, Stanisavljević et al. (2018) demonstrated that the use of Gum Arabic improved the stability of phenolics throughout the heating process, which was most likely due to the gum carrier qualities, which established a good matrix structure and created a barrier between phenolics and heat. A high amount of phenolics was attained in a previous study utilizing gum Arabic as a coating material, which could be due to strong

polyphenol affinity for the polysaccharide type carrier. Furthermore, gum Arabic is a completely soluble compound with excellent emulsification and film-forming characteristics, allowing phenolic chemicals to be trapped throughout the spray drying process. Tolun, Altintas et al. (2016).

4.4.3 Total flavonoid content of marigold flower microcapsules

Flavonoids, which have a polyphenolic structure, are a significant class of secondary metabolites. Flavonoids, which are related with the category of phenolic chemicals, are the major component of marigold flower extract. Flavonoids have antioxidant and anti-inflammatory properties. Because of their antioxidative, anti-inflammatory, anti-mutagenic, and anti-carcinogenic qualities, as well as their ability to alter important cellular enzyme processes, flavonoids are linked to a variety of health benefits Panche, Diwan et al. (2016). The effect of several types of surface coatings and varied encapsulant ratios on the amount of flavonoid content in marigold flower microcapsules. The amount of total flavonoid content was ranging from 209.87 ± 4.60 to 389.56 ± 9.58 mg QCE/100 g db. The results demonstrated that increasing the ratio of encapsulants reduced the flavonoid concentration of marigold flower extract microcapsules, according to this study. It was also discovered that microcapsules containing marigold flower extract with gum Arabic at a 1:2 ratio had the highest amount of flavonoid content (389.56 ± 9.58 mg QCE/100 g db), while microcapsules with maltodextrin at a 1:2 ratio had the lowest amount of flavonoid content (209.87 ± 4.60 mg QCE/100 g db) as shown in Table 23.

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Table 23 Total flavonoid content of marigold flower extract microcapsule from spray drying

Encapsulation type and Concentration (w/v)	**Total flavonoid content (mg QCE/100g db)**
45% Maltodextrin (MD) 1:1	282.90 ± 1.53^{c}
45% Maltodextrin (MD) 1:2	209.87 ± 4.60^{d}
20% gum Arabic ratio 1:2	389.56 ± 9.58^{a}
20% gum Arabic ratio 1:3	376.73 ± 10.37^{b}

*Average ± Standard Deviation

*Average with different characters (a, b, c, ….). There was significant statistical difference at a confidence level of 95 % ($P \leq 0.05$).

In the study of Rajabi, Ghorbani et al. (2015), the flavonoid content and antioxidant activity of saffron extract were boosted when gum Arabic was used as an encapsulating agent because of its high solubility and emulsion characteristics in gum Arabic microcapsules. Furthermore, gum Arabic is a charged molecule that can interact with both water-soluble and fat-soluble substances. Gum Arabic can protect bioactive chemicals from degradation caused by heat during spray drying.

4.4.4 Antioxidant activity by DPPH of marigold flower microcapsules

DPPH is a stable free radical due to the delocalization of the spare electron over the molecule. The antioxidants that contribute an electron or a hydrogen radical to the stable DPPH free radical are known as DPPH. The impact of different types of encapsulating agents and varied encapsulant ratios on the formation of antioxidant properties of marigold flower extract microcapsules with spray drying, according to the study. The antioxidant activity of marigold flower extract microcapsules measured by DPPH was between 794.50 ± 15.61 and 1756.66 ± 28.99 mM Trolox equivalent/100 g db in this study. Gum Arabic was used in a 1:2 ratio to achieve the greatest DPPH (1756.66 ± 28.99 mM Trolox equivalent/100 g db). As indicated in Table 24, gum Arabic encapsulation at a ratio of 1:3 (1469.70 ± 31.02 mM Trolox equivalent/100 g db) had the second highest antioxidant activity by DPPH, followed by maltodextrin encapsulation at 1:1 and 1:2 with 1217.83 ± 22.68 mM Trolox equivalent/100 g db and 794.50 ± 15.61 mM Trolox equivalent/100 g db, respectively.

Table 24 Antioxidant activity by DPPH of marigold flower extract microcapsule from spray drying

Encapsulation type and	**Antioxidant activity by DPPH**

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Concentration (w/v)	(mM Trolox equivalent/100g db)
45% Maltodextrin (MD) 1:1	1217.83 ± 22.68^{b}
45% Maltodextrin (MD) 1:2	794.50 ± 15.61^{c}
20% Gum arabic ratio 1:2	1756.66 ± 28.99^{a}
20% Gum arabic ratio 1:3	1469.70 ± 31.02^{b}

*Average ± Standard Deviation

*Average with different characters (a, b, c, ….). There was significant statistical difference at a confidence level of 95 % (P≤0.05).

Gum Arabic, rather than maltodextrin, could be a better carrier for bioactive powder protection. Furthermore, gum Arabic is linked to the carbohydrate chain, making it an efficient film-forming agent with a high entrapping effectiveness, lowering bioactive chemical degradation sensitivity Zhang, Khoo et al. (2020). Pitalua, Jimenez et al. (2010) studied the antioxidant activity of beetroot juice powder with spray drying using gum Arabic as the encapsulating agent. It was found that gum Arabic protein helps to encourage Maillard reaction. The Maillard reaction, whose products serve as precursors to the polyphenols that improve antioxidant activity, may have been encouraged by the extraction of a protein portion of the gum Arabic. The increase in antioxidant activity in all the samples could be attributed to the release or production of novel compounds with antioxidant activity capable of quenching radicals, such as the intermediary chemicals created during Maillard's reaction; they are formed as a result of food storage and processing, or the production of polyphenols during processing or storage.

4.4.5 Antioxidant activity by FRAP of marigold flower microcapsules

Medical plants contain antioxidant compounds. Plants naturally contain substances called antioxidants that can prevent serious damage from free radicals. Oxidation, which can destroy cells, can be stopped with the help of antioxidants. They could boost the immune system and lower the risk of cancer, heart disease, and infections. However, these substances are susceptible to environmental factors (temperature, heat and light). Modern techniques have been developed as a result to preserve the extract's purity and increase its shelf life. Because it is adaptable,

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economical, effective, simple to scale up, and produces high-quality powder, spray drying is a preferred microencapsulation and drying method in the food applications I Ré (1998). This study looked at the antioxidant effects of FRAP on marigold flower extract microcapsules. The research showed that marigold flower extract microcapsules created with a 1:2 ratio of gum Arabic had the best antioxidant properties (4837.89 ± 27.85 mM Trolox equivalent/100 g db), followed by gum Arabic microcapsules made with a 1:3 ratio. The antioxidant activity of FRAP with gum Arabic of 4837.89 ± 27.85 and 4308.07 ± 26.49 mM Trolox equivalent/100 g db with the relationship between the bioactive compounds of phenolic and flavonoid compounds with the use of maltodextrin as 1:2 and 1:3 ratio encapsulation with the antioxidant activity of FRAP of 3076.49 ± 16.08 and 2665.96 ± 21.91 mM Trolox equivalent/100 g db with the relationship between the bioactive compounds of phenolic and flavonoid compounds. According to the findings of this investigation, marigold flower extract microcapsules with a 1:2 ratio of gum Arabic as encapsulating agents had the highest antioxidant and flavonoid content, which is consistent with the total phenolic compound values. In addition, microcapsules with gum Arabic had the higher antioxidant properties than maltodextrin in the encapsulation process. Ramakrishnan, Adzahan et al. (2018) demonstrated that the antioxidant content of gum Arabic was higher than that of maltodextrin and resistant maltodextrin. It could be because gum Arabic interacts with polarized phenolic chemicals and has a structure that provides superior light shielding and heat resistance during the spray drying process.

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Considering the antioxidant property, it was showed that marigold flower extract microcapsules with gum Arabic as the encapsulating agents at a ratio of 1:2 had the highest antioxidant activity with DPPH (1756.66 ± 28.99 mM Trolox equivalent/100 g db) and FRAP (4837.89 ± 27.85 mM Trolox equivalent/100 g db) (Table 25).

Table 25 Antioxidant activity by FRAP of marigold flower extract microcapsule from spray drying

Encapsulation type and Concentration	**Antioxidant activity by FRAP**

(w/v)	(mM Trolox /100g db)
45% Maltodextrin (MD) 1:1	3076.49 ± 16.08[c]
45% Maltodextrin (MD) 1:2	2665.96 ± 21.91[c]
20% gum Arabic ratio 1:2	4837.89 ± 27.85[a]
20% gum Arabic ratio 1:3	4308.07 ± 26.49[b]

*Average ± Standard Deviation

*Average with different characters (a, b, c, ….). There was significant statistical difference at a confidence level of 95 % (P≤0.05).

4.4.6 Total carotenoid content of marigold flower microcapsules

Carotenoids are pigments (coloring agents) that are found in both animals and plants. The red, orange, and yellow colors are caused by fat-soluble pigments, which contain about 700 compounds. Carotenoids are mostly hydrocarbons with 40 carbon atoms and two terminal rings. They can also be employed in food fortification due to their potential activity as provitamin A and biological functions that improve health, such as immune system strengthening, decreasing the chance of degenerative diseases, antioxidant characteristics, and ant obesity/hypolipidemic activities Mezzomo and Ferreira (2016). The total amount of total carotenoid content in marigold flower extract microcapsules was investigated with the variations of coating materials and various ratio of encapsulating agents (Table 26).

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Table 26 Total carotenoid content of marigold flower extract microcapsule from spray drying

Encapsulation type and Concentration (w/v)	Total carotenoid content (mg carotenoid/100g db)
45% Maltodextrin (MD) 1:1	55.22 ± 1.94[c]
45% Maltodextrin (MD) 1:2	44.62 ± 2.63[c]
20% gum Arabic ratio 1:2	208.45 ± 2.36[a]
20% gum Arabic ratio 1:3	162.75 ± 1.69[b]

*Average ± Standard Deviation

*Average with different characters (a, b, c, ….). There was significant statistical difference at a confidence level of 95 % ($P \leq 0.05$).

The amount of total carotenoid content was discovered to be between 44.62 ± 2.63 and 208.45 ± 2.36 mg carotenoid/ 100 g db. The highest carotenoid content was obtained with the microcapsules using gum Arabic at a ratio of 1:2 with 208.45 ± 2.36 mg carotenoid/100 g db and followed by gum Arabic at a ratio of 1:3 and the use of maltodextrin 1:1 and 1:2 respectively. According to this study Gong, Hou et al. (2012), the encapsulation method using maltodextrin destroyed carotene because of the high temperature used throughout the process. Because of their unsaturated structure, carotenoids are highly susceptible to heat, oxidation, and light, and so deteriorate.

4.4.7 Study on the yield percentage and storage efficiency of marigold flower extract microcapsules

Encapsulation can increase bioavailability while also altering the time and/or location of release following injection. As a result, the encapsulation technique could be improved to improve carotenoid pigment stability. The use of a variety of carrier materials allows for the creation of encapsulates in the form of powders with improved physical properties and yields. Encapsulation has proven to be an efficient approach for limiting carotenoid degradation by avoiding oxygen-mediated auto-oxidation processes, albeit the results are reliant on the carrier material properties (Šeregelj, Tumbas Šaponjac et al. (2019). Spray drying is being used in a variety of industries, including pharmaceuticals, food & beverage, and animal feed. Alvim, Stein et al. (2016) studied the application of microcapsules of ascorbic acid in biscuits using spray drying. It was discovered that microcapsules made from this procedure can prevent dark spots forming on biscuits during the baking process. Spray-dried ascorbic acid microcapsules also showed a high level of stability, indicating that this method could be used to protect active ingredients in products. The purpose of food and beverage applications is to encapsulate chemicals and regulate therapeutic food distribution. The ideal percentage of marigold flower extract microcapsules is determined by the inlet temperature of 155 °C. The sample flow rate is set to 3 per min, and the output temperature is set at 90 °C. It was found that the percentage of

yield obtained from marigold flower extract microcapsules from spray drying was between 53.59 ± 0.70 and 79.2 ± 0.56. There is a significant difference found in the percentage of output. It was discovered that the use of maltodextrin gave the highest yield percentage (79.2%) whereas the samples with gum Arabic provided the lowest yield percentage (53.59%) (Table 27). It could be due to the differences in the configuration of the carrier agents. Gum Arabic is a highly soluble and surface-active encapsulation matrix for oils and flavors that is widely utilized. Because of its high protein concentration, gum Arabic can produce gels. Low viscosity results in better flow during atomization and higher yields; hence viscosity content is critical for spray drying (LÓPEZ and Millan 2009). Maltodextrin has a lack of emulsifying capacity and lead to low volatile retention. This is in line with a study by (Kalušević, Lević et al. 2017), who investigated the production of microcapsules from anthocyanin-rich black soybean coat extract by spray drying with maltodextrin, gum Arabic, and skimmed milk powder and discovered that maltodextrin (MD) based microcapsules yielded 71.2% which more than gum Arabic (GA) based microcapsules (63.7%). The feed emulsion's higher viscosity resulted in larger particles that were more prone to settle and stick in the chamber, lowering the process yield. When larger droplets are created, the overall area available for water evaporation is reduced. As a result, powder stickiness is one of the elements that influences process yield (Borompichaichartkul, Hamad et al. (2020).

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Table 27 Yield percent and efficiency of marigold flower extract microcapsule from spray drying

Encapsulation type and Concentration (w/v)	Yield percent (%)	Efficiency (%)
45% Maltodextrin (MD) 1:1	61.15 ± 0.58^{b}	88.00 ± 1.15^{a}
45% Maltodextrin (MD) 1:2	79.2 ± 0.56^{a}	78.05 ± 1.04^{b}
20% gum Arabic ratio 1:2	53.59 ± 0.70^{c}	80.79 ± 1.12^{b}

20% gum Arabic ratio 1:3	56.15 ± 0.73^{c}	80.49 ± 1.85^{b}

*Average ± Standard Deviation
*Average with different characters (a, b, c, ….). There was significant statistical difference at a confidence level of 95 % (P≤0.05).

Samples with high efficiency can be obtained because each encapsulating substance has various qualities in the process of microencapsulation, spray drying is linked to the type and ratio of the encapsulating agent. The encapsulation efficacy of marigold flower extract microcapsules varied depending on the type and ratio of the encapsulating agent, according to the findings of this investigation. The encapsulation efficiency of the marigold flower microcapsules ranges from 78.05 ± 1.04 to 88.00 ± 1.15. It was found that marigold flower microcapsules using maltodextrin as the encapsulating substance at the ratio of 1:1 have the highest efficiency. Santos, Silveira et al. (2020) studied the effects of different types of encapsulating substances on the quality of pequi oil microparticles from spray drying. It was found that the microcapsules using maltodextrin showed the highest encapsulation efficiency (84.20%) while the use of gum Arabic as encapsulant resulted in (79.17%). The viscosity of the emulsion can affect encapsulation efficiency. Increased emulsion viscosity reduces the encapsulation effectiveness of gum Arabic. Because of its superior solubility in water and low viscosity at high concentrations, maltodextrin as a coating material improves encapsulating efficiency. High emulsion viscosity increases encapsulation efficiency (EE) by decreasing internal circulations in the droplets and accelerating the creation of a semi-permeable membrane. However, if the emulsion's viscosity reaches the optimum threshold, it can cause a reduction in their retention by subjecting the volatiles to high temperatures during atomization. Additionally, this condition may cause the gradual development of separate droplets during atomization. Moreover, the viscosity will have to be low enough to inhibit particle air absorption. The maltodextrin (MD) emulsion presented the lowest viscosity and high emulsion stability and this formulation gave the highest encapsulation efficiency (EE) (Ozdemir, Bayrak et al. 2021). Similar results were obtained by (Ramakrishnan, Adzahan et al. 2018) for microcapsules prepared by tamarillo carotenoids and

maltodextrin as encapsulant showed the highest encapsulation efficiency (84.77%). The low encapsulation efficiency may be as a result of the high viscosity of gum Arabic. Viscous liquid feeds apparently cannot form well-coated microcapsules and are not appropriate for spray drying compared to other less viscous liquid feeds (Zokti, Sham Baharin et al. 2016).

4.4.8 Study on moisture content and water activity of marigold flower extract microcapsules

Water activity and moisture content are important physicochemical parameters for dried powder product quality, stability, and shelf life, whereas solubility refers to a powder's ability to reconstitute. Moisture content is a measure of food solids production and quantity, and it can be used as a direct indicator of a product's economic value, stability, and quality Park (2008). Moisture in the food can be occurred into two forms: (1) bound water to the food ingredients such as proteins, salt and sugar, and (2) free or unbound water which can be easily changed from liquid state to vapor.

The availability of free water in the food product, which is responsible for biological reactions, is represented by water activity (a_w). As a result, one of the most critical characteristics for dried food products, particularly for long-term preservation, is water activity. For the products with water activity (a_w) less than 0.6 is microbiologically stable (Stajčić, Ćetković et al. (2020). In intermediate-moisturized foods, the effect of a_w on microbial development is critical. Most bacteria require water activity in the range of 0.90–1.00 at normal temperatures for microbial development. To reduce the water activity, salt, sugar, or other ingredients can be added into the products. Depending on the bacteria, the minimal a_w below which most critical food bacteria will not grow is around 0.90. The water activity is also associated with chemical and biochemical reactions in the food products, such as Maillard reaction, while the moisture content refers to the composition of water in the food. Generally, products with a_w less than 0.6 is microbiologically safe. However, when the water activity drops below 0.4, most of the undesirable changes during storage such as lipid oxidation, enzymatic reactions and non-enzymatic browning will be observed. In the lipid oxidation of microencapsulated compounds, water plays a

crucial role. Because of changes in the kinetic control mechanism, lipid oxidation rate increases in food products when moisture content drops below the monolayer value (Park 2008).

According to the analysis of moisture content and water activity of marigold flower extract microcapsules by spray drying, the effect of the type of coating material and different ratio of encapsulating agent was investigated in Table 28. The results showed that the moisture content was in the range between 3.19 ± 2.05 and 4.35 ± 0.13 while the water activity was between 0.10 ± 0.04 and 0.15 ±0.07. Water activity for dried food products should be lower than 0.6 and moisture content should be less than 8%. According to the findings, all encapsulated marigold flower extract powder samples exhibited microbiological safe food products since the water activity (a_w) was less than 0.6 (Petruzzi, Corbo et al. (2017). In this study, it was discovered that the increased concentration of maltodextrin resulted in a decrease in the moisture content than that with gum Arabic. The influence of coating materials on the physicochemical properties of microcapsules were investigated Cid-Ortega and Guerrero-Beltrán (2020). When compared to gum Arabic, it was discovered that using maltodextrin as an encapsulating agent resulted in a lower moisture content. It could be due to variances in the coating materials' chemical structures. Gum Arabic contains a fully organized heteropolysaccharide with excellent solubility. The results are also consistent with the study of (Cano-Higuita, Vélez et al. 2015). It was discovered that the use of gum Arabic resulted in a higher amount of moisture content than the use of maltodextrin and modified starch. Gum Arabic's high hygroscopicity, since it contains many hydrophilic ramifications and can boost higher water adsorption from the ambient air.

Table 28 Moisture content and water activity of marigold flower extract microcapsule from spray drying

Encapsulation type and Concentration (w/v)	Moisture content (%)	Water activity (%)

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45% Maltodextrin (MD) 1:1	4.35 ± 0.13^{a}	0.10 ± 0.04^{b}
45% Maltodextrin (MD) 1:2	3.19 ± 2.05^{b}	0.12 ± 0.03^{a}
20% gum Arabic ratio 1:2	4.42 ± 0.18^{a}	0.12 ± 0.03^{a}
20% gum Arabic ratio 1:3	3.45 ± 0.13^{b}	0.15 ± 0.07^{a}

*Average ± Standard Deviation

*Average with different characters (a, b, c, ….). There was significant statistical difference at a confidence level of 95 % ($P \leq 0.05$).

4.4.9 Study of L*, a*, b* color of marigold flower extract microcapsules

The color properties of marigold flower extract micro capsules were investigated utilizing spray drying and different types and ratios of encapsulating agent. The results revealed that the color values of L*, a*, and b* were strongly impacted by variations in coating material and encapsulating agent ratio. As shown in Table 29, the color values L*, a*, and b* ranged from 58.16 ± 3.81 to 73.18 ± 1.51, -3.17 ± 0.13 to -5.99 ± 0.14, and 19.02 ± 0.73 to 30.45 ± 1.59, respectively. Table 4. 8 shows the color values of marigold flower extract microcapsules after spray drying for L* (lightness), a* (green-red), and b* (blue-yellow). L * Indicates brightness from 0-100, which 0 means black, and 100 means white (very bright), where marigold flower extract microcapsule with maltodextrin at a ratio of 1:1 described the highest brightness with 73.18 ± 1.51 while marigold flower extract microcapsule that uses gum Arabic with a ratio of 1:2 showed the smallest brightness (L* = 58.16 ± 3.81). Furthermore, a* denotes greenness (-a*) and red (+a*), and all samples with a* that are negative denote the sample's greenness. The values of a* and b* decreased with the concentration of encapsulating material, provoking a reduction in the yellow and green tonalities, also associated to the addition of the encapsulating agents. The addition of maltodextrin decreased the yellowness of the encapsulated marigold flower extract powder and a darker color.

Table 29 Color value of L*, a*, b* of marigold flower extract microcapsule from spray drying

Encapsulation type and Concentration (w/v)	**L***	**a***	**b***

45% Maltodextrin (MD) 1:1	73.18 ± 1.51^{a}	-5.99 ± 0.14^{a}	24.46 ± 0.61^{b}
45% Maltodextrin (MD) 1:2	71.27 ± 1.39^{a}	-5.23 ± 0.11^{a}	19.02 ± 0.73^{c}
20% gum Arabic ratio 1:2	61.46 ± 1.55^{b}	-4.47 ± 0.17^{b}	30.45 ± 1.59^{a}
20% gum Arabic ratio 1:3	58.16 ± 3.81^{b}	-3.17 ± 0.13^{b}	25.09 ± 1.53^{b}

*Average ± Standard Deviation

*Average with different characters (a, b, c, ….). There was significant statistical difference at a confidence level of 95 % ($P \leq 0.05$).

4.4.10 Study of solubility of marigold flower extract microcapsules

Solubility is a feature of powders that determines how easily they may be reconstituted in water at room temperature before being employed as a food component or for food coloring. Furthermore, the carrier type and concentration had a significant impact on powder solubility (Suravanichnirachorn, Haruthaithanasan et al. (2018). The solubility of marigold flower extract microcapsules was investigated using various encapsulating agents and coating material ratios, according to the study. It was discovered that the solubility of marigold flower extract microcapsules with diverse coating material types and ratios was affected. Table 30 shows that the solubility of marigold flower extract microcapsules ranged between 88.41 ± 2.91 and 92.98 ± 4.22%. According to the findings, using maltodextrin at a 1:2 ratio had the highest solubility (92.98%).

Table 30 Solubility of marigold flower extract microcapsule from spray drying

Encapsulation type and	**Solubility (%)**

Concentration (w/v)	
45% Maltodextrin (MD) 1:1	89.31 ± 1.31[b]
45% Maltodextrin (MD) 1:2	92.98 ± 4.22[a]
20% gum Arabic ratio 1:2	88.41 ± 2.91[b]
20% gum Arabic ratio 1:3	92.73 ± 3.20[a]

*Average ± Standard Deviation

*Average with different characters (a, b, c, ….). There was significant statistical difference at a confidence level of 95 % ($P \leq 0.05$).

Maltodextrins are nonsweet nutritional saccharide combinations of oligomers and polymers made up of D-glucose units joined together in chains of varying lengths and mostly linked by -1-4 bonds. Maltodextrin is made by heating and gelatinizing a food-grade starch, most commonly corn or wheat starch in the United States or Europe, and then partially hydrolyzing it with safe and acceptable acids and enzymes. Maltodextrins done entirely by alkaline hydrolysis have a high concentration of linear chains that are easily retrograded. Maltodextrins with low hygroscopicity and high-water solubility are produced by combining acid catalysis with amylase catalyzed hydrolysis. Marigold flower extract powder with maltodextrin had a higher water solubility index than with gum Arabic which has emulsifying properties as it has low protein content. The study's findings are consistent with the Suravanichnirachorn, Haruthaithanasan et al. (2018) findings, which found that Mao powder with maltodextrin had a greater water solubility index than gum Arabic, which possesses emulsifying capabilities because to its low protein concentration. Even at high concentrations, maltodextrin has good water solubility and low viscosity. The water solubility index (WSI) rose as the concentration of maltodextrin increased. The water solubility index increased as the maltodextrin concentration was increased from 30% to 35% (95.44% and 96.87%, respectively). Ahmed, Akter et al. (2010) also discovered that encapsulated purple sweet potato powder with a higher maltodextrin concentration showed higher solubility than powder with a lower concentration.

4.4.11 Study of surface structure characteristics of microcapsule marigold flower extract microcapsules

The necessity to determine the encapsulating capabilities of various polymers is one of the reasons for employing SEM in microencapsulation research. The degree of integrity and porosity of the microcapsule are indicators of this ability. Morphologic investigation demonstrated the size, form, and common characteristics of microcapsules made from various wall materials, as well as a tendency for the tiniest particles to clump together. Dents are found on the exterior surfaces of spray-dried microcapsules, and these dents are produced by particle shrinkage during drying and cooling; similar dents were reported in a study of milk powder (Finotelli and Rocha-Leão (2005). The morphological modifications (size, structure, and appearance) of the particles during the drying process were described by Sheu and Rosenberg (1998). Due to internal creation of lipid droplets and dents, depression, and external breakage, many of these spherical droplets create particles with irregular surfaces (folds). Vacuole formation results from the shrinking process that occurs after the hardening of the outer surface followed by the expansion of the air bubbles trapped inside the droplet. The temperature and liquid dispersion are related to the expansion and distortion of the spray-dried particles because prolonged drying times cause the structure to deform, shrink, and disintegrate (which results in breakage) Cano-Higuita, Vélez et al. (2015). Figures 45 and 46 illustrate the results of the external shape of microcapsules with scanning electron microscope (SEM) with magnification of 500 and 1000 times. The shape of a marigold flower microcapsule made with a 1:2 ratio of gum Arabic was discovered to be spherical and wide diameter distribution. This could be related to moisture loss causing fast shrinkage. As a consequence, the microcapsule's surface features crimps and dents. When the encapsulation ratio was increased to 1:3, the surface area of the microcapsule was found to be more spherical and smoother. The shape of the microcapsule became smoother and more spherical as the thickness of the encapsulating layer increased in gum Arabic (Figure 46 a, b). There were globular capsules with rough surfaces. This result was most likely brought on by the cooling that followed the expansion from the water vapor escape. The particles from gum Arabic were spherical in shape and had several concavities, according to numerous investigations. Concavities may develop

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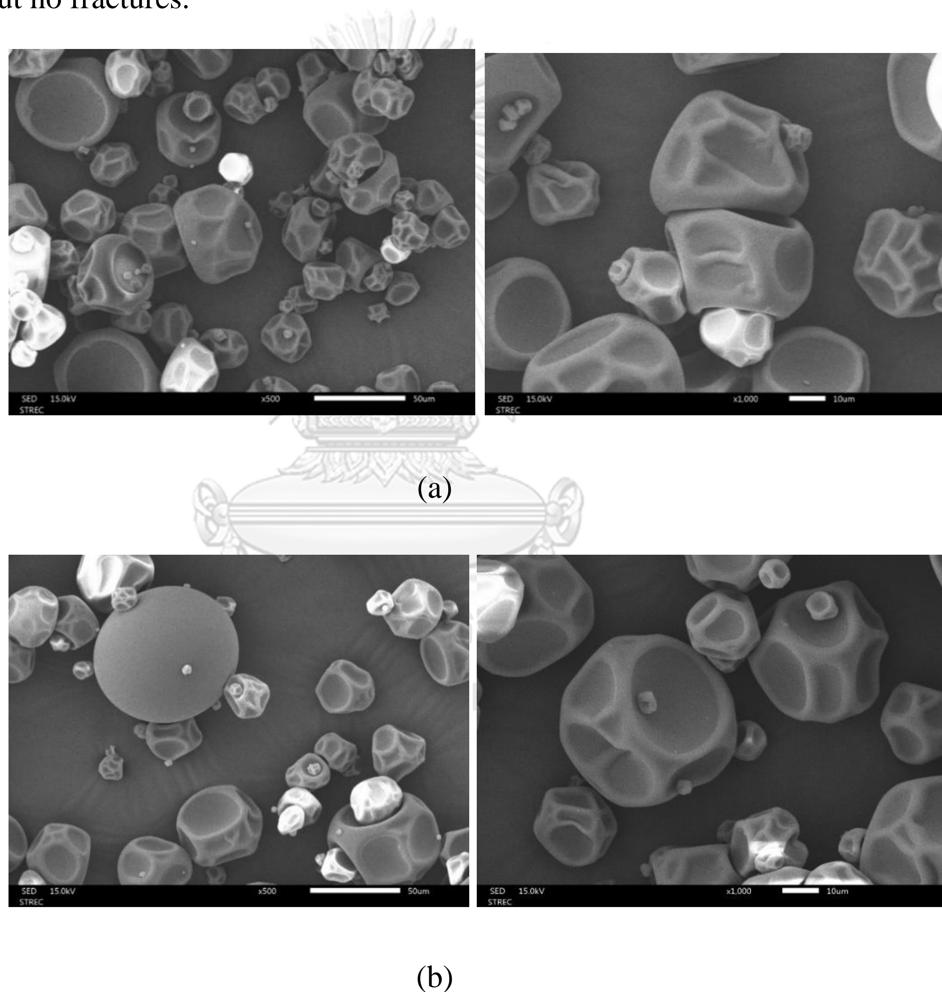
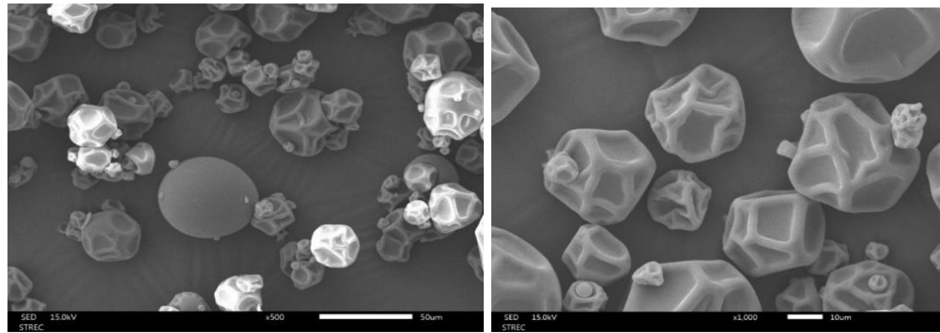
in gum Arabic powders as a result of the particles shrinking brought on by the drying process' fast evaporation (Bernstein and Noreña 2015). The microcapsules obtained from maltodextrin were slightly circular and wrinkles can be observed on the surface in Figure 45 a, b. The microcapsules of marigold flower extract with gum Arabic were smaller as compared to the maltodextrin. In the study of (Cano-Higuita, Vélez et al. 2015), the microcapsules made entirely gum Arabic had smooth surfaces but some teeth on the surface that exhibited shrinkage, whereas the microcapsules made from maltodextrin and modified starch had somewhat round surfaces with wrinkles on the surface but no fractures.



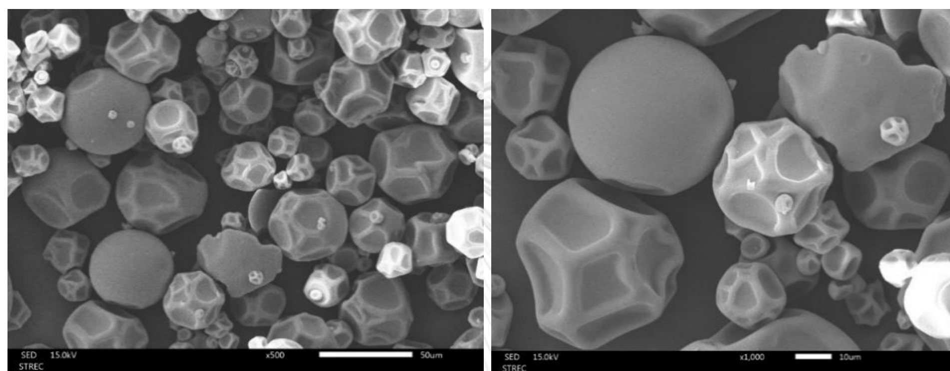
(a)

(b)

Figure 45 Photo with a scanning electron microscope that is expanding 500 times and 1000 times (left to right, respectively) of marigold flower extract microcapsules. a) Maltodextrin with an encapsulation ratio of 1:1, b) Maltodextrin with an encapsulation ratio of 1:2



(a)



(b)

Figure 46 Photo with scanning electron microscope that is expanding 500 times and 1000 times (left to right, respectively) of marigold flower extract microcapsules. c) Gum Arabic with an encapsulation ratio of 1:2, d) Gum Arabic with an encapsulation ratio of 1:3

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Chapter 5
Summary of Experimental Results

The consumer demand for value-added and/or functional products to enhance health benefits has increased. Marigold flower contains high content of phenolic compounds, and antioxidant capacity. Ultrasound-assisted extraction affects the total phenolic compounds, total flavonoids, antioxidant activity and carotenoid content. The optimized extraction conditions in the current study were ethanol concentration (68%) (v/v), temperature (40 °C) and time (15min) with higher bioactive compounds and antioxidant activities. Marigold flower extract contains total phenolic compounds (75.699 mg QAE/100g db), total flavonoid content (68.740 mg QCE/100g db), antioxidant activity by DPPH and FRAP (630.369 mM TE/100g db and 3226.171 mM TE/100g db), and total carotenoid content (234.741 mg carotenoid/100g db), respectively. Depending on the optimum condition, antimicrobial analysis was determined using disc diffusion method and minimum inhibition concentration against *Escherichia coli* and *Staphylococcus aureus*. The optimized extract had an inhibitory zone of 9.66 and 8.33 mm against *E. coli* and *S. aureus*, respectively. The MIC value of the optimized extract was also 25 mg/mL. The effects of encapsulation by spray drying using maltodextrin (MD) and gum Arabic (GA) as the coating materials on the physical properties of the microcapsules were significant. It discovered that the yield (%) from spray dried marigold flower microcapsules was between 53.59 % and 79.2 % and the encapsulation efficiency was ranging from 78.05 to 88.00 % and thus the highest production yield and efficiency was obtained using maltodextrin microcapsules at a ratio of 1:2. But the type and ratio of encapsulating agent did not affect the solubility of the microcapsules. Additionally, the moisture content and water activity of all microcapsules were lower than 8% and 0.6 respectively. For the color, it was found that the type and ratio of the encapsulating agent affected L*, a* and b* values and all samples had a* negative color value and b* had positive color value. Moreover, the study of the surface structure characteristics of microcapsules with SEM demonstrated that the type and ratio of coating material affected the shape and texture of the microcapsules. Microcapsules with maltodextrin showed spherically bigger shaped than with gum Arabic. However, the microcapsules using gum Arabic at a ratio of 1:2 demonstrated

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the total phenolic compound (627.91 mg GAE/100g db), total flavonoid content (389.56 mg QCE/100g db), antioxidant activity by DPPH and FRAP assay (1756.66 mM TE/100g db and 4837.89 mM TE/100g db) and total carotenoid content (208.45 mg carotenoid/100g db), respectively are the highest.

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Appendix A

How to analyze chemical properties

A.1 Analyze the total phenolic compounds by Folin-Ciocalteau colorimetry method

A.1.1 Equipment & Tools

1. UV-visible spectrometer (Thermo Fisher Scientific, GENESYSTM20 Visible, U.S.A)
2. Analytical balance (Mettler Toledo, New Classic MF, Switzerland)

A.1.2 Chemicals

1. Ethanol (CH_3CH_2OH) (EMSURE®, Germany)
2. Folin-Ciocalteau reagent (Carlo Erba, France)
3. Gallic acid ($C_7H_6O_5$) (Fluka, Spain)
4. Sodium carbonate (Na_2CO_3) (A.R. grade, Ajax Finechem, Australia)

A.1.3 Preparation of solution

1. 1000 mg/L concentration of gallic acid solution

0.1 g of gallic acid was dissolved in distilled water and then adjusted with 100 mL volume of distilled water.

2. 10% (w/v) concentration of Folin–Ciocalteau's phenol solution

10 mL of Folin–Ciocalteau's phenol was dissolved in distilled water and then adjusted with 100 mL volume of distilled water.

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3. 10% (w/v) concentration of sodium carbonate solution

10 g of sodium carbonate was dissolved in distilled water and then adjusted with 100 mL volume of distilled water.

A.1.4 How to analyze

For Marigold Flower Extract

The total phenolic compound of the sample extract was measured using the Folin–Ciocalteau method. 0.5 mL of sample was pipetted into test tube and 10 mL of distilled water was added into test tube. After that 0.5 mL of 10% concentration of Folin–Ciocalteau's phenol solution was added, and the solution was then mixed well

with vortex mixer. The solution was incubated at room temperature for 5 min. 2 mL of 10% (v/v) sodium carbonate solution was added into test tube and then mixed the solution well with vortex mixer. After that the solution was incubated at room temperature for 10 min. Finally, the absorbance was measured at 765 nm with microplate reader using distilled water as a blank.

Y-value was substituted with absorption value in the standard curve equation of gallic acid solution. The total phenolic content in the sample was calculated and the result was expressed in mg gallic acid equivalent (GAE)/100 g dry db.

For Marigold Flower Extract Microcapsules

Dissolve 1 g of marigold flower extract microcapsules in 10 mL of distilled water and mix well with vortex mixer for 3 min. The sample is put in a hot tub, controlling the shaking temperature to 30 °C up to 30 min. Centrfuge the sample at a speed of 4000 rpm for 20 min. 0.5 mL of sample was pipetted into test tube and 10 mL of distilled water was added into test tube. After that 0.5 mL of 10% concentration of Folin–Ciocalteau's phenol solution was added, and the solution was then mixed well with vortex mixer. The solution was incubated at room temperature for 5 min. 2 mL of 10% (v/v) sodium carbonate solution was added into test tube and then mixed the solution well with vortex mixer. After that the solution was incubated at room temperature for 10 min. Finally, the absorbance was measured at 765 nm with microplate reader using distilled water as a blank.

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Calculate the total amount of phenolic compound using absorption values compared to standard graphs. The value was expressed in mg GAE/100 g dry db.

A.1.5 Standard curve of a gallic acid solution

0.1 g of gallic acid was dissolved in distilled water and then adjusted with 100 mL of distilled water to get 1000 mg/L of gallic acid concentration. After that 0.1, 1, 2.5, 5- and 10-mL of 1000 mg/L of gallic acid concentration was pipetted and dissolved in distilled water. After that adjusted with 100 mL volume of distilled water by using volumetric flask to get 1,10, 25, 50 and 100 mg/L of gallic acid concentration. 0.5 mL of each gallic acid concentration was pipetted into the test tube and 10 mL of distilled water was poured into test tube. After that 0.5 mL of 10%

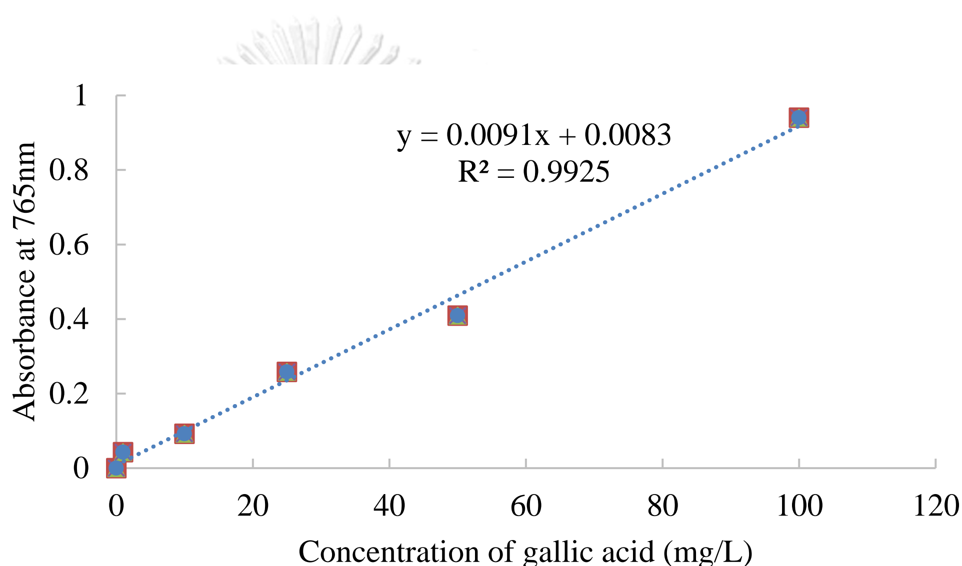

Figure A.1 Gallic acid standard curve for total phenolic content

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concentration of Folin–Ciocalteau’s phenol solution was added and then mixed the

solution well.

with vortex mixer. The solution was incubated at room temperature for 5 min. 2 mL of 10% (w/v) concentration of sodium carbonate solution was added into test tube and the solution was then mixed well with vortex mixer. After that the solution was incubated at room temperature for 10 min. Finally, the absorbance was measured at 765 nm with microplate reader by using distilled water as a blank and made a standard curve of gallic acid solution. The concentration of gallic acid is x axis and absorbance of 760 nm is y axis.

A.2 Analysis of the total flavonoid content with aluminum chloride colorimetry method

A.2.1 Equipment & Tools

1. UV-visible spectrophotometer (Thermo Fisher Scientific, GENESYSTM20 Visible, U.S.A)
2. Analytical balance (Mettler Toledo, New Classic MF, Switzerland)

A.2.2 Chemicals

1. Aluminum chloride ($AlCl_3$) (Ajax Finechem, New Zealand)
2. Methaonol (CH_3OH) (Fisher Scientific, UK)
3. Quercetin ($C_{15}H_{10}O_7$) (Sigma-Aldich, Germany)
4. Sodium nitrite ($NaNO_2$) (Ajax Finechem, New Zealand)

A.2.3 Preparation of solution

1. 1000 mg/L concentration of quercetin solution

0.1 g of quercetin was dissolved in distilled water and then adjusted with 100 mL volume of distilled water.

2. 2% concentration of aluminum chloride solution

2 g of aluminum chloride was dissolved in 95% ethanol solution (w/v) and then adjusted with 100 mL volume of 95% ethanol solution.

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A.2.4 How to analyze

For Marigold Flower Extract

The aluminum tri-chloride method with slight modification was used for total flavonoid content (TFC) determination of the marigold flower extract. 1 mL of sample was pipetted into test tube. 1 mL of 2% of concentration aluminum chloride solution (w/v) was pipetted into test tube and the solution was then mixed well with vortex mixer. After that the solution was placed in a dark place at room temperature for 30 min. Finally, the absorbance was measured at 430 nm with microplate reader by using distilled water as a blank. Y-value was substituted with absorption value in

the standard curve equation of quercetin. The total flavonoid content in the sample was calculated and expressed in mg quercetin equivalent (QE)/100 g dry db.

For Marigold Flower Extract Microencapsules

Dissolve 1 g of marigold flower extract microcapsules in 10 mL of distilled water and mix well with vortex mixer for 3 min. The sample is put in a hot tub, controlling the shaking temperature to 30 °C up to 30 min. Centrfuge the sample at a speed of 4000 rpm for 20 min. 1 mL of sample was pipetted into test tube. 1 mL of 2% of concentration aluminum chloride solution (w/v) was pipetted into test tube and the solution was then mixed well with vortex mixer. After that the solution was placed in a dark place at room temperature for 30 min. Finally, the absorbance was measured at 430 nm with microplate reader by using distilled water as a blank. Calculate the total amount of phenolic compound using absorption values compared to standard graphs. The value was expressed in mg QE/100 g dry db.

A.2.5 Standard curve of a quercetin solution

0.1 g of quercetin was dissolved in distilled water and then adjusted with 100 mL of distilled water to get 1000 mg/L of concentration of quercetin solution. After that 0.1, 1, 2.5, 5- and 10-mL of 1000 mg/L quercetin solution was pipetted and dissolved in distilled water. After that adjusted with 100 mL volume of distilled water by using volumetric flask to get 1,10, 25, 50 and 100 mg/L of quercetin concentration. 1 mL of each quercetin concentration was pipetted into test tube. 1 mL of 2% of aluminum chloride (w/v) was pipetted into test tube and the solution was then mixed well with vortex mixer. After that the solution was placed in a dark place at room temperature for 30 min. Finally, the absorbance was measured at 430 nm with microplate reader by using distilled water as a blank and made a standard curve of quercetin solution. The concentration of quercetin is x axis and absorbance of 430 nm is y axis.

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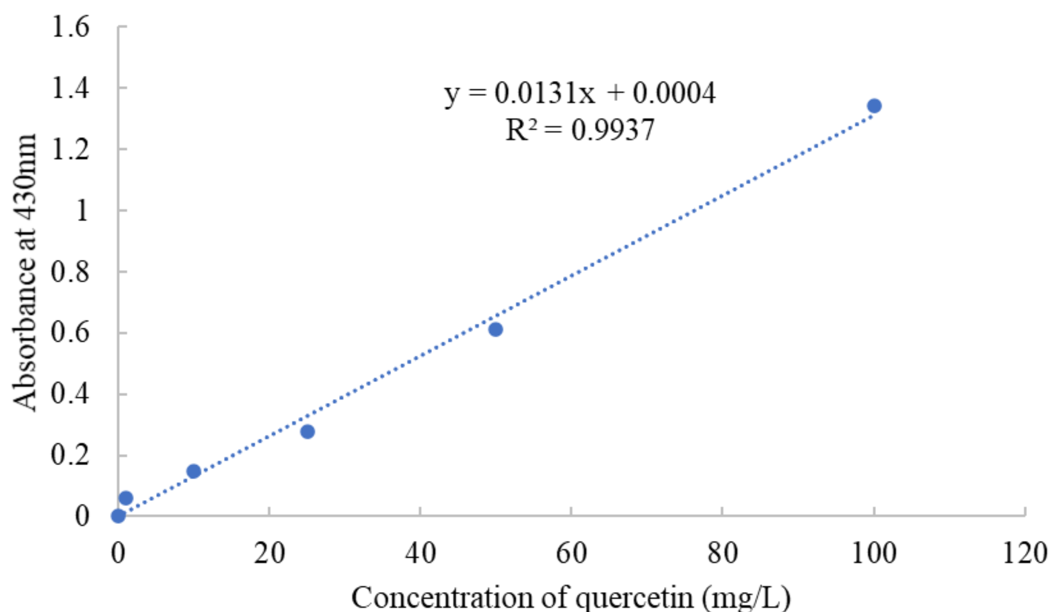

Figure A.2 Quercetin standard curve for total flavonoid content

A.3. Determination of antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl or DPPH assay

A.3.1 Equipment & Tools

5. UV-visible spectrophotometer (Thermo Fisher Scientific, GENESYSTM20 Visible, U.S.A)

6. Analytical balance (Mettler Toledo, New Classic MF, Switzerland)

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A.3.2 Chemicals

1. 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Fluka, U.S.A)

2. 6-hydroxy-2,5,7,8-tretamethylchroman-2-carboxylic acid (Trolox) (Fluka, Denmark)

3. Methanol (CH_3OH) (Fisher Scientific, UK)

A.3.3 Preparation of solution

1. 10,000 µm concentration of Trolox solution

0.025 g of Trolox was dissolved in 10 mL of methanol to get 10,000 µm concentration of Trolox solution. After that 10,000 µm concentration of Trolox

solution was diluted as shown in table to build standard graph, Trolox standard solution was used at a concentration 82.415 µm.

A.3.4 Preparation of DPPH solution

0.024 g of DPPH was dissolved in 50 mL of methanol and then adjusted with 100 mL volume of methanol. 6×10 M of DPPH solution was stored not more than 5 days at a temperature of 4°C. Daily solution was prepared for further analysis, 50 mL volume of DPPH stock solution was then adjusted with 50 mL volume of methanol to get 1.2×10 M of DPPH concentration. The daily solution ($A_{initial}$) value should be approximately 1.1. If this solution is more than 1.1, DPPH or methanol solution was added with adjust ($A_{initial}$) value.

Table A.1 Method for preparation of Trolox standard solution

Initial concentration of Trolox (µm)	Trolox volume (mL)	Methanol volume (mL)	Final concentration of Trolox (µm)
10,000	5	5	5,000
5,000	5	5	2,500
2,500	5	5	1,250
1,250	5	5	625
625	4	2	417
417	4	2	278
278	4	2	185
185	4	2	123
123	4	2	82

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A.3.5 How to analyze

For Marigold Flower Extract

The assessment of the antioxidant activity was measured based on the inhibition of the DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical. 250 µL of sample extract was mixed with 4.75 mL of DPPH daily solution (1.1) and stored in a dark place at a room temperature for 15 min. And then sample extract solution was measured at 515 nm with microplate reader by using methanol as a blank. The absorbance of DPPH daily solution ($A_{initial}$) should have approximately 1.1 and the absorbance of sample (A_{final}) should have from 0.6 to 0.7.

$$A_{difference} = A_{initial} - A_{final}$$

$A_{difference}$ was calculated in the above equation and the result was expressed in mM Trolox/ 100 g dry wt.

For Marigold Flower Extract Microcapsules

Dissolve 1 g of marigold flower extract microcapsules in 10 mL of distilled water and mix well with vortex mixer for 3 min. The sample is put in a hot tub, controlling the shaking temperature to 30 °C up to 30 min. Centrfuge the sample at a speed of 4000 rpm for 20 min. 250 µL of sample extract was mixed with 4.75 mL of DPPH daily solution (1.1) and stored in a dark place at a room temperature for 15 min. And then sample extract solution was measured at 515 nm with microplate reader by using methanol as a blank. Calculate the difference of the absorption value (A_{diff}) of the absorption value of the solution and the value was expressed as mM Trolox/ 100 g dry wt.

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A.3.6 Standard curve of a Trolox solution

250 µL of Trolox was mixed with 4.75 mL of DPPH daily solution (1.1) and stored in a dark place at a room temperature for 15 min. And then Trolox was measured at 515 nm with microplate reader by using methanol as a blank. The absorbance of DPPH daily solution ($A_{initial}$) should have approximately 1.1 and the absorbance of Trolox (A_{final}) should have from 0.6 to 0.7.

$$A\ difference = A_{initial} - A_{final}$$

A $_{difference}$ was calculated in the above equation and the result was expressed in mM Trolox/ 100 g dry wt.

The concentration of Trolox is x axis and absorbance of 515 nm is y axis.

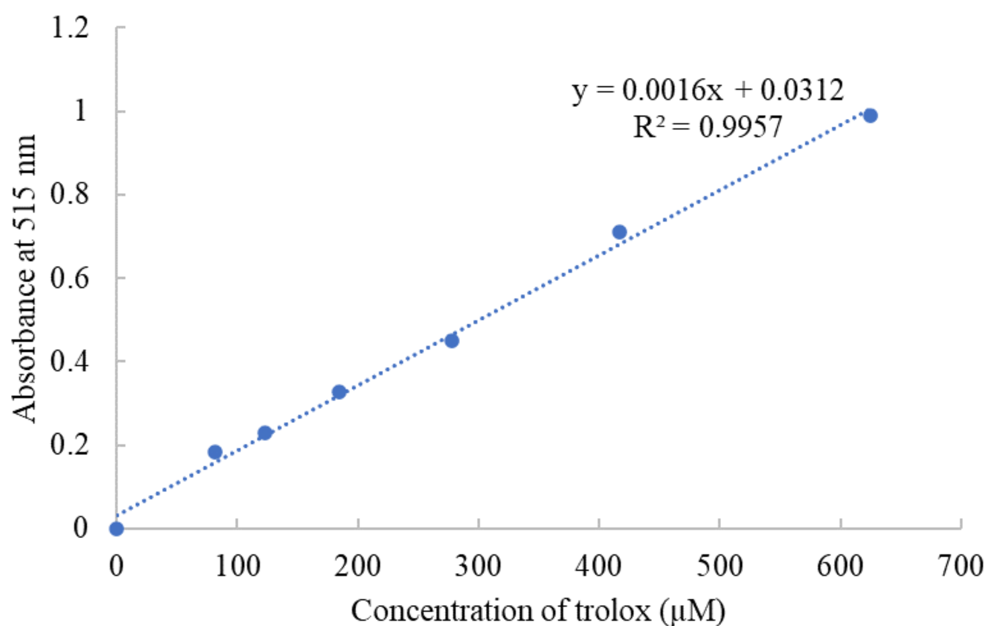

Figure A.3 Trolox standard curve for antioxidant activity by DPPH assay

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A.4. Antioxidant activity by ferric reducing antioxidant power (FRAP) assay

A.4.1 Equipment & Tools

4. UV-visible spectrophotometer (Thermo Fisher Scientific, GENESYSTM20 Visible, U.S.A)

5. Analytical balance (Mettler Toledo, New Classic MF, Switzerland)

A.4.2 Chemicals

1. 6-hydroxy-2,5,7,8-tretamethylchroman-2-carboxylic acid (Trolox) (Fluka, Denmark)

2. Sodium acetate treta-hydrate (CH3COONa.3H20) (A.R. grade, KemAus, Australia)

3. Tripyridyltriazine (TPTZ) (Merck, Germany)

4. Ferric chloride ($FeCl_3$) (Fisher Scientific, UK)
5. Gallic acid ($C_7H_6O_5$) (Fluka, Spain)
6. Hydrochloric acid 0.1 M (HCl) (A.R grade, Ajax Finechem, Australia)
7. Methanol (CH_3OH) (Fisher Scientific, UK)

A.4.3 Preparation of FRAP

25 mL of acetate buffer and 2.5 mL of ferric chloride was mixed with 2.5 mL of TPTZ, and the solution was then mixed thoroughly with vortex mixer. This solution was then heated with water bath at temperature 37°C for 5-10 min and turned brown color. The absorbance of FRAP ($A_{initial}$) was measured at 593 nm with microplate reader by using distilled water as a blank.

A.4.4 How to analyze

For Marigold Flower Extract

The FRAP assay was done according to Benzie and Strain (1996) with some modifications. 50 µL of sample extract was pipetted and then was mixed with 950 µL of FRAP. After that this solution was placed at room temperature for 4 min. The absorbance of sample extract solution (A_{final}) was measured at 593 nm with microplate reader by using distilled water as a blank.

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$$A\ difference = A_{final} - A_{initial}$$

$A_{difference}$ was calculated in the above equation and the result was expressed in mM Trolox/ 100 g dry wt.

For Marigold Flower Extract Microcapsules

Dissolve 1 g of marigold flower extract microcapsules in 10 mL of distilled water and mix well with vortex mixer for 3 min. The sample is put in a hot tub, controlling the shaking temperature to 30 °C up to 30 min. Centrfuge the sample at a speed of 4000 rpm for 20 min. . 50 µL of sample extract was pipetted and then was mixed with 950 µL of FRAP. After that this solution was placed at room temperature

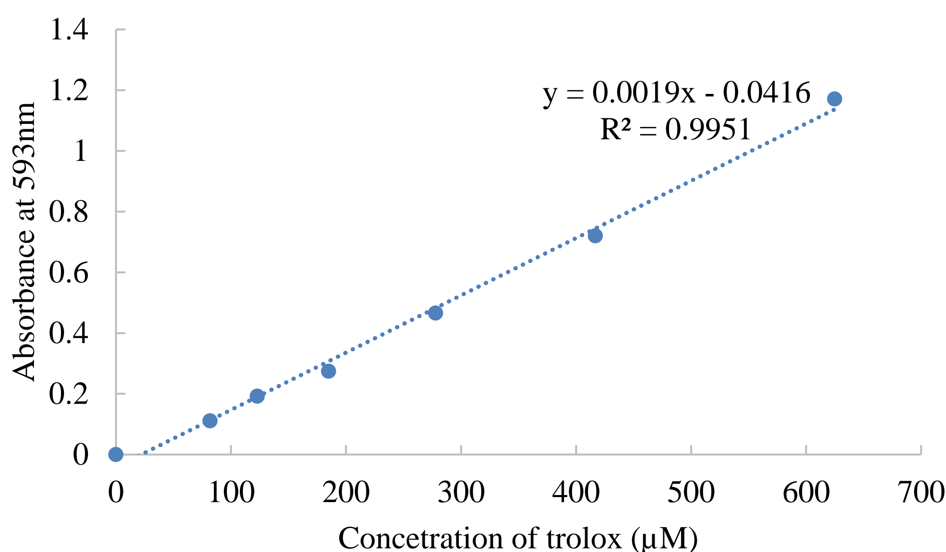
for 4 min. The absorbance of sample extract solution (A_{final}) was measured at 593 nm with microplate reader by using distilled water as a blank. Calculate the difference of the absorption value (A_{diff}) of the absorption value of the solution and the value was expressed as mM Trolox/ 100 g dry wt.

A.4.5 Standard curve of a Trolox solution

50 µL of Trolox was pipetted and then was mixed with 950 µL of FRAP. After that this solution was placed at room temperature for 4 min. The absorbance of Trolox (A_{final}) was measured at 593 nm with microplate reader by using distilled water as a blank.

$$A_{difference} = A_{final} - A_{initial}$$

$A_{difference}$ was calculated in the above equation and the result was expressed in mM Trolox/100 g dry wt.



The concentration of trolox is x axis and absorbance of 593 nm is y axis.

Figure A.4 Trolox standard curve for antioxidant activity by FRAP assay

A.5 Determination of total carotenoid content

For Marigold Flower Extract

Total carotenoid content was done according to the method of Biswas et al. with some modifications. For the extraction, sample extract (1g) was weighed and placed in the glass test tube. 5 mL of acetoned was then mixed with the sample and vortexed at high speed for 10 min, and centrifuged at 1370×g for 10 min. After that the supernatant was collected and the remained content was done extraction again using 5 mL of acetone. Finally, both of the supernatant was collected together and then filtered with Whatman filter paper No.1. The absorbance value was measured at 450 nm in a UV-Vis spectrometer and the results were revealed as mg carotenoid/100 g db.

For Marigold Flower Extract Microcapsules

Dissolve 1 g of marigold flower extract microcapsules in 10 mL of distilled water and mix well with vortex mixer for 3 min. The sample is put in a hot tub, controlling the shaking temperature to 30 °C up to 30 min. Centrfuge the sample at a speed of 4000 rpm for 20 min. sample extract (1g) was weighed and placed in the glass test tube. 5 mL of acetoned was then mixed with the sample and vortexed at high speed for 10 min, and centrifuged at 1370×g for 10 min. After that the supernatant was collected and the remained content was done extraction again using 5 mL of acetone. Finally, both of the supernatant was collected together and then filtered with Whatman filter paper No.1. The absorbance value was measured at 450 nm in a UV-Vis spectrometer and the results were revealed as mg carotenoid/100 g db.

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A.5.1 Standard curve of β-carotene solution

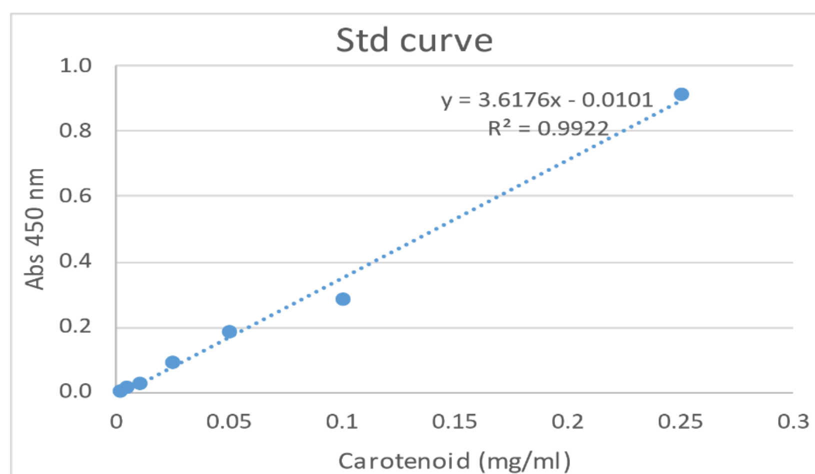

Figure A.5 Standard curve for carotenoid content

A.6: Antimicrobial activity experiment

A.6.1 Preparation of test microorganisms

Microbial cultures including *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 were obtained from Thailand Institute of Scientific and Technological Research, TISTR (Pathum thani, Thailand). Inoculum of each microorganism was prepared by inoculating with 10 mL sterile growth medium (Muller Hinton Broth). The test tubes were incubated for 18-24 h at 37 °C. The initial concentration of test microorganism was approximately 6.0 log CFU/mL by measuring the optical density (OD) at 600 nm.

A.6.2 Antimicrobial activity by disc diffusion method

Antimicrobial activity was measured using the disc diffusion method. To obtain the final concentration, a stock extract solution was made by dissolving with relative solvents (ethanol). The sterile, blank discs were dotted with 20 µL of extract and then dried. These discs were placed on Mueller-Hinton agar plates that had previously contained 10^8 CFU/ml of the target bacteria. To test the inhibitory zone of the solvent, distilled water or ethanol were utilized as negative controls and chloramphenicol was employed as a positive control in each disc. The discs were then

placed in an incubator at 37 °C for 24 h. The diameter of the inhibitory zone that developed around the discs was used to measure antibacterial activity.

A.7 Minimum inhibitory concentration (MIC) by broth microdilution method

The extract that demonstrates inhibition zones against microbial strains was used to calculate the Minimum Inhibitory Concentration (MIC) values using the disc diffusion method. The broth microdilution method was employed with minor modifications to determine the minimum inhibitory concentration (MIC) of extracts. The extracts were put into the plates in two-fold serial dilutions using 96-well culture plates. A two-fold serial dilution of chloramphenicol was utilized as a control. Positive control (antibiotic, Mueller-Hinton broth, and test organism) and sterile control (distilled water and Mueller-Hinton broth) wells were kept the same. The inoculated petri dishes were then placed in an incubator at 37 °C for 24 h before being assessed. And the color changes caused by bacterial growth were assessed. The MIC values were determined by looking at the turbidity in the wells plate. The minimal inhibitory concentration was defined as the lowest concentration of the extract that inhibited the microorganism's growth (MIC).

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Appendix B

Physical Properties

B.1 Analysis of moisture content according to AOAC method (2000)

1. Dry the aluminum cup at 105 °C for 2-3 h and put it in the desiccant jar until the temperature of the container is equal to the room temperature and then weigh. Repeat the procedure until the weight is constant.
2. 0.2 g of sample was put into the aluminum cup and dried at 105 °C for 4-5 h. Remove the cup from the incubator contained in the desiccant jar and weigh the container with the samples, and then put it back into the incubator. Repeat this process until the moisture content is constant. The moisture content can be calculated according to the following equation:

Moisture (%) = (sample weight before drying – Sample weight after drying)/ (Sample weight before drying) * 100%

B.2 Water activity analysis (a_w) according to AOAC method (2000)

The water activity (a_w) of marigold flower extract encapsulated powder was analyzed by water activity analyzer (MODEL MS1, Novasina, Switzerland) at 25 °C.

B.3 Color analysis CIE LAB system with chroma meter

The color value of the encapsulated marigold flower extract powder was determined at room temperature with CIE LAB system by Minolta Chroma Meter CR-400 colorimeter, which uses illuminant D65 displaying the color values in the CIE color space (L*, a* and b*).

- a* is the color ranging from green (-a*) to red (+a*)
- b* indicates the blue value (-b*) through the yellow (+b*)
- L* value consider as the lightness ranges between 0-100 (where 0 is black and 100 is white)

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B.4 Analysis of the percentage of encapsulation yield according to the (Ramakrishnan, Adzahan et al. 2018)

Encapsulation yield (%) was determined based on the method of Ramakrishnan, Adzahan, Yusof, and Muhammad (2018) and calculated according to the equation:

Encapsulation yield (%) = Weight of marigold flower extract microcapsule at spray dryer/ Weight of dry compounds in mixture before spray drying * 100%

B.5 Analysis of percentage of encapsulation efficiency adapted from (Saénz, Tapia et al. 2009)

Total bioactive compounds

1. 0.1 g of marigold flower extract encapsulated powder was weighed and dissolved in 1 mL of mixed solution (Ethanol: Acetic acid: Water) at a ratio of (50: 8: 42) and then shaken with vortex mixer for 1 min.
2. Centrifuge the sample at the speed of 10,000 rpm for 5 min and then filter. Analyze the total amount of phenolic compound using Folin-Ciocalteau colorimetry (Slinkard and Slingleton (1997))

Surface bioactive compounds

1. 0.1 g of marigold flower extract encapsulated powder was weighed and dissolved in the mixed solution (ethanol and methanol at a ratio of 1:1) and then shaken with vortex mixer for 1 min.
2. Filter the samples and analyze the total amount of phenolic compounds using Folin-Ciocalteau colorimetry (Slinkard and Slingleton (1997).

Efficiency % = TO – SO/ TO * 100

Where, TO is the total bioactive compound and SO is the surface bioactive compound

B.6 Analysis of the characteristics of external shape of marigold flower extract encapsulated powder with scanning electron microscope (SEM)

The microstructure of marigold flower extract microcapsule was analyzed by Scanning Electron Microscope (SEM) and energy dispersive X-ray spectrometer

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(JEOL, JSM-IT300 Oxford, X-Max N 20) at 30 kV magnifications for 500 and 1000 times.

B.7 Water solubility index (WSI) analysis adapter from (Drozłowska, Bartkowiak et al. 2021)

1. Weigh 0.2 g of marigold flower extract and mix with 10 mL of distilled water and shake with vortex mixer for 3 min.
2. Heat the sample using water bath with shaker at 30 °C for 30 min.
3. Centrifuge the sample at 4000 rpm for 20 min. The supernatant was transferred to the aluminum cup with a certain weight and dry with oven at 105 °C until the weight is constant. After that the cup left in the desiccant jar before weighing.

Water solubility (%) = Solid content in supernatant/Total solid content *100%

B.8 Total solid content analyzed according to AOAC method (2006)

1. Dry the aluminum cup at 105 °C for 2-3 h and put it in the desiccant jar until the temperature of the container is equal to the room temperature and then weigh. Repeat the procedure until the weight is constant.
2. 0.2 g of sample was put into the aluminum cup and dried at 105 °C for 4-5 h. Remove from the desiccant jar and weigh the cup with the sample and then put it back into the incubator. This process was repeated until the weight is constant and calculate the total amount of solid with the following equation:

Total solid percentage = (Weight of sample after drying – cup weight/ Weight of sample before drying – empty aluminum cup weight) * 100

B.9 Preparation of a solution of maltodextrin encapsulation with a concentration of 45% w/v in the marigold flower extract

Maltodextrin solution with a concentration of 45% w/v at the ratio of extracts to encapsulants 1:1

1. Weigh maltodextrin DE 10-12 225 g and distilled water 500 mL dissolved using an agitator (SCILODEX, SCI550-S, USA) for 30 min to obtain a 45 % w/v maltodextrin solution.

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2. Using a mixer, combine 500 mL of pure marigold flower extract and 500 mL of 45 % w/v maltodextrin solution for 5 min. Then, for 10 min, homogenize with a high-speed blender (Ystral, X10, Germany).

Maltodextrin solution with a concentration of 45% w/v at the ratio of extract to encapsulants 1:2

1. Weigh maltodextrin DE 10-12 297 g and distilled water 660 mL treated with an agitator (SCILODEX, SCI550-S, USA) for 30 min to obtain a 45 % w/v maltodextrin solution.
2. In a mixer, combine 330 mL concentrated marigold flower extract and 660 mL maltodextrin solution at a 45 % w/v concentration for 5 min. Then, for 10 min, homogenize with a high-speed blender (Ystral, X10, Germany).

B.10 Preparation of a solution of 20% w/v concentration of Gam Arabic encapsulation in the marigold flower extract microcapsules

20% w/v concentration of gum Arabic solution at the ratio of the extract to encapsulant 1:2

1. Weigh 132 g of gum Arabic and 660 mL of distilled water and then dissolved with a mixer (SCILOGEX, SCI500-S, U.S.A) for 30 min to get 20% w/v concentration of gum Arabic solution.
2. Combine 330 mL of concentrated marigold flower extract with 660 mL of 20% w/v concentration of gum Arabic solution and then mix with the mixer for 5 min. After that, homogenize with a high-speed blender (Ystral, X10, Germany) for 15 min.

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20% w/v concentration of gum Arabic solution at the ratio of the extract to encapsulant 1:3

3. Weigh 150 g of gum Arabic and 750 mL of distilled water and then dissolved with a mixer (SCILOGEX, SCI500-S, U.S.A) for 30 min to get 20% w/v concentration of gum Arabic solution.
4. Combine 250 mL of concentrated marigold flower extract with 750 mL of 20% w/v concentration of gum Arabic solution and then mix with the mixer

for 5 min. After that, homogenize with a high-speed blender (Ystral, X10, Germany) for 15 min.

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