MICROENCAPSULATION AND SPRAY DRYING OF *ORTHOSIPHON STAMINUES*

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Thesis submitted in fulfillment
of the requirements for the award of the degree of
Bachelor of Chemical Engineering (Biotechnology)

Faculty of Chemical Engineering and Natural Resources
UNIVERSITI MALAYSIA PAHANG

FEBRUARY 2013

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LIST OF ABBREVIATIONS

DPPH	1,1-diphenyl-2-picrylhydrazyl
HPLC	High performance liquid chromatography
MD	Maltodextrin
UPLC	Ultra performance liquid chromatography
UV-VIS	Ultraviolet-visible spectrophotometer
WPI	Whey protein isolate

LIST OF SYMBOLS

g	gram
mg	milligram
µg	microgram
ml	milliliter
L	Liter
K	Kelvin
℃	Degree of Celcius
hz	Hertz
nm	nanometer
mm	milimeter
µm	micrometer

PEMIKROKAPSULAN DAN PENGERINGAN SEMBURAN *ORTHOSIPHON STAMINEUS*

ABSTRAK

Orthosiphon Stamineus adalah tergolong dalam keluarga Lamiaceae dan dikenali sebagai misai kucing. Daun *Orthosiphon Stamineus* mengandungi pelbagai sebatian bioaktif yang berguna (flavonoid) dengan ciri-ciri terapeutiknya yang baik. Walaupun demikian, kandungan flavonoid dalam *Orthosiphon Stamineus* akan berkurangan apabila terdedah kepada suhu yang tinggi semasa semburan pengeringan untuk menghasilkan serbuk *Orthosiphon Stamineus*. Pemikrokapsulan adalah salah satu kaedah untuk melindungi kandungan flavonoid dalam *Ortosiphon Stamineus* daripada degradasi. Penyelidikan ini dijalankan dengan objektif untuk meminimumkan degradasi sebatian *Orthosiphon stamineus* menggunakan kaedah semburan pengeringan selain merumuskan sejenis konsep mikroenkapsulan yang organic dan stabil dengan tujuan untuk memelihara sebatian bioaktif dalam *Orthosiphon Stamineus* daripada degradasi. Penyelidikan ini dijalankan dengan mengekstrakan daun *Orthosiphon Stamineus* dengan 70% methanol. Kepekatan ejen pengkapsulan, maltodekstrin (MD) dan protein whey isolasi (WPI) yang berbeza telah digunakan dalam proses pengeringan. Kandungan kelembapan, ujian kandungan pepejal, ujian kandungan flavonoid, dan ujian UPLC dianalisis untuk mengkaji degradasi kandungan flavanoid dan kandungan bioaktif dalam *Orthosiphon Stamineus.* Melalui kajian tersebut, campuran WPI 5% dengan larutan ekstrak mencatatkan kandungan asid rosmarinic yang tinggi iaitu 2.48 mg / ml dan 0.00714 mg / ml untuk sinensetin manakala MD 5% pula mencatatkan jumlah asid rosmarinic 2.38 mg / ml dan 0.00719 mg / ml untuk sinensetin.. Walau bagaimanapun, 5% WPI lebih baik berbanding dengan maltodekstrin 5% dengan teknik pemikrokapsulannya yang cekap dengan pengekalan asid Rosmarinic dan Sinensetin sebanyak 98.7% dan 92.5% berbanding dengan 5% maltodekstrin yang mencatatkan pengekalan rosmarinic yang rendah (94.8%) dan pengekalan Sinensetin sebanyak 93.1%.Justeru itu, *Orthosiphon Stamineus* dengan penggunaan kepekatan 5% WPI berkesan untuk melindungi komponen bioaktif dalam *Orthosiphon Stamineus* daripada degradasi ketika pengeringan.

MICROENCAPSULATION AND SPRAY DRYING OF *ORTHOSIPHON STAMINUES*

ABSTRACT

The leaves of *Orthosiphon Stamineus* contained many useful bioactive compounds (flavonoid) that exhibit excellent therapeutic properties. However, the flavonoid contain is prone to degradation when expose to high temperature during spray drying to produce *Orthosiphon Stamineus* in powder form. Microencapsulation is one of the methods to preserve the flavonoids from degrading. This research is undertaken to achieve the objectives to minimize the thermal degradation of *Orthosiphon Stamineus* compound using spray drying method and microencapsulation method besides formulating a stable organically derived micorencapsulat to preserve the bioactive compounds from degrading. This research was conducted by extracting the bioactive compound from *Orthosiphon Stamineus* leaves using 70% methanol. Different concentration of the encapsulating agent, maltodextrin (MD) and whey protein isolate (WPI) were studied. The moisture, total solid and total flavonoid content test besides UPLC were analyzed. From the result obtained, 5% WPI added to the extract solution recorded the highest concentration of rosmarinic acid with amount 2.48 mg/ml and 0.00714 mg/ml. of sinensetin whereas 5% MD recorded high concentration of rosmarinic acid with amount 2.38 mg/ml and 0.00719 mg/ml sinensetin. However, 5% of WPI was a better encapsulating agent when compare to maltodextrin with its capability to achieve highest rosmarinic acid and sinensetin retention at 98.7% and 92.5% respectively. On the other hand, usage of 5% maltodextrin recorded lower rosmarinic retention (94.8%) and sinensetin (93.1%). Thus, minimize degradation of the useful bioactive components in *Orthosiphon Stamineus* were able to be prevented by applying the spray drying method using 5% microencapsulating agent whey protein isolate.

CHAPTER 1

INTRODUCTION

1.1 Background of Proposed Study

Orthosiphon Stamineus, the perennial herb plant which belongs to the *Lamiaceae* family is locally known as "Misai Kucing' or cats whiskers due to its pale purple flowers with long wispy stamens shaped like cats whiskers (Indubala and Ng, 2000). The leaves of *Orthosiphon Stamineus* are discovered to be the most important parts of the plant and traditionally used as treatment for diuresis, rheumatism, diabetis, urinarylithiasis, oedema, eruptive fever, influenza, hepatitis, jaundice, biliary lithiasis, and hypertension (Sumaryono et al., 1991, Tezuka et al., 2000 and Shibuya et al., 1999).

Orthosiphon Stamineus is used as a medicinal plant because of its diuretic, anti-fungal and bacteriostatic properties of leaves (Olah et al., 2003). *Orthosiphon Stamineus* is widely used in South East Asia as the tea prepared from the extraction of the leaves that taken as beverage to improve health and to treat illness such as

kidney disease, bladder inflammation, gout and diabetes (Hegnauer, 1996, and Wagner, 1982). Besides, the leaves of *Orthosiphon Stamineus* are also used as remedy for urinary system ailments in Malaysia (Liu et al., 2003).

Orthosiphon Stamineus is a medical plant native to tropical Asia that contains chemical components such as terpenoids (diterpenes and triterpenes), polyphenols (lipophilic flavonoids and phenolic acids), and sterols (Tezuka et al., 2000). The previous scientific studies revealed that extracts of *Orthosiphon Stamineus* contained many useful bioactive compounds that exhibit excellent antibacterial, antifungal, antimicrobial and antitumor properties (Saravanan et al., 2006 and Hossain et al., 2001).

The therapeutics effects of this herb are mainly due to its polyphenol (flavonoid), which has the effect on enzyme inhibition and antioxidant activity. There are six flavonoid compounds isolated from the leaves of this medicinal plant which are eupatorin, sinensetin, 5-hydroxy-6,7,30,40-tetramethoxyflavone, salvigenin, 6-hydroxy-5,7,40-trimethoxyflavone and 5,6,7,30-tetramethoxy-40-hydroxy-8-C-prenylflavone (Hossain, and Rahman, 2011). This flavonoids content and the antioxidant properties of *Orthosiphon Stamineus* have to be protected from any degradation or loss during industrial processes to ensure that the highest content of this therapeutic components in this medical herbs can be preserved from degrading when pack in tablet or capsule form before consumed by the consumers.

Microencapsulation is one of the methods to preserve the antioxidant properties and the flavonoid content in *Orthosiphon Stamineus*. Microencapsulation technique is an effective way to protect the food ingredients from being deteriorated or having any volatile losses. Microencapsulation is defined as a process in which tiny particles or droplets are surrounded by a coating, or embedded in a homogeneous or heterogeneous matrix which providing a physical barrier between the core compound and the other components of the product. The protective mechanism works by forming a membrane wall that encloses the droplets or particles of the encapsulated material (Gharsallaoui, Roudaut, Chambin, Voilley and Saurel, 2007).

There are various kinds of microencapsulation techniques which applied to microencapsulate food ingredients in food industries such as solvent dispersion, phase separation, co-crystallization, interfacial polymerization, liposome entrapment, molecular inclusion, spray cooling, freeze drying, spray drying and many other methods (Desai and Park, 2005; Gibbs, Kermasha, Alli and Mulligan, 1999; Gouin, 2004; King, 1995; Shahidi and Han, 1993). Different particles can be obtained due to the different type of microencapsulation technique used and the different physic-chemical properties of the core.

Among all those microencapsulation techniques, spray drying is the most common method and the cheapest technique used to produce microencapsulated food material. Microencapsulation by spray drying has been successfully used in the food industry for several decades (Goiun, 2004), and this process is one of the oldest

encapsulation methods used since 1930s for preparing the first encapsulated flavors by using gum acacia as the wall material (Shahidi and Han, 1993).

Spray drying is the most popular method used in food industry since the equipment is readily available in the market and the production costs are lower for using spray drying than most of the other methods. Desobry, Netto and Labuza stated that the cost needed for spray drying is 30 to 50 times cheaper than freeze drying method. Spray drying is not only efficiently forming micro particles but also easy to industrialize and allow for continuous production (Su et al, 2008). Spray drying is stated to be a beneficial technique for turning liquids into solid powder form that improves the shelf life of the product, stabilizing the product besides of making it easier to be handled (Porrarud, and Pranee, 2010).

Microencapsulation efficiency and microcapsules stability during storage are largely dependent on the type of wall material or coating agent used. Carbohydrates such as maltodextrins are usually used in microencapsulation of food ingredients. Maltodextrins are mainly used to reduce the stickiness and agglomeration problems during storage besides improving the product stability (Bhandari et al., 1993). However, maltodextrin have poor interfacial properties and must be modified to improve their surface activity. In contrast, protein such as whey protein isolates has an amphiphilic character which having the physicochemical and functional properties that require for encapsulating hydrophobic core materials (Gharsallaoui, Roudaut, Chambin, Voilley, and Saurel, 2007). Thus, in order to obtain the best encapsulating agent, the encapsulating agents, maltodextrin and whey protein isolate with different

concentration were used. However, this research has not been developed in previous studies on *Orthosiphon Stamineus.*

1.2 Problem Statement

Orthosiphon Stamineus is the medical plant that has widely taken as beverage to treat different kinds of illness and may have greater market potential. There are high demands in the market for obtaining this medical plant due to its potential uses in pharmaceutical industry, in medical as well as in the food processing. Therefore, this is the main reason this research is done with the purpose to fulfill the demand and urgency from the consumers or from various industries to obtain the high quality neutraceutical product of *Orthosiphon Stamineus*.

Nevertheless, the product in solid powder form is desirable for easier handling during storage and transportation besides having a longer shelf life. A solid pharmaceutical dosage in the form of tablets is also desirable for convenience of administration by consumers. However, the method to produce *Orthosiphon Stamineus* capsule with efficient preservation of bioactive compounds has not been established.

Due to the benefits that this medical capsule brings to the consumers, research of this study will be done in order to obtain the *Orthosiphon Stamineus* in capsules form by using spray drying method. However, the bioactive compounds in *Orthosiphon Stamineus* tend to suffer from a thermal degradation process when

exposed to high temperature during spray drying process. The thermal degradation is undesirable because the degraded product is of low nutritional value and consequently, hampers the intention to produce a nutraceutical product. Thus, microencapsulation technique has to be applied without degrading the flavonoid content and the antioxidant activity in the product.

Furthermore, a very limited study concerning the microencapsulation of flavnonoids from *Orthosiphon Stamineus* extract is available in the literature. The drying process and the encapsulating conditions (concentration of encapsulating agent needed) for these products have not been determined for preservation purposes since none of the research had ever been done on this method to obtain *Orthosiphon Stamineus*. This research had be conducted to determine the the concentration of the encapsulating agent suitable to be used to preserve the bioactive components in the spray drying process to obtain the highest quality of *Orthosiphon Stamineu*s tablet.

1.3 Research Objectives

There are two main objectives to be conducted in this research:

1. To minimize the thermal degradation of *Orthosiphon Stamineus* compound using spray drying method and microencapsulation method.
2. To formulate a stable organically derived microencapsulant to preserve the bioactive compounds from degrading.

1.4 Scope of proposed study

In this research, the extracted leaves of *Orthosiphon Stamineus* is dry using spray drying method to produce the herbal powder with high contain of bioactive compounds, phenolic, flavonoids and antioxidant activities. The purposes to conduct this study are to prepare the powders using spray drying method and to evaluate the effects of drying on physico-chemical properties especially on the type and the concentration of the encapsulating agents, maltodextrin and whey protein that had been applied on this process. The moisture content, the determination of the structure of the powders tests and the level of nutrient retention of *Orthosiphon Stamineus* had been investigated when spray drying method is applied. Spray dryer is one of the highest nutrient retention dryer compare to freeze drying, foam mat drying and tunnel drying. (Hchopra, 2005)

The scopes of the study are stated as:

1. To develop spray drying process to convert the extract *Orthosiphon Stamineus* into powder solid form.
2. To perform microencapsulation technique by encapsulating the bioactive compounds in *Orthosiphon Stamineus* extract using microencapsulating agent such as whey protein isolate (WPI) and maltodextrin (MD) during spray drying.
3. To develop spray drying process to extract *Orthosiphon Stamineus* using 70% methanol assisted with ultrasonic extraction method.

1.5 Significance of research

This research conducted is focusing on preserving the bioactive compounds in *Orthosiphon Stamineus* from being degrading when expose to high temperature during spray drying process. The application of microencapsulation technique will be applied to solve this problem with the purpose to produce capsule with high nutrientional value for the consumers. Thus, this will increase the health of the consumers for taking the nutraceutical capsule with the highest quantity and quality of bioactive compounds that has the therapeutic effect to treat illness.

Besides, it is crucial to dry Orthosiphon Stamineus to produce a stable, a long storage life and easily handled form of the powder which easily being transported and exported to local industries or various other countries. Dried powders of Orthosiphon Stamineus will be used mainly as convenience products with long storage life at ordinary temperatures. This eventually will increase the availability of high quality nutraceutical product no matter to industries dealing with pharmaceutical or food processing. For pharmaceutical field, powder form of Orthosiphon Stamineus is packed into capsule or tablets as supplements or medicine to treat kidney diseases, bladder inflammation, gout and diabetes.

In addition, the production of Orthosiphon Stamineus powder will be beneficial for the government in increasing the economic through exportation. *Orthosiphon Stamineus* is in great demand in the market especially from various industries since this herbal plant is taken as beverage or as diuretic tea to treat

different kinds of diseases. Since there are high demands in various industries on the product of *Orthosiphon Stamineus*, this research is done to fulfill the need of all the consumers as well as to ensure that the products produced are high with nutritional value and containing high quality of bioactive compounds which beneficial for the health of the consumers.

CHAPTER 2

LITERATURE REVIEW

2.1 *Orthosiphon Stamineus*

2.1.1 Characteristics of *Orthosiphon Stamineus*

Orthosiphon Stamineus is an herbal plant which named as Cat's Whiskers or "Misai kucing" by the Malaysian due to its long wispy stamens shaped like cat whiskers (Figure 2.1) (Indubala and Ng, 2000). *Orthosiphon Stamineus* (OS), the native plant of South East Asia are also known as poosai meesai by the locals or named as Java Tea in Indonesia. It is a well known perennial herb from the family of L*amiaceae* (Wiart, 2000). *Orthosiphon Stamineus* comes from little oval, green leaves that are finely toothed and rolled like ordinary tea (Figure 2.1). *Orthosiphon Stamineus* is widely found growing in tropical areas and turns to become one of the important medicinal plants in Southeast Asia that can be consumed as herbal tea (Doan et al., 1992; Beaux et al., 1999; Olah et al., 2003).

Orthosiphon Stamineus had great potential for commercialization in Malaysia due to its easy cultivation and its validated health benefits in treating the ailments associated with the kidney and urinary system (Jagarath and Ng, 2000). *Orthosiphon Stamineus* (OS) had been one of the most popular medicinal plants in Southeast Asia since it has been used for treating a variety of diseases related to the urinary tract, diabetes mellitus, hypertension, tonsillitis, rheumatism and menstrual disorders (Englert and Harnischfeger, 1992; Beaux et al., 1997; Matsubara et al., 1999; Sriplang et al., 2007; Awale et al., 2003). *Orthosiphon Stamineus* have been reported to possess anti inflammatory (Masuda et al., 1992), anti hypertensive (Ohasi et al., 2000) hypoglycaemic or anti-hyperglycaemic activities (Mariam et al., 1999) and having diuretic effect (Galyuteva et al., 1990; Dona et al., 1992).

Besides, this plant has been used as traditional medicine for centuries to improve health or to treat kidney diseases, bladder inflammation, gout and diabetes (Wagner, 1982; Akowuah et al., 2005). This medical plant is also known worldwide and widely used in India for treatment of eruptive fever, urinarylithiasis, edema, hepatitis and jaundice (Mahesware, Maryammal and Venkatanarayanan, 2008). The popularity of these plants is due to its extracts for medicinal purposes or as traditional drinks. *Orthosiphon Stamineus* extracts have been proven of containing anti-diabetic and lipid which able to minimize the diabetes effects in diabetic rats (Mencherini et al., 2007; Mencherini et al., 2010; Kim et al., 2006; Park et al., 2008).

2.1.2 *Orthosiphon Stamineus* leaves

Orthosiphon Stamineus leaves are identified as the most important parts of this medical plant that containing many useful bioactive compounds which showing the therapeutic properties such as antibacterial, anti-inflammatory, antifungal, antimicrobial antitumor anti-allergic and antithrombotic actions to prevent from cardiovascular diseases and cancer (Hossain and Rahman, 2011). *Orthosiphon Stamineus* (OS) leaves are usually extracted and used for producing diuretic tea to treat illnesss such as kidney and bladder inflammation (PT EisaiIndonesia, 1995). *Orthosiphon Stamineus* leaves are also used traditionally for the treatment of diuresis, rheumatism, diabetis, oedema, eruptive fever, jaundice, hepatitis, urinarylithiasis,influenza and hypertension (Tezuka et al, 2000).

Figure 2.1 The leaves and the flower of *Orthosiphon Staminues.*