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EFFECT OF TEA EXTRACT ANTIOXIDANT ACTIVITY ON INHIBITION OF MELANOSIS AND LIPID OXIDATION IN SHRIMP

TAN CHIW LING

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF AGRICULTURE SCIENCE WITH HONOURS

CROP PRODUCTION PROGRAMME
FACULTY OF SUSTAINABLE AGRICULTURE
UNIVERSITI MALAYSIA SABAH
2015

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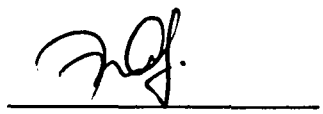

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ABSTRACT

This study was conducted to investigate the effects of farming practices on tea polyphenol content and antioxidant activity. Comparison was also made between the extraction methods (rotary evaporation and spray drying). Besides that, the effect of organic tea extract on melanosis and lipid oxidation inhibition in shrimp over 12 days of iced storage also evaluated. All the experiments were arranged in a completely randomized design (CRD). Results revealed that there was a significant difference between tea leaves types and extraction methods in terms of total phenolic content ($p<0.05$). Highest total phenolic content (252.13 ± 1.11 mg GAE/100 g) was observed in organic extract prepared by rotary evaporation. Meanwhile, present study showed that stronger antioxidant activity was observed in the organic extract prepared by rotary evaporation with the lowest IC_{50} value (0.027 ± 0.008 mg/mL), whereas conventional extract prepared by spray drying with high IC_{50} value (0.126 ± 0.005 mg/mL). The strong negative correlation ($r= -0.931$) was found between polyphenol content and IC_{50} ($p<0.05$). Among the treatment, shrimp treated with sodium metasulfide and 15 g L^{-1} of tea extract is effectively control the development of melanosis and lipid oxidation respectively. Results from current study suggested that total polyphenol content and antioxidant activity tends to be different with different farming practices and extraction method. Furthermore, use of tea extracts can be a safe alternative instead of using sodium metabisulfide in order to retard melanosis and lipid oxidation apart maximize the storage stability of shrimp.

KESAN AKTIVITI ANTIOKSIDAN TEH EKSTRAK UNTUK PERENCATAN MELANOSIS DAN PENGOKSIDAAN LEMAK DALAM UDANG

ABSTRAK

Tujuan kajian ini dijalankan untuk mengkaji kesan amalan pertanian (organik dan konvensional) kepada teh aktiviti antioksidan. Perbandingan juga dibuat antara teknik rotary evaporasi dan spray drying. Penggunaan teh ekstrak dalam perencatan melanosis serta pengoksidaan lemak pada udang juga dikajikan. Semua eksperimen dijalankan dengan rekabentuk rawak secara keseluruhan. Hasil kajian menunjukkan bahawa terdapat perbezaan yang signifikan antara daun teh organik dan konvensional dari segi jumlah kandungan fenolik ($p < 0.05$). Jumlah kandungan fenolik tertinggi (252.13 ± 1.11 mg GAE / 100 g) terdapat dalam ekstrak organik dengan rotary evaporasi. Sementara itu, kajian ini juga menunjukkan bahawa aktiviti antioksida tertinggi adalah ekstrak organik rotary evaporasi dengan IC 50 nilai yang paling rendah (0.027 ± 0.008 mg / mL), manakala ekstrak konvensional spary drying menunjukkan nilai IC50 tinggi (0.126 ± 0.005 mg / mL). Kolerasi yang tinggi (r= -0.931) *didapati antara kandungan polifenol dan* IC_{50} ($p<0.05$). *Antara rawatan, udang dirawat dengan natrium metasulfide dan 15* gL^{-1} *ekstrak teh adalah berkesan menghalang melanosis dan aktiviti pengoksidaan. Hasil daripada kajian ini telah menunjukkan bahawa jumlah kandungan polifenol teh berbeza dengan amalan pertanian yang berbeza. Justeru, penggunaan teh ekstrak merupakan alternatif yang selamat untuk melambatkan melanosis dan lemak pengoksidaan selain memaksimumkan kestabilan penyimpanan udang berbanding dengan penggunaan natrium metalsulfide.*

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LIST OF SYMBOLS, UNITS, AND ABBREVIATIONS

AA	Antioxidant Activity
Abs	Absorbance
Anova	Analysis of Variance
BHT	Butylated hydroxytoluence
CFS	Conventional Farming System
DPPH	2,2-diphenyl-1-picrylhydrazyl
EDTA	Ethylenediaminetetraacetic acid
FA	Fatty acid
FAO	Food and Agricultural Organization
FSA	Faculty of Sustainable Agriculture
FYP	Final Year Project
G	Gram
GAE	Gallic Acid Equivalent
HCI	Hydrochloric acid
HPLC	High Performance Liquid Chromatography
IC_{50}	Inhibitory concentration
km	Kilometer
L	Liter
MDA	Malonyldialdehyde
mg	Milligram
mm	Millimeter
mM	Millimolar
OFS	Organic Farming System
PPO	Polyphenoloxidase
R^2	Coefficient of determination
ROS	Reactive oxygen species
SPSS	Statistical Package for the Social Sciences
STP	Sabah Tea Plantation
TAC	Total antioxidant capacity
TBA	Thiobarbituric acid
TBARS	Thiobarbituric acid reactive substances
TCA	Trichloroacetic acid
TE	Tea extract
TPC	Total phenolics content
w/v	Weight per volume

LIST OF FORMULAE

CHAPTER 1

INTRODUCTION

1.1 Introduction

Sabah Tea Plantation (STP) is the largest single commercial tea plantation in Borneo with approximated area of 1,000 acres endowed with *Camellia Sinensis*. It is also one of the very few tea plantations in the world that certified by Control Union World Group. There are two types of farming systems practice in Sabah Tea plantation which are organic and conventional farming systems. Organic farming system is a farming system that based on sustainable productivity without harming the natural environment or human being. While, conventional farming system utilizes high-yield crop cultivars, chemical fertilizers and pesticides, irrigation, and mechanization. Thus, it usually brings negative impacts to the environment such as soil erosion, nutrient runoff, loss of organic matter, and pollution of natural water by agricultural chemicals.

Tea is one of the most widely consumed beverages in the world, next to water (Vinson, 2000). It is prepared as an infusion with the leaves of *Camellia sinensis* (L.), a plant which has been cultivated in over 30 countries across the world that belongs to the Theaceae family (Lopez and Calvo, 2011). Generally, tea contains abundant of phenolic compound which acts as natural antioxidant and responsible for anti-carcinogenic and anti-mutagenic properties. Polyphenols in tea regulate various biochemical processes which are involved in carcinogenesis inhibition of cellular proliferation, angiogenesis, and blockage of tumor cell cycle progression (Han *et al.*, 2007). Basically, antioxidant is defined as substances that can inhibit or delay the oxidation of lipids or other molecules by inhibiting the initiation or propagation of oxidative chain reactions (Velioglu *et al.*, 1998). Generally, mechanism of antioxidant includes transition metal chelating, reducing peroxide, and stimulation of *in vivo* anti oxidative enzyme activities. The health advantage of diets, which rich in antioxidants

degeneration of the body associated with the aging process (Miyachi, 1995).

Recently, plant phenolics have been paid attention as potential natural additives with antioxidant and microbial activities (Pereira *et al.*, 2006). Therefore, phenolic compound is often used to protect food quality by preventing oxidative deterioration of its lipids. Several studies have been conducted on use of plant phenolic compound such as tocopherols, flavonoid compounds, cinnamic acid derivatives, and coumarins to retard or prevent oxidative effect in a peroxidation model system (Jayaprakash *et al.*, 2001).

In shrimp, melanosis is a main cause of deleterious changes in the organoleptic properties, resulting in shorter shelf life, poor quality, as well as financial loss (Montero *et al.*, 2006). Melanosis is caused by the action of polyphenoloxidase (PPO), which oxidizes phenols to quinine and followed by non-enzymatic polymerization, which results as high molecular weight and very dark or black coloring pigment (Benjakul *et al.*, 2005). Additionally, lipid oxidation is another common deteriorative reaction occurs in shrimp apart of melanosis. Lipid oxidation is defined as metabolic process which reactive oxygen species (ROS) cause the deterioration of lipids which may significantly affect cell membrane structure and function.

In order to control of melanosis or lipid oxidation in shrimp, reducing agents such as sulfilting agents and their derivatives are widely used in food industry (Gokoglu and Yerlikaya, 2008). This has increased the consumer awareness in creating safe and effective alternatives to control the melanosis and lipid oxidation. One of the alternatives is by using natural extract of plant origin. Among the natural extracts, tea extract has been considered as excellent melanosis inhibitor due to its phenolic compounds (Banerjee, 2006). Besides that, they are capable of inhibiting lipid oxidation in foods and provide protection against oxidative damage to membrane functions in biological systems.

1.2 Justification

This study is conducted to help in understanding the impact of organic and conventional farming practices on antioxidant activity of tea as well as quality of tea.

Teas are cultivated in diverse environments causing yield and quality differences in their beverage. The presence of active chemical compounds in tea is a main factor to influence the quality of tea (Mudau *et al.*, 2007). Antioxidant activity in teas tends to be different with using different agronomic practices since total amount of tea polyphenol content is vary.

Oxidation processes are among the primary reasons behind the deterioration of product life and quality during storage. Hence, natural antioxidant widely used to protect food quality by inhibiting oxidation besides extending shelf life (Naerin, 2012). It should be emphasized that oxidation is not only give rise quality deterioration in food, but also affect human organism, triggering a number of illnesses and increasing aging. Secondary oxidation of lipid products can cause cross-linking and oxidative modification of proteins hence, adversely affecting the texture of the muscle tissue. Additionally, production of strong rancidity, physiochemical change, and off-flavors, greatly influence consumer acceptance of stored seafood.

Shrimps consider as popular seafood which has excellent nutritional value in terms of lipid profile, as high content of health-promoting nutrients such as polyunsaturated fatty acids, phospholipids and carotenoids. In addition, carotenoids commonly found in shrimps tend to have significant antioxidant activity; thus consumption could bring a beneficial dietary effect. On the other hand, shrimp is a very perishable product, postmortem changes occur rapidly compared with fish, and has limited shelf life due to melanosis. Iced storage or refrigerating does not inhibit, but it only can slow down the development of melanosis (Montero *et al.*, 2004). Therefore, by using tea extracts may become promising melanosis inhibitor especially in conjunction with synergists, retardation or preventing the development of melanosis in shrimp. With this also, it is consider as safe and effective alternatives to maximize the storage stability of shrimp.

1.3 Objectives

This study is conducted to:

1. Compare the effects of farming practices and extraction method on antioxidants content of Sabah tea leaves.

2. Evaluate the effect of organic tea extract on melanosis and lipid oxidation inhibitions on shrimp stored in ice.

1.4 Hypothesis

H_0: There is no significant difference on antioxidants activity of tea extract between the farming practices and extraction method, as well as melanosis and lipid oxidation inhibitions effect in shrimp by using tea extract.

H_A: There is a significant difference on antioxidants activity of tea extract between farming practices and extraction method as well as melanosis and lipid oxidation inhibitions effect in shrimp by using tea extract.

CHAPTER 2
LITERATURE REVIEW

2.1 Classification of Tea

Tea generally can be categorized based on botanical varieties, geographical origin and processing (Mejia *et al.*, 2009). It can be classified into three major types, not fermented (green and white tea), partially fermented (oolong tea) and completely fermented (black tea) according to the level of fermentation. Each kind of tea has its own characteristic flavor and appearance.

2.1.1 Green Tea

Green tea is a non-fermented tea. It acts as the main beverage in China and Japan, however rarely consumed by western people. It is rich in monomeric polyphenols. The major polyphenols compounds found in green tea are flavonoids such as catechin, epicatechin, epicatechin gallate, epigallocatechin gallate and proanthocyanidins. The catechins content in green tea is higher than that in black tea (Cheng, 2004). In recent years, catechins have attracted much attention in relation to their physiological potential as anti-mutagenic and anti-tumorigenic agents (Wang *et al.*, 1989). In addition, catechins have been recognized as efficient antioxidants by scavenging oxygen radicals and chelating metal ions (Chen *et al.*, 1990; Shahidi *et al.*, 1992). The leaves are rolled and steamed in green tea production for minimizing oxidation and denatures polyphenoloxidase. The usual concentration of total polyphenols in dried green tea leaves is around 8 to 12 percent. Other compounds interest in dried green tea leaves include caffeine (3.5%), an amino acid known as theanine (4%), lignan (6.5%), organic acids (1.5%), protein (15%), and chlorophyll (0.5%). One cup of green tea contains approximately 300-400 mg of polyphenols, but only 8-12% of the entire cup will be polyphenols and a smaller portion will be beneficial polyphenol (epigallocatechin gallate).

2.1.2 White Tea

White tea is one of the less studied teas but its flavor is more accepted in Europe than that of green Tea (Almajano *et al.*, 2008). It is the least processed tea, and less ascribed to have the highest content of phenolic compounds (Dias *et al.*, 2013).

White tea is produced from very young tea leaves or buds covered with tiny, silvery hairs, which are harvested only once a year in the early spring. In white tea production, plants materials are steamed and dried immediately after picking to avoid the oxidation occur, and a light and delicate taste had produced. The main catechins present in white tea are epicatechin, epigallocatechin, collectively known as flavanol monomers, while, epicatechin 3-gallate, and epigallocatechin 3-gallate, are known as flavanol gallates (Mejia *et al.*, 2009).

2.1.3 Oolong Tea

Oolong tea is an intermediate variant between green and black tea on a continuum of flavor, color, fermentation and antioxidant content. It is a semi fermented tea with special flavor and quality. Generally, it is sold commercially in the United States and is often served in Chinese restaurant.

Oolong tea possesses four primary polyphenols, which are epicatechin, epicatechin gallate, epigallocatechin, and epigallocatechin gallate. Oolong tea has low concentration of polyphenols than green tea, but a higher concentration than black tea. Oolong polyphenols with lowered blood sugar that can aid in diabetes prevention and treatment, though this test were not conclusive. Besides that, polyphenols in oolong tea may lower cholesterol by inhibiting cholesterol absorption.

2.1.4 Black Tea

Black tea is one of the most common beverages and makes up about 75 % of world tea consumption (Krishnan and Maru, 2006). Black tea is a fully fermented black tea and more popular in North America and Europe. Production of black tea leaves involved extensive enzymatic oxidation of the leaf polyphenols to form dark products such as theaflavins and thearubigens.

The black tea resulted composition mainly depends on the tea process of its production. Therefore, it is difficult to state a definitive composition for black tea beverage, as it varies with different preparations. The major theaflavins in black tea are theaflavin (TF1), theaflavin monogallate A (TF2A), theaflavin monogallate B (TF2B) and theaflavin digallate (TF3).

Black tea has low amount of theaflavin (2-6%) and high thearubigin (20%). Drinking black tea showed similar benefit as in green tea from the perspective of antioxidant capacity since presence of theaflavin in black tea having the similar amount of catechins as present in green tea (Leung *et al.*, 2001).

2.2 Antioxidants

Antioxidants are broadly defined as substances, synthetic or naturally occur that can neutralize free radicals by donating one of the electrons and exist in stable form without transforming to new free radicals after electron donation. These substances are normally found in forms of vitamins, minerals, or enzymes. In simple words, antioxidant activity associated with compounds is capable of protecting a biological system against the harmful effect from the excessive oxidation, which involving reaction of oxygen and nitrogen species (Mogotlane *et al.*, 2007). The antioxidant can found in form of nutrient (vitamins A, C and E), or non-nutrient (lycopene, flavonoid and anthocyanin).

2.2.1 Free Radicals

Free radicals are defined as reactive chemical species with at least one unpaired electron in an outermost shell (Riley, 1994). This unstable configuration created energy and readily reacted with adjacent molecules, such as proteins, lipids, carbohydrates, and nucleic acids. Additionally, free radicals initiated autocatalytic reactions in which molecules that were reacted with free radicals converted themselves into other types of free radicals, thereby propagating a chain of damage.

Oxygen free radicals, also known as reactive oxygen species (ROS) are the oxygen-derived free radicals that damage in biological systems. For example,

superoxide anion radical, hydroxyl radicals and singlet oxygen are the various forms of activated oxygen (Gulcin *et al.*, 2003). In fact, ROS can be both harmful and beneficial in biological systems depending on the environment (Glade, 2003). ROS mediate damage to cell structures at high concentrations, such as lipids and membranes, proteins and nucleic acids. This damage is usually referred as oxidative stress (Poli *et al.*, 2004). The harmful effects of ROS can be balanced with action of antioxidants, some of which are enzymes present in the body (Halliwell, 1996). Oxidative damage is accumulated during the life cycle since the antioxidant defense system hinders oxidative damage from ROS, thus, results as aging and age-dependent diseases such as cardiovascular disease, cancer, neurodegenerative disorders and other chronic conditions (Rahman, 2003).

2.2.2 Mechanism of Antioxidants

Antioxidants can be categorized into primary (chain breaking antioxidant) and secondary antioxidants (synergist) based on action of mechanism. It is important to understand this mechanism in order to classify the food antioxidant.

Primary antioxidants are chain breaking antioxidants such as tocopherols, butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). It is able to inhibit lipid oxidation by interfering at the propagation or initiation phase or in β-scission reactions by accepting free radicals to form stable free radicals. In simple words, it acts as free radical acceptor or hydrogen donors to inhibit the chain reaction. For example,

$$ROO\bullet + AH \longrightarrow ROOH + A\bullet$$

Generally, the primary antioxidant is effective at extremely low concentrations and may become pro-oxidant in high concentration due to their involvement in the initiation reaction.

Secondary antioxidant, or also known as preventive antioxidant does not break free radical chain but are able to act through various mechanisms such as chelators (citric acid, phosphoric acid, EDTA), oxygen scavengers (ascorbic acid, ascorbyl

palmitate, sulfites) and singlet oxygen quenchers (carotenoids). These antioxidants are considered as synergist which can reduce the rate of oxidation by scavenging initiating radical (Frankel, 2005).

2.2.3 Plant Based Antioxidant Compounds

In recent, natural phenolics antioxidants derived from plants such as sage, rosemary, tea and grape seeds have received much interest in their incorporation into foods to control oxidation. Some plant derived antioxidants such as carotene, vitamin C, flavonoids, zinc and selenium can be found in different sources as shown in Table 2.1.

Table 2.1 Some examples of plant based antioxidants with their sources

Antioxidant	Food Source
B-carotene	Carrot, spinach, tomato, and pumpkin,
Vitamin C	Peppers, orange, lemon, and grapefruit, kale
Vitamin E	Aspagarus, lettuce, peas, purslane, and sunflower seed
Lycopene	Tomato, red watermelon
Glutathione	Garlic, potato, and broccoli
Flavonoids	Tea, wine, apple, garlic, onion, and soybeans
Zinc	Celery, asparagus, eggplant and peaches
Selenium	Oatmeal, brown rice and peaches
Anthocyanins	Blackgrapes, cherries, and berries
Resveratrol	Red wine, peanut, red and purple grape juice

Source: International Food Information Council, 2006

Generally, fruit and vegetables are predominately rich in sources of polyphenols, such as carotenoids, vitamins and minerals that can against the oxidative stress and chemoprevention of disease. According to epidemiological studies, consumption of vegetables and fruits can protect humans against oxidative damage by inhibiting free radicals and reactive oxygen species (Ames *et al.*, 1993). For example, phenolic compound in mushroom has been found to be an excellent antioxidant and synergist that is not mutagenic.

Various herbs and spices have been reported possess to antioxidant activity,

such as rosemary, peppermint, onion, ginger, garlic and peppers due to its photochemical compound. For instances, peppers rich of lutein, while ginger contain allicin, oxalic acid and gingerol. Based on ethnopharmacological surveys, the herbs and spices and their purified constituents have shown beneficial therapeutic potentials. Besides that, rosemary extract (Cadun *et al.*, 2008) have been used as the natural safe additives in food industry. Generally, fresh herbs and spices contain higher antioxidant levels compared with after processed. For example, fresh garlic has 150% more antioxidant activity than dry garlic powder.

Antioxidant activity of tea is primarily contributed by its high catechin content. Green tea and black tea leaves are derived from dried leaves of *Camellia sinensis* Linn., which is belonging to the family Theaceae. Green tea has abundant of polyphenols, up to 30% of the dry weight, which include flavonols, flavandiols, flavonoid and phenolic acids. Caffeine, theobromine and theophylline, the principle alkaloids, account for about 4% of the dry weight. Therefore, green tea has been reported to have antioxidant, anti-inflammatory, antimutagenic, antidiabetic, and antibacterial activities (Cabrera *et al.*, 2006).

Beverages such as coffee, wines and fruit and vegetable juices are also sources of phenolic compound. Alcoholic drinks and red wine have high phenolic content due to the presence flavanols, flavonols and anthocyanins (Beecher, 2003). In addition, fruit and vegetable juice blends can aid for hyperglycaemia since the vegetable juice products can deliver natural antioxidants and other bioactive phyto-chemicals in a small volume and offer a convenient method of consumption which is better than overall vegetable consumption (Wootton-Beard *et al.*, 2011).

2.2.4 Applications of Antioxidants

Various applications of antioxidants are being widespread particularly in food industry. For example, it is used as additives in cosmetics, foodstuffs (McCarthy *et al.*, 2001), beverages (Yamaguchi *et al.*, 1998), baking products (Rafecas *et al.*, 1998) as well as dietary supplements (Prior and Cao, 2000). Besides that, it is also used to prevent polymers from oxidative degradation, lubricant from sludge formation, rubber and plastic from losing strength, gasoline from autoxidation, synthetic and natural pigments from discoloration.

Currently, many food manufactures use the commercial antioxidants such as proxyl gallate (PG), T-butyl hydroquinone (TBHQ), butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) as food preservatives. It is typically used for preventing oxidative deterioration of products, maintaining the nutritional value, as well as extending their shelf life. Effectiveness of antioxidants varies depending on the food and conditions of processing and storage. For example, BHA is an effective antioxidant in fats and oils, fat-contained foods, confectionary, essential oils, food-coating materials, and waxes. While, BHT is very effective in animal fats, low-fat food, fish products, packaging materials, paraffin, and mineral oils but is less effective in vegetable oils.

As some of the synthetic antioxidants carcinogenic, thus, many studies are focused on utilizing more effective antioxidants from natural sources. For example, plant phenolic compound such as tocopherols, flavonoid, and cinnamic acid derivatives is used as effective antioxidant in a peroxide model system. Besides that, catechin from tea is recognized as an efficient antioxidant by scavenging oxygen radicals and chelating metal ions (Shahidi *et al.*, 1992). Furthermore, natural extracts rich in antioxidant compounds such as rapeseed oil by-products extracts and red grape marc extracts (Bonilla *et al.*, 1999) have been reported as endogenous antioxidants to stabilize refined oils.

In terms of medical, antioxidants are widely used as ingredients in dietary supplements for health purposes. Although antioxidant supplements can benefit to health, however, excess supplementation may even be harmful. Dietary supplementation has less specific antioxidants as compared to broad diet that rich in phyto-nutrients, which will yield thousands of different polyphenol antioxidants available for metabolism. Besides that, antioxidant is used as alternatives for controlling obesity and promoting weigh loss due to its phenolic compound. Based on study conducted by Hsu and Yen, (2007) o-coumaric acid and rutin have the highest inhibition on intracellular triglyceride (61.3 and 83.0 percent, respectively) among 15 phenolic acids and six flavonoids tested. Therefore, it had showed that antioxidants flushing out of the bad cells that are stored in fat cells and resulted weight loss.

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