THERMAL DEGRADATION KINETICS OF BIOFLAVONOIDS FROM *ORTHOSIPHON STAMINEUS*

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LIST OF ABBREVIATIONS

USD	United State Dollor
SEN	Sinensetin
EUP	Eupatorin
TMF	30-hydroxy-5,6,7,40-tetramethoxyflavone
RA	Rosmarinic Acid
HPLC	High Performance Liquid Chromatography
UPLC	Ultra Performance Liquid Chromatography
H_2O	Water
UV-Vis	Ultraviolet-Visible
$NaNO_2$	Sodium Nitrite
$AlCl_3$	Aluminium Chloride
NaOH	Sodium Hydroxide
Na_2CO_3	Sodium Carbonate
GAE	Gallic Acid Equivalent
QE	Quercetin
DW	distilled water
DPPH	2,2-diphenyl-1-picrylhydrazyl
TPC	Total phenolic content
TFC	Total Flavonoid Content
MS	Microsoft
TFA	Trifluroacetic Acid
ACN	Acetonitrile

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LIST OF SYMBOLS

n.d.	no date
%	Percentage
m	Meter
cm	Centimeter
&	and
℃	Degree Celcius
h	Hours
C_t	Concentration at time t
C_0	Initial Concentration
k/k_0	Reaction rate constant
A_0	Pre-exponential Factor
E_a	Activation Energy of the reaction
R	Gas Cosntant
T	Absolute Temperature
t	time/holding time
n	order of reaction
k_{ref}	Reference rate constant
T_{ref}	Reference Temperature
mm	milimeter
i.d.	inner diameter
µm	micrometer
µl	microliter
ml/min	mililiter per minute
nm	nanometer
g	gram
ml	milimeter
mg/ml	milligram per mililiter
mg/g	milligram per gram
min^{-1}	per minute
min	minute
cal M^{-1}	catalyst per mol

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cal $M^{-1}K^{-1}$	catalyst per mol per Kelvin
K	Kelvin

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DEGRADASI KINETIK OF BIOFLAVANOIDS DARI *ORTHOSIPHON STAMINEUS*

ABSTRAK

Orthosiphon stamineus juga dikenali sebagai Misai Kucing digunakan untuk meningkatkan kesihatan umum dan rawatan penyakit. Walaubagaimanapun, proses penyediaan dan penyimpanan produk menyebabkan degradasi dan mengurangkan nutrien produk akhir. Objektif kajian ini adalah untuk menentukan pelarut yang sesuai untuk pengekstrakan *Orthosiphon stamineus* dan menjelaskan kinetik degradasi bagi bioflavonoids menggunakan persamaan Hinrichs Rademacher dan Arrhenius untuk mendapatkan parameter kinetic degradasi. Penyelidikan ini melindungi bereksperimen pelarut yang berlainan semasa pengekstrakan dan degradasi. Sampel akan dianalisis oleh UPLC dan UV-Vis Spectrophotometer. Selepas itu, eksperimen akan terus dengan mencari pola degradasi pada suhu 60, 90 dan 120 ℃ dan linkungan masa 0, 6, 12, 18, 23, 33 minit. Kemudian, eskperi data dimodel dalam persamaan Hinrichs-Rademacher dan Arrhenius. Hasil daripada pengekstrakan pelarut yang berlainan menunjukkan pelarut yang sesuai digunakan adalah 70% metanol. Komponen aktif dalam *Orthosiphon stamineus* dimusnah semasa suhu dan linkungan masa eskperimen meningkat. Eupatorin menunjukkan kemerosotan yang besar manakala Asid Rosmarinic dan Sinensetin menunjukkan kemerosotan yang kecil. Eskperi data yang diperolehi menunjukkan sesuai dipakai dalam kedua-dua persamaan menggunakan “Microsoft Excel Solver” dan nilai kadar degradasi yang dapat dari penyelesaian persamaan menunjukkan meningkat semasa suhu pemanasan dan linkungan masa eskperimen meningkat.

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THERMAL DEGRADATION KINETICS OF BIOFLAVANOIDS FROM *ORTHOSIPHON STAMINEUS*

ABSTRACT

Orthosiphon stamineus which also known as Misai Kucing are traditional used for improve general health and treatment of disease. However, degradation of bioflavonoids occurred and the nutritional value of the final product decrease while product processing and storage. The objectives of this research are to determine the suitable solvent for *Orthosiphon stamineus* extraction and to elucidate the thermal degradation kinetics of bioflavonoids content from *Orthosiphon stamineus* using Hinrichs-Rademacher and Arrhenius equation to obtain the kinetic parameters.. The research focused on experimenting different solvent type for the extraction and thermal degradation of bioflavonoids *Orthosiphon stamineus* which analyzed by UPLC and UV-Vis Spectrophotometer. The experiment will be conducted to find the degradation pattern for 60, 90 and 120℃ and 0, 6, 12, 18, 23, 33 minutes. Then, model the results with Hinrichs-Rademacher degradation kinetics approach and Arrhenius equation. The result of solvent extraction showed the suitable solvent used was 70% methanol. The active components showed degraded while temperature and residence time increased. Eupatorin showed larger degradation while Rosmarinic Acid and Sinensetin showed less degradation. The experiment data obtained showed fitted well by both equation using Microsoft Excel Solver and degradation rate constant showed increasing while heating temperature and residence time increased.

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CHAPTER 1

INTRODUCTION

1.1 Background of Research

Recently, both Eastern and Western cultures have paid highly attention on herbal products due to its' traditionally used as therapeutic agents and dietary supplements. This herbal market normally includes the processed herbs that used as food or food additives, cosmetic ingredients and herbal medicines (Aziz et al., n.d.). A World Health Organization survey found out that 70 -80% of the world population are relies on herbal-based traditional medicine for primary healthcare (Chan, 2003, as cited in Muhammad et al., 2010). The market for herbal and natural products in Malaysia has been currently estimated to be worth USD 1.4 million (Jamia, 2006, as cited in Muhammad et al., 2010). Since the market demand of the herbal product is increasing around the world, it is important to have more research on this field.

Orthosiphon stamineus also known as "Cats whiskers" or "Misai Kuching" (in Malay) is a type of very common herbs plant which can easily found in Malaysia.

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Since 1930s, *Orthosiphon stamineus* has gained the interest of many researchers from different parts of the world for phytochemical and pharmacological studies (Arafat et al., 2008). *Orthosiphon stamineus* belongs to the Lamiaceae family and were reported to contain several chemically active constituents, such as terpenoids (diterpenes and triterpenes), polyphenols (lipophilic flavonoids and phenolic acids), and sterols by Tezuka et al., (2000, as cited in Akowuah et al., 2005). Different bioactive compounds content in *Orthosiphon stamineus* leading to various activities such as antibacterial, antifungal, antimicrobial and antitumor (Hossain et al., 2008). There are three main polymethoxylated flavones in *Orthosiphon stamineus* leaves, sinensetin (SEN), eupatorin (EUP), 30-hydroxy-5,6,7,40-tetramethoxyflavone (TMF) and the major phenolic acid, rosmarinic acid (RA) (Akowuah et al., 2005).

Orthosiphon stamineus is commonly become a traditional medicine used to improve general health and treatment of kidney diseases such as bladder inflammation, gout, diabetes, rheumatism, tonsillitis and menstrual disorder (Wagner, 1982; Akowuah et al., 2005; & Awale et al., 2003, as cited in Adam et al., 2009). Since there are many advantages, peoples like to produce food supplement product by using this plant for health improvement. This was proved by the statement of previous researchers about *Orthosiphon stamineus* are widely used in South East Asia which prepared from the extraction of the leaves into tea and taken as beverage (Wagner, 1982).

Extraction is an important contributing element for the development of processed foods and nutraceutical food ingredients. Extraction is a separation process that functions as to separate a solute from one phase into another. For the

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nutraceutical products produce from *Orthosiphon stamineus*, the bioactive compounds are extracted out from the plant and this extraction for the leaves powder of *Orthosiphon stamineus* can be performed by using different kind of extraction methods such as maceration extraction, ultrasound extraction and microwave assisted extraction (Akowuah et al., 2005). Different type of solvents also used for example water, methanol, 50% methanol, 70% methanol and 50% propanol (Trusheva et al., 2007).

However, during the final production process of those food supplement from *Orthosiphon stamineus*, it consist the step of drying which involved higher temperature (Silva et al., 2011). According to Miranda (2010), the high temperature of operating condition reduces the quality of the final product due to the physical, chemical, organoleptic and nutritional changes. The operation condition of hot drying process for solution to produce final product in a dry powder form is not suitable and unfavorable in preserving the bioactive compound. The bioactive compounds contain in *Orthosiphon stamineu*s suffer from degradation problem (Hung et al., 2012).

The thermal degradation of the bioflavonoids reduced the nutritional value of these products and this had become a major problem for the industry (Cisse et al., 2009). In order to predict the quality loss of the product during storage and thermal processing of the product, it is vital to know its kinetic degradation mechanism. Therefore, this research is important to identify the effect of different solvents during the extraction of *Orthosiphon stamineus* and to elucidate the thermal degradation kinetics of bioflavonoids content from *Orthosiphon stamineus* using

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Hinrichs-Rademacher degradation kinetics approach and Arrhenius type kinetics to determine the kinetic parameter of the degradation of *Orthosiphon stamineus.*

1.2 Problem Statement

Orthosiphon stamineus that content a bioactive compounds-flavonoid is proved as a very useful plant which not only used to improve general health and for treatment of diseases and also bringing beneficial to the plant itself. These flavonoids are normally found in most of the plants and also playing a significant role in the growth, reproduction and defence mechanism in the plant (Routray & Orsat, 2012). Besides that, the products of *Orthosiphon stamineus* are previously produced in the form of tea as a beverage to improve general health and for the treatment of kidney disorders, bladder inflammation, gout and diabetes (Hegnauer, 1996, & Wagner, 1982). The market demand of this food supplement product is increasing around the whole world with an average annual growth rate of 15 to 20% (Merican, 2003 & Exim Bank 2003, as cited in Aziz et al., n.d.). Therefore, it is worth for carrying out this research for the production of a high quality of nutraceutical product from *Orthosiphon stamineus* due to the highly demand from consumers.

For the production of this nutraceutical product from *Orthosiphon stamineus*, it is desire to produce it in powder form in order for convenience of consumers, easier handling during storage and transportation. The process of producing such a powder form product usually involved drying process. However, the drying process by high temperature in product preparation causes the degradation of bioflavonoids

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occurred and decrease the nutritional value of the final product (Miranda, 2010). As a result, the low quality of nutritional values of the product limits the commercialization of this health product.

The degradation amount and the degradation trends during the production of these nutraceutical products from *Orthosiphon stamineus* is still an unknown. Therefore, it is relevant to carry out this research to determine the degradation pattern or trends of the *Orthosiphon stamineus* and elucidate kinetics parameters of flavonoids from *Orthosiphon stamineus* using Hinrichs-Rademacher degradation kinetics approach and Arrhenius type kinetics equation so that can be used for further study on the enthalpy and gibbs energy so that it possible to predict the food quality loss.

1.3 Research Objectives

The research is done to achieve the following objectives:

(i) To determine the best solvent that used for extraction of *Orthosiphon stamineus* by Ultrasonic Extraction.

(ii) To elucidate the thermal degradation kinetics of bioflavonoids content from *Orthosiphon stamineus* using Hinrichs-Rademacher degradation kinetics approach and Arrhenius type kinetics equation.

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1.4 Scope of Research

To achieve the research objectives, the scopes of this research are listed as below:

(i) Ultrasonic extraction of *Orthosiphon stamineus* by using different type of solvents- pure methanol, pure propanol, pure water, 50% Methanol and 70% Methanol.

(ii) Study the degradation pattern for various temperature and residence time.

(iii) Applying Hinrichs-Rademacher degradation kinetics approach and Arrhenius equation used to elucidate the kinetics parameter of flavonoid.

1.5 Significance of Research

The research of determination of degradation mechanism of bioflavonoids from *Orthosiphon stamineus* is significant to be study to able the prediction of product quality loss during the storage and thermal processing of the product. This research on kinetic degradation of bioflavonoids from *Orthosiphon stamineus* is not studied yet by other researcher.

From this research, by elucidate the degradation kinetic of bioflavonoids from *Orthosiphon stamineus*, kinetic parameters of the thermal degradation of *Orthosiphon stamineus* can be found. Further study can be extended to determine the

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thermodynamics parameter i.e. enthalpy and gibbs energy, which can be further used for predict the quality loss of the product during the thermal processing. The nutritional value of the final product can be maintained and being produced in good quality by operates at certain operation which will give less degradation of the product. The productions of good quality products will bring benefits to the society and nutraceutical industry. Hence, it will help to increase the applicability and commercial values of these nutraceutical product and even more strengthen the research or production by nutraceutical industry.

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CHAPTER 2

LITERATURE REVIEW

2.1 *Orthosiphon staminues*

Figure 2.1 The leaves and the flower of *Orthosiphon staminues*

Orthosiphon stamineus (Misai Kucing) is one of the most popular medicinal herbs found in South East Asia. Figure 2.1 shows the appearance of the leaves and the flower of *Orthosiphon staminues. Orthosiphon staminues* is a perennial herb with 0.3 - 1 m high and having stem 4 - angled. The leaves of *Orthosiphon staminues* are simple, opposite, ovate-oblong-lanceolate, elliptic or rhomboid, 2 - 4 cm wide, 4 - 7

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cm long, white or pale lilac flowers and stamens exceed more than 2 cm from the corolla-tube (Wiart, 2000, as cited in Adam et al., 2009). According to Ahamed and Abdul (2010), the appearance and characteristic of the plant, *Orthosiphon stamineus* is looks similar to peppermint and it has dry, salty and bitter taste.

Orthosiphon stamineus is a very common plant which can be grown anywhere in Malaysia and it brings benefits towards humans' health. Therefore, *Orthosiphon stamineus* usually been produced into a supplement products in nutraceutical industry for provides health benefits including prevention and treatment of diseases.

2.2 Uses and Advantages of *Orthosiphon stamineus*

Orthosiphon stamineus is a kind of herbs which has been widely used as a food supplement for maintain good health and treatment of diseases. It brings beneficial on diuresis, treat rheumatism, diabetes, urinary lithiasis, oedema, eruptive fever, influenza, hepatitis, jaundice, biliary lithiasis and hypertension (Hossain & Rahman, 2011). Wagner (1982) stated that *Orthosiphon stamineus* is always performed in the form of tea and taken as beverage for the improvement of health and treatment of diseases.

According to research done by Sriplang et al. (2007), *Orthosiphon stamineus* aqueous extract is effective for alleviating hyperglycemia and improving lipid profile in diabetic rats. During their study, extract was given to normal and diabetic rats and

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it is significantly reduced plasma glucose concentration in diabetic rats at days 7 and 14. Therefore, from the testing on rats, it is clear that *Orthosiphon stamineus* can be used to help in treating of diabetic.

Next, *Orthosiphon stamineus* can also be used as a remedy for kidney stones. There is an experiment tested on rats showed the benefits of taking product of *Orthosiphon stamineus* in kidney disorder. Kidney stone is a condition with the deposition of precipitates of calcium oxalate crystals and is difficult to dissolved and expelled successfully (Ahamed & Abdul 2010). They also mentioned that, the disease can be caused by abnormality in uric acid metabolism. In the study of Arafat et al. (2008), the extract of *Orthosiphon stamineus* used to test on a rate and it showed in reducing uric acid level. The use of *Orthosiphon stamineus* will enhance the activity of adenosine A receptor antagonists and stimulate the kidney for excessive flow of urine and thus sodium and other ions excretion (Ahamed & Abdul, 2010).

2.3 Bioactive Compounds in *Orthosiphon stamineus*

Orthosiphon stamineus is the plant which belongs to the Lamiaceae family (Adam et al., 2009). The study of Tezuka et al., (2000, as cited in Akowuah et al., 2005) found out that the plant contain many chemically active constituents, such as terpenoids (diterpenes and triterpenes), polyphenols (lipophilic flavonoids and phenolic acids), and sterols. Among the listed active constituents, the most important classes of compounds is phenolic group (Akowuah, 2004).

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The phenolic compounds that have been isolated from *Orthosiphon stamineus* are such as lipophilic flavones, caffeic acid derivatives (rosmarinic acid and 2,3-dicaffeoyltartaric acid) (Sumaryono et al., 1991; Akowuah et al., 2004), sinensetin and methoxy flavones (Pietta et al., 1991), diterpenes, betulinic acid, oleanolic acid and sitosterol (Tezuka et al., 2000). The compounds that possess the potential therapeutic properties are flavonoids and caffeic acid (Loon et al., 2005).

2.3.1 Flavonoids or Bioflavonoids (Bioactive Compound in *Orthosiphon stamineus*)

Flavonoids are a type of low molecular weight polyphenolic secondary metabolic compounds which universally distributed in the green plant and located in cell vacuoles (Samanta et al., 2011). Flavonoids that contain in the plants not only possess the potential therapeutic properties for improvement of health and treatment of diseases but also play an important role to the plant itself by protecting and bringing benefits towards the plants. According to Samanta et al. (2011), flavonoids that distributed in the plants will help to absorb the harmful UV radiation which induced cellular damage. Besides that, flavonoids are bioactive and influence the transport of the plant hormone, auxin (Buer et al., 2010) and also responsible for the colors of flowers and protecting the plant from microbes and insects (Griesbach, 2005; Bohm, 1998; Yao et al., 2004).

Flavonoid in the plants can be used to act as antioxidants and their role in the prevention of coronary heart diseases are the most important actions of these compounds (Patel, 2008). From the review journal of Routray and Orsat (2012), there are many classes of flavonoids in food or plants such as flavones, isoflavones,

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flavanones, flavandiols, anthocyanins, proanthocyanidins, catechin and their chemical structures of the classes of flavonoids are shown in Figure 2.2. In *Orthosiphon stamineus* leaves, there are three main polymethoxylated flavones which are sinensetin (SEN), eupatorin (EUP), 30-hydroxy-5,6,7,40-tetramethoxyflavone (TMF) and the major phenolic acid, rosmarinic acid (RA) (Figure 2.3) (Akowuah et al., 2005). Difference bioactive compounds content in *Orthosiphon stamineus* leading to various activities such as antibacterial, antifungal, antimicrobial and antitumor (Hossain et al., 2008). These activities will help to maintain the general health of the people.

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